

Supply chain losses of vegetables in Central Philippines

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Abstract

This study was designed to benchmark interventions on fresh vegetable losses through key informant interviews, a detailed survey to determine practices and loss estimates, and actual loss measurement. The study used cabbage as a model system and tracked product from the leading vegetable growing area in Cebu, Philippines to retailers 100 km away. Both wet markets (traditional chain) and supermarkets (modern chain) were studied. Traditional and modern retail chains had similar players: farmers, commission agents, wholesalers and retailers. The total losses for cabbages were estimated at 26 to 27% in both chains. However, farmers in the modern chain incurred higher losses (13%) than farmers in the traditional chain (10%) due to more stringent quality requirements. In contrast, retailers in the traditional chain incurred higher losses (10%) than in supermarkets (7%). Wholesalers in both chains had losses of 6 to 7%. Losses were mainly due to insect damage and rotting at the farm level, with weight loss and physical damage the major issues at the wholesale and retail level. Much of this loss was due to poor packaging and handling techniques. The total loss of edible material was calculated based on the sum of weight loss and trimming of damaged, wilted and/or rotten parts. This revealed much higher losses of 34% in the traditional chain. Nearly half of this (16%) occurred during retail, which could take 3 days at ambient temperatures. In contrast, only 9% loss occurred during retail in the modern chain. In both cases, transport resulted in 12% of total loss. Partly because of these losses, wholesale prices were about 40% higher than farm gate price in both chains, with retail prices about 60% and 190% higher than farm gate prices in the traditional and modern chain, respectively. Based on these results, technological improvements for testing and organizational interventions are recommended.

Keywords: cabbage, postharvest loss, traditional chain, modern chain

INTRODUCTION

Cabbage is considered one of the top twenty vegetables in the Philippines. It is the fifth leading vegetable and the number one leafy vegetable with production approaching 125,310 tonnes from an area of 8,550 hectares (BAS, 2013). However, cabbages have to be transported over long distances from the production areas to the market. Poor handling consequently results in significant product losses. The high perishability of cabbage exacerbates the problem.

The extremely perishable nature of vegetables results in the inability of producers to manage supply in the assembling markets. Furthermore, the long distances that separate the production area and markets, and poor postharvest management contribute to the huge postharvest losses of vegetables which are estimated to exceed 30 to 40%.

To effectively reduce postharvest losses, it is recommended that a supply chain approach be adopted, in which the whole supply chain must be considered in determining appropriate interventions, rather than a piecemeal approach in which only one stage in the chain is targeted for intervention. The supply chain starts from the collection of the harvested produce at the farm gate, through processing or preparation, distribution and



finally display for retail sale to consumers (Batt and Cadilhon, 2007).

In many developing countries, the fresh produce industry is hampered by the lack of efficient and effective supply chains. In these countries, supply chains are generally fragmented, unorganized and losses in product quality and quantity are usually high. These losses are most often absorbed by farmers as reduced farm gate prices and by consumers through an increased purchase price (Acedo, 2010; Rapusas and Rolle, 2009).

For cabbages produced in Mantalongon, Dalaguete, Cebu, traditional and modern supply chains have not been assessed. This study was conducted to map the traditional and modern supply chains for locally produced cabbage and to assess postharvest losses in traditional and modern chains in Cebu.

MATERIALS AND METHODS

An upstream approach (from retailers to farmers) was employed (Genova et al., 2006). The selected markets were the Carbon market, the biggest wholesale-retail market in the Visayas for the traditional chain, and selected supermarkets for the modern chain. A key informant survey using reliable reference points (agricultural technicians and key traders/wholesalers, retailers and farmers) was conducted to construct the supply chain map. A detailed interview was then conducted using a pretested structured questionnaire. This approach enabled the collection of qualitative and quantitative data (Prinsloo, 2000).

Following the sample size principle (Van Der Vorst, 2000), 35 respondents (15 farmers, 5 collectors, 5 wholesalers and 10 retailers) were randomly selected for the traditional chain and 28 respondents (15 farmers, 5 traders/collectors, 5 wholesalers and 3 supermarkets) for the modern chain (farm to supermarkets).

Survey results were analyzed using the STAR program and reported as frequencies and averages. Product losses were estimated at each stage of the supply chain.

RESULTS AND DISCUSSIONS

Supply chain map

Cabbages produced in Mantalongon, Dalaguete, Cebu, were transported to the Mantalongon Trading Centre and retailed at the Carbon market in Cebu City. The traditional supply chain involved five players: farmers in Mantalongon and nearby barangays; commission agents and wholesalers in the Mantalongon Trading Centre; and wholesalers and retailers in the Carbon market. Farmers brought the harvested cabbages to the trading centre, about 2.7 km away from their farms. Upon arrival, cabbages were weighed by commission agents and transferred to wholesalers. The wholesalers arranged transport of the packed cabbages, usually by big truck and/or by mini bus, to retailers in the Carbon market, about 98 km away, or 3.1 hours travel on average. Upon arrival in the Carbon market, wholesalers distributed the cabbages to retailers and nearby wet markets in the city. Aside from Cebu City retailers, wholesalers also catered to retailers in other municipalities of Cebu province and other provinces outside Cebu.

The modern supply chain had four main players: the farmers in Mantalongon and nearby barangays; commission agents and wholesalers in the Mantalongon Trading Centre; and supermarkets within Cebu City. Farmers delivered the cabbages to the trading centre from an average distance of 3.8 km away. The cabbages were usually packed in sacks and bamboo baskets with the capacity of 125-130 kg and 90-120 kg, respectively. Produce was transported mostly by multicab, big trucks, or manually carried, and partly by motorcycle and tricycle. In the trading centre, the commission agents handled the cabbages and sold them to wholesalers who bought the cabbages for distribution to retailers in the city. The wholesaler shouldered the cost for trimming, cleaning, packaging, and the transportation of the produce. The packed cabbages were delivered to the Carbon market. Upon arrival in Cebu City, trimming and sorting were done before the cabbages were individually wrapped with cling wrap. The wholesalers then transported the cabbages to supermarkets using public transport. The distance was some 2.3 km travel and 8 minutes travel time from the Carbon market on average (Figure 1).

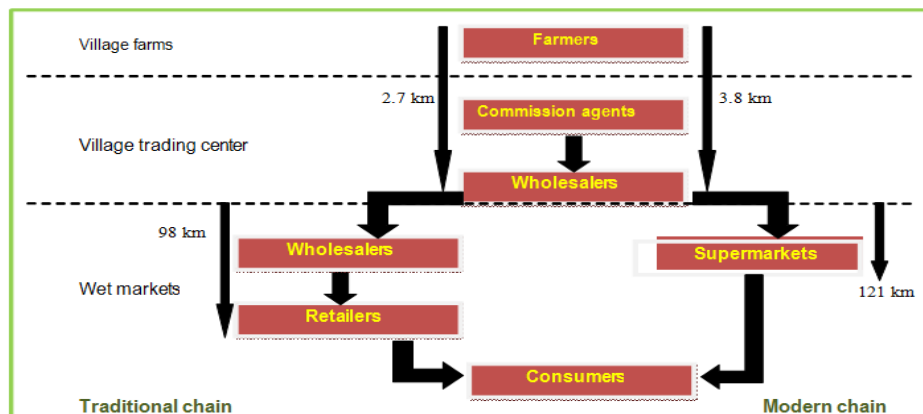


Figure 1. Traditional and modern supply chain map of cabbage in Cebu, Philippines.

Demographics of supply chain actors

Farmers in both traditional and modern supply chains were female-dominated. The average farm size was 0.7-1.02 ha, with farmers producing more than 5 tonnes of cabbage per crop. Farmers in modern supply chains were older and had more farming experience, but were less educated than those in the traditional supply chain.

On the other hand, commission agents, wholesalers and retailers in the traditional chain were female-dominated, relatively younger, with college education or a college level education. They generally had more business experience than those in the modern chain, except the supermarket retailers who were college graduates. Commission agents traded the biggest volume of produce in both traditional and modern chain with about 2 tonnes per day because they dealt with a large number of wholesalers. Retailers traded the smallest volume of produce, with an average of 173.6 kg per day in the traditional market and 66.7 kg per day in the supermarkets.

In the modern supply chain, commission agents and supermarket retailers were male dominated, while wholesalers were female dominated. Commission agents and wholesalers were older and less educated (40% had not finished elementary school) as compared to the supermarket retailers who were young (23 years old on average) and had finished college. The modern supply chain had been in existence for almost twenty years (Table 1).

Postharvest practices

Harvesting cabbages was based on the age of the plant, firmness of the head, or a combination thereof (Figure 2). Where harvesting was delayed, cabbages stayed for 4.1-4.5 days in the field, which caused over maturity and head cracking. Harvesting was done by cutting the butt-end of the cabbage with an ordinary knife. The frequency of harvesting did not vary greatly between the traditional and modern chain. However, traditional chain farmers harvested the cabbage more frequently (4.5 times) than modern chain farmers (4.1 times). Immediately after harvesting, farmers in both chains sold the cabbages in the trading centre.

Postharvest operations

Postharvest operations in traditional and modern supply chains included trimming, cleaning, sorting, packaging, storage and transport (Table 2). In the traditional chain, farmers trimmed/cleaned (80%) and sorted (40%) the harvested produce in the field before bringing it to the market. Trimming/cleaning was done by the removal of all outer leaves and trimming the butt-end of the cabbage using an ordinary knife. Cabbages were sorted based on freedom from pest damage and over maturity on the ground, which could provide a rich source for spoilage and human pathogens. After trimming/cleaning and sorting, the cabbages were packed into 90-125 kg capacity bamboo baskets, which were then delivered to the Mantalongon Trading Centre using motorcycles ('habal-habal'), tricycles, or they were

manually carried. No refrigerated vehicle was used to transport the produce in both traditional and modern chains. Upon arrival, the packed cabbages were weighed, which served as the basis of payment to farmers. Commission agents then sold the produce to wholesalers. There was no postharvest operation at the commission agent level.

Upon receipt, wholesalers re-trimmed the cabbages by removing damaged leaves and any protruding butt, and sorted them visually based on freedom from pest damage and physical damage. After trimming and sorting, cabbages were individually wrapped with newsprint, placed in bamboo baskets or sacks, and transported to the Carbon market by truck ('trak de karga'). Upon arrival in the Carbon market, wholesalers either distributed the cabbages by hired labour to adjacent retailers, usually just a few metres from their stall, or retailers purchased the cabbages from wholesalers and brought the produce to their respective selling area. In the retail market, cabbages were re-trimmed, packed using bamboo baskets and sacks, and stored under ambient conditions while waiting for buyers.

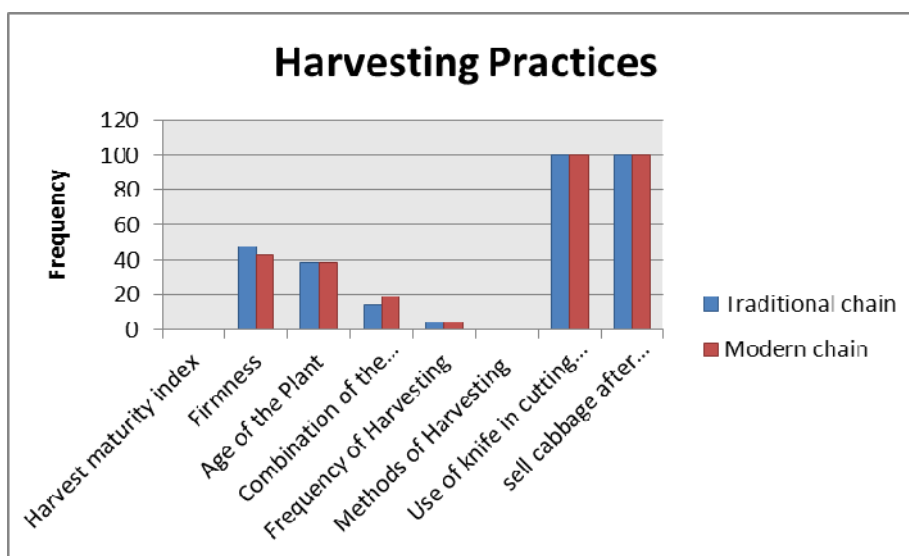


Figure 2. Postharvest practices of farmers in traditional and modern supply chains of cabbage (frequency, %).

Table 1. Demographic characteristics of traditional and modern supply chain actor for cabbage in Cebu, Philippines.

	Traditional chain					Modern chain				
	Farmers	Commission agents	Wholesalers	Retailers	Farmers	Commission agents	Wholesalers	Retailers	Wholesalers	Retailers
Gender (male:female ratio)	27:73	20:80	20:80	40:60	47:53	60:40	40:60	40:60	40:60	100:0
Age (years)	48.5	45.8	43.6	45.6	49.1	48.8	47.8	47.8	47.8	23
Elementary graduate	20				7					
Elementary level	53	60	40	60	80	40	40	40	40	
High school graduate	7	20				20				
High school level	20		40	30	13	20	40	40	40	
College level				10						
College graduate		20	20						20	100
Vocational										
None						20				
Years in farming/business	22.1	23.2	23.4	11.4	28.5	19.4	16.2	16.2	16.2	16.3
Farm size	1.02				0.7					
Traded volume	6.6 t/season	1.9 t/day	310 kg/day	173.6 kg/day	5.2 t/season	1.8 t/day	224 kg/day	224 kg/day	224 kg/day	66.7 kg/day

Table 2. Postharvest operations of traditional and modern supply chain actors (frequency, %) and description of operation.

Post-harvest operations	Commission agents		Wholesalers		Retailers	
	Farmer	Traditional chain	Wholesalers	Traditional chain	Retailers	Retailers
Trimming/cleaning	80	0	100	100	100	100
Sorting	Trim butt, remove outer leaves 40	0	Trim butt, remove damaged leaves 62.5	Trim butt, remove damaged leaves 40	Trim butt, remove damaged leaves 40	Trim butt, remove damaged leaves 40
Special treatments	No pest damage (100%) Overmature (33%)	0	No pest damage (60%) No physical damage (40%)	No pest damage (60%) No physical damage (40%)	No pest damage (53%) No physical damage (47%)	No pest damage (53%) No physical damage (47%)
Packaging	0	0	0	0	0	0
	100	0	100	100	100	100
	Bamboo basket		Bamboo basket (60%) Sacks (40%)	Bamboo basket (40%) Sack (60%)	Bamboo basket (40%) Sack (60%)	Bamboo basket (40%) Sack (60%)
Transport	86.7	0	100	100	100	100
	Motorcycle (33%) Multicab (7%) Large truck (7%) Carry by man (33%) Tricycle (20%)		Wrapped newspaper (100%) 100	Large truck	Carry by man	Carry by man
Storage	0	0	0	0	100	100
					Ambient temperature until sold	
Modern chain						
Trimming/cleaning	80	0	100	100	100	100
Sorting	Trim butt, remove outer leaves 53.3	0	Trim butt, remove damaged leaves 0	Trim butt, remove damaged leaves 40	Trim butt, remove damaged leaves 40	Trim butt, remove damaged leaves 40
Special treatments	No pest damage (100%) Overmature (20%)	0	0	No pest damage (40%) No physical damage (60%)	No pest damage (40%) No physical damage (60%)	No pest damage (40%) No physical damage (60%)
Packaging	0	0	0	0	0	0
	100	0	100	100	100	100
	Bamboo basket (93%) Sack (7%)		Bamboo basket (60%) Sack (40%)	Bamboo basket (60%) Sack (40%)	Individually wrapped (foodwrap)	Individually wrapped (foodwrap)
Transport	93.3	0	100	100	100	100
	Motorcycle (13%) Multicab (27%) Large truck (27%) Carry by man (20%) Tricycle (13%)		Wrapped newspaper (100%) 100	Large truck	Public car	Public car
Storage	0	0	0	0	100	100
					Refrigerated storage until sold	

In the modern supply chain, the same postharvest operations were performed as in the traditional chain: the butt was trimmed and all outer leaves were removed; sorting was based on freedom from pest damage and over maturity; and the heads were packed mostly into bamboo baskets and in a few cases into sacks. The produce was then transported to Mantalongon Trading Centre by multi-cab, truck or manually carried (Table 2). Upon arrival, the cabbages were weighed. After the commission agents and wholesalers had agreed on a price, the wholesalers re-trimmed the cabbages which were then wrapped individually in newsprint before packing in bamboo baskets or sacks. The packed cabbages were then transported to the Carbon market by truck. Refrigerated transport was absent.

Neither the traditional nor the modern supply chain had any special treatment to control bacterial soft rot. In the Carbon market, wholesalers re-trimmed, re-sorted and re-packed the cabbages using individual shrink wrap before delivery to supermarkets, usually by public transport. In the supermarkets, cabbages were displayed for sale under refrigerated conditions.

Cabbage prices were dictated by commissioners, wholesalers and retailers in both chains. Pricing was based on whether the cabbages were of good quality with no damage, wilted and/or rotten parts, insect damage and physical damage. In transporting the cabbages, both traditional and modern chains employed big trucks, which contributed to high losses due to mechanical damage, weight loss and the build-up of micro-organisms inside the package due to high temperatures and humid conditions. A major marketing constraint was the poor infrastructure that contributed to huge losses of produce.

Postharvest loss

The total loss of cabbage was similar in both traditional and modern supply chains (26%) (Table 3). However, farmers in modern supply chains incurred a higher loss of 13% compared to traditional chain farmers who had a 10% loss. Losses were primarily due to pre-harvest origin, including insect damage and rotting/decay due to rain. No losses were recorded at the commission agent's level, for no postharvest operation was performed: the cabbages were merely transferred from the farmers to the wholesalers at the trading post.

Losses at the wholesaler and retailer level differed with the supply chain. In the traditional chain, wholesalers incurred a 7% loss due to weight loss and mechanical damage as a result of improper handling and poor packaging during transportation. Retailers incurred a 9.6% loss on average, much higher compared to the wholesaler's, due to physical damage and weight loss resulting from the improper handling of fresh produce and poor temperature and humidity control. Loss occurred in the form of trimmings or whole cabbage rejection at the farmer level, while at the wholesaler and retailer levels, trimming contributed more to losses.

In the modern chain, wholesalers incurred an average loss of 6% and retailers an average loss of 7.2% (Table 3). Poor transport was the main contributory factor to the loss in both chains. Losses at the wholesaler level were due to weight loss and mechanical damage as a result of improper handling and poor packaging during transportation. Physical damage and soft rot were among the main causes of loss at the retailer level.

Cabbage prices along the chain varied greatly. The farm gate price was slightly higher in the modern chain (PhP 27.3 kg⁻¹) than in the traditional chain (PhP 26.7 kg⁻¹). Commission agents in both chains received a PhP 1.0 kg⁻¹ commission for facilitating the transaction between growers and wholesalers. No postharvest operations were performed. Wholesalers in both chains added about PhP 11 kg⁻¹ to the price given to commission agents, however, they shouldered the costs of postharvest operations and transport to retailers. The biggest discrepancy in price was obtained at the retail level, where the price in the traditional chain (PhP 42.7 kg⁻¹) was half that in the modern chain (PhP 78.3 kg⁻¹).

Table 3. Postharvest loss of cabbage in the traditional and modern supply chains in Cebu, Philippines.

Supply chain actor	Postharvest loss (%)	Causes of loss	Reason for loss	Nature of loss	Price of cabbage (PhP)
Farmers	10.1	Insect damage Rotting/decay	Traditional supply chain Lack of reliable maturity indices	Trimming/cleaning Rejection	26.7
Commission agent Wholesaler	0 6.8	Weight loss Mechanical damage	Poor use of pesticides Improper handling	Trimming/cleaning	27.7 37.7
Retailer	9.6	Weight loss Physical damage	Poor packaging during transport Improper handling Poor temperature and humidity control	Trimming/cleaning	42.7
Total loss	26.5				
Farmers	13.2	Insect damage Rotting/decay	Modern supply chain Poor use of pesticides	Trimming/cleaning rejection	27.3
Commission agent Wholesaler	0 6.0	Weight loss Mechanical damage	Improper handling Poor packaging during transport	Trimming/cleaning	28.3 38.3
Retailer	7.2	Physical damage Soft rot	Improper handling	Trimming/cleaning	78.3
Total loss	26.4				

CONCLUSION

Farmers received the lowest profit in both chains. Postharvest losses of cabbages were about one quarter of the total volume. These losses were passed onto farmers in the form of low farm gate prices and onto consumers in the form of a high retail price. Appropriate technological interventions to reduce losses should translate to higher farm gate prices with increased financial returns of farmers.

The traditional chain was more complex than the modern chain. Nevertheless, practices in both chains were far from desirable. Postharvest losses did not differ much between the two chains, but the modern chain had more stringent quality requirements with a retail price almost two times higher than the traditional chain.

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Efficacy of guava and mangosteen extracts in reducing soft rot (*Pectobacterium carotovorum*) in harvested Chinese cabbage

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Abstract

The effect of mature guava leaf and mangosteen rind extracts was evaluated in soft rot (*Pectobacterium carotovorum*)-inoculated butt-ends of Chinese cabbage heads. Chemical extraction (with methanol and ethanol) exhibited higher antibacterial properties in the bioassay compared to the mechanically produced extracts. The inhibition zones observed with five concentrations (100, 250, 500, 750 and 1000 µg mL⁻¹) of guava leaf and mangosteen rind extracts did not vary but were significantly better than distilled water. Newly-trimmed Chinese cabbage heads were inoculated with the soft rot organism 24, 48 and 72 h followed by air-drying for 30 min before treatment with 10% alum (positive control); distilled water (negative control); and extracts from mature guava leaf (1000 µg mL⁻¹) or mangosteen rind (500 µg mL⁻¹). In the bioassay, the mangosteen rind extract exhibited a greater diameter of inhibition than the guava extract but this was lesser *in vitro*. The two extracts very slightly extended shelf life of heads held under ambient conditions (26.2±0.30°C, 84.8±2.0%RH) by one day. On the other hand, the 10% alum treatment extended shelf life to twice that of the control. Delaying inoculation on butt-ends trimmed 72 h earlier delayed onset of disease by one day. This study confirms efficacy of 10% alum in reducing soft rot in Chinese cabbage butt-ends. Further assessment is needed to evaluate other concentrations of the two plant extracts.

Keywords: alum, guava leaves, mangosteen rind, *Pectobacterium carotovorum*, soft rot

INTRODUCTION

Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) is an annual and cool-season crop (Stephens, 2015). It is considered as one of the most valuable crops in East Asia including Philippines, as it is known to be a good source of nutrients. In the Philippines, it contributed around P 1.08 B revenue to local economy (PCAARRD, 2003).

Various problems may arise during postharvest operations, one of which is soft rot infection. Soft rot is a disease commonly caused by the bacterium *Pectobacterium carotovorum* (Nazerian et al., 2011). Infection occurs when conditions become favorable for bacterial growth, such as high relative humidity, high temperature and a wet environment. *Pectobacterium carotovorum*, formerly known as *Erwinia carotovorum*, can effectively induce cellular maceration by releasing enzymes that can degrade the cell walls (Davidsson et al., 2013).

Natural agents with antibacterial properties to inhibit infection could be a practical way to mitigate this postharvest disease. Two potential agents are guava leaf and mangosteen rind extracts. The use of these natural plant extracts as an alternative antibacterial control may satisfy consumer demand for food nutrition (Tiwari et al., 2009). Guava (*Psidium guajava*) leaf extract is known to exhibit antibacterial activity (Gnan and Demello, 1999; Biswas et al., 2013). One component that provides this property of guava leaf is tannin. Tannins are known to have antiseptic properties that can inhibit the growth of

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disease-causing microorganisms (Godstime et al., 2014). On the other hand, mangosteen (*Garcinia mangostana*) has broad-spectrum antibacterial components such as α -mangostin and xanthones, which are known to exert potent antimicrobial activity in human pathogens (Torrungruang et al., 2007; Priya et al., 2010). Among plant extracts, only lemon grass, garlic, *Aloe vera*, neem (leaf, bark seed) are commonly studied in controlling plant pathogens (Simeon and Abubakar, 2014; Nweke and Ibiam, 2012; Bdliya and Dahiru, 2006). Mangosteen leaf extracts were reported to display antibacterial activity against plant pathogens such as *X. oryzae* and *P. syringae* (Alsultan et al., 2016). The extracts of the guava leaf and mangosteen rind extracts are not well reported as a biocontrol to plant pathogens. The antibacterial properties of the two extracts could be used as potential bioagents in controlling plant pathogens.

Hence, in this study, guava leaf and mangosteen rind extracts were assessed for their control of soft rot in Chinese cabbage compared with postharvest alum treatment. The effect of time of inoculation of the soft rot organism followed by drying and application of extracts after 24, 48 and 72 h from harvest on soft rot development in the Chinese cabbage butt-end was evaluated.

MATERIALS AND METHOD

Chinese cabbage heads and mangosteen fruit were obtained from Bankerohan Public Market, while the guava leaves of the native variety were picked from ALCON Farm, Los Amigos, Tugbok District, Davao City. For the mechanical extraction, an HA100W Hurom Masticating Slow Juicer was used to mechanically extract guava leaf and mangosteen rind. The extracts were each diluted with water to produce various concentrations (10, 25, 50, 75 and 100%). For the chemical extraction, mature guava leaves (130.5 to 191.0 mm long, dark green), and mangosteen rind from reddish purple fruit were washed separately with water, dried and powdered. A Soxhlet apparatus and a rotary evaporator were used to obtain the crude extracts prior to the preparation of dilutions to produce concentrations of 100, 250, 500, 750 and 1000 $\mu\text{g mL}^{-1}$. Methanol and ethanol were used as solvents for the guava leaf and the mangosteen rind, respectively.

An assay for antibacterial activity was performed on the extracts at each concentration. A bacterial suspension of *P. carotovorum* was prepared using the method described by D'Amato and Hochstein (1982). Disc diffusion assay, the method of Murray et al. (1995) was employed. The diameters of inhibition were measured and compared. The concentrations with higher inhibitory action were tested next on butt-ends of Chinese cabbage heads.

Seventy-two Chinese cabbage heads of uniform quality and size were selected. After harvest, three to four leaves of Chinese cabbage were trimmed to remove senescing or damaged outer leaves. Butt-ends were uniformly trimmed prior to the experiment. The samples were divided into three groups, for the three periods (24, 48 and 72 h) of inoculation followed by application of extracts. At each period (24, 48 and 72 h after harvest) the butt-ends were inoculated with the 24-h old *P. carotovorum*. This was followed by treatment with extracts 30 min after inoculation. Ten percent alum and distilled water served as controls. The concentrations used for guava and mangosteen rind extracts were 1000 and 500 $\mu\text{g mL}^{-1}$, respectively. The Chinese cabbage samples were stored for eight days at 25.5-26.6°C and 76.3-88.7% relative humidity. The Chinese cabbage leaves and butt-end were separately assessed daily on four parameters: the degree of decay (DoD) scale, where 1 = no infection, 2 = 1-10% infection (slight), 3 = 11-25% infection (moderate), 4 = 26-50% infection (moderately severe), and 5 = more than 50% infection (severe); days to initial occurrence of decay; visual quality using the scale: 1 = excellent (fresh), 2 = very good (slight defects), 3 = good (limit of saleability, defects progressing), 4 = fair (useable but not saleable), and 5 = poor; and shelf life, where the sample was terminated when the butt-end or leaves reached a visual quality rating of 4. The experiment was laid out in a completely randomized design (CRD) with six replications. The data were analysed using analysis of variance (ANOVA) and least significant difference (LSD) test for comparison of means.

RESULTS AND DISCUSSION

Table 1 compares young and mature guava leaves and mangosteen rind or pericarp in a laboratory assay. Young and mature guava leaf extracts exhibited similar antibacterial activity because they contain almost the same amount of alcohol-and water-soluble active compounds (Shruthi et al., 2013).

Table 1. Diameter of inhibition exhibited by different extracts obtained using mechanical and chemical extraction methods.

Solvent	Diameter ² (mm)			
	Young guava leaf	Mature guava leaves	Guava (mean)	Mangosteen
Water	0.69	0.25	0.47 ^b	5.06 ^b
Alcohol ¹	1.56	2.53	2.05 ^a	7.33 ^a
Mean	1.12 ^A	1.39 ^A		

¹Methanol was used on guava leaf; ethanol on mangosteen rind.

²Means with common letter in a column (solvent) or in a row (source of extract) indicate no significant difference at $P \leq 0.05$ using LSD.

The aqueous extract used water as a solvent, while the chemical extraction used alcohol. Chemically-obtained extracts exhibited a larger diameter of inhibition (2.05 mm for guava extracts and 7.33 mm for mangosteen) compared to the mechanical extracts (Table 1). Alcohol extracts performed better than those mechanically extracted by 77.07% (for guava leaf extract) and 30.97% (for mangosteen rind extract).

Unlike water, which only has affinity for polar substances, alcohol (i.e., methanol and ethanol) is a better solvent for bioactive components because it exhibits affinity for both polar and non-polar substances (Sanches et al., 2005). This suggests that higher concentrations of polyphenols and other metabolites may have been retained through chemical extraction compared to mechanical extraction. Polyphenols may have contributed to the action of extracts against soft rot. Another study has shown that in guava leaf extracts, the activity of antibacterial agents is dependent on its concentration (Mailoa et al., 2014).

However, among the five concentrations (100, 250, 500, 750 and 1000 $\mu\text{g mL}^{-1}$) of guava leaf and mangosteen rind extracts, no significant differences were observed because of the narrow differences between concentrations (Table 2). Distilled water and 10% alum served as the negative and positive control, respectively. Both extracts were significantly better than water. Soft rot-infected cabbage heads produced soft, water-soaked lesions with a strong foul odor (Bhat et al., 2010). The diameter of inhibition in distilled water was 0 mm. Alum, the positive control, on the other hand, yielded a diameter of 13.02 mm. This was 262% higher than the 1000 $\mu\text{g mL}^{-1}$ guava leaf extract and 55% higher than 500 $\mu\text{g mL}^{-1}$ mangosteen rind extract. Compared to mangosteen, guava leaf extract showed weak inhibition. The guava leaf extract was reported to show weak antibacterial activities against gram negative (*E. coli* and *P. aeruginosa*) compared to gram positive (*B. subtilis* and *S. aureus*) (Maria et al., 2013). *Pectobacterium carotovorum* is a gram negative bacteria which may have contributed to the weak inhibition of guava extract.

As days in storage increased, soft rot progression was minimized when treated with 10% alum (Table 3) Soft rot was severe in the distilled water treatment. There was no decay observed in days 0 and 1 in 48 h; and days 0, 1 and 2 in 72 h as these samples have not been inoculated yet with soft rot. Within 24 h of inoculation, infection in the butt-ends of Chinese cabbage was observed as shown in days 1, 2 and 3 for 24, 48 and 72 h of inoculation, respectively. Among treatments, only alum and mangosteen showed decay at day 1. Unlike guava and mangosteen extracts, alum was able to effectively minimize decay in later days of storage. At day 5, all Chinese cabbages in the distilled water control were observed to exhibit severe decay. Also, heads were discarded from day 5, hence only the remaining Chinese cabbages were rated for decay. The interaction between the time of inoculation and treatment was only significant for days 2 and 8. This indicates that if the two factors (time of inoculation and treatment) were combined, there would be a significant difference in their

effects towards soft rot progression. This could be expected as time of inoculation could affect the response of Chinese cabbage to soft rot. Starting at day 5, decay ratings were those of the remaining heads of Chinese cabbage.

Table 2. Diameter of inhibition as influenced by different concentrations of chemically extracted mature guava leaf and mangosteen rind extracts.

Treatment	Diameter ¹ (mm)	
	Mature guava leaf	Mangosteen rind
Water (negative control)	0.00 ^c	0.00 ^c
10% Alum (positive control)	13.02 ^a	13.02 ^a
100 µg mL ⁻¹	2.15 ^b	6.55 ^b
250 µg mL ⁻¹	1.86 ^b	7.44 ^b
500 µg mL ⁻¹	2.21 ^b	8.38 ^b
750 µg mL ⁻¹	2.84 ^b	7.64 ^b
1000 µg mL ⁻¹	3.59 ^b	6.65 ^b

¹Means with a common letter in a column indicate no significant difference at P≤0.05 using LSD.

Table 3. Degree of decay of the butt-end of the Chinese cabbages.

Factor	Degree of decay ¹								
	Days of storage								
	0	1	2	3	4	5	6	7	8
Inoculation time (h)									
After 24 h	1.00 ^a	1.13 ^a	2.42 ^a	3.50 ^a	4.04 ^a	3.33 ^a	3.00 ^a	1.00 ^a	1.00 ^b
After 48 h	1.00 ^a	1.00 ^b	1.54 ^b	2.75 ^a	4.04 ^a	3.55 ^a	2.83 ^a	2.33 ^a	2.67 ^a
After 72 h	1.00 ^a	1.00 ^b	1.00 ^c	1.96 ^b	2.86 ^b	3.46 ^a	3.61 ^a	2.89 ^a	1.67 ^{ab}
Treatment									
10% Alum	1.00 ^a	1.06 ^a	1.28 ^b	1.72 ^c	2.33 ^b	1.27 ^b	1.08 ^b	1.33 ^b	1.00 ^b
Water	1.00 ^a	1.00 ^a	2.28 ^a	3.89 ^a	4.61 ^a	5.00 ^a	5.00 ^a	-	-
Guava	1.00 ^a	1.00 ^a	1.44 ^b	2.56 ^{bc}	3.83 ^a	3.82 ^a	4.17 ^a	2.83 ^a	2.50 ^a
Mangosteen	1.00 ^a	1.11 ^a	1.61 ^b	2.78 ^b	3.83 ^a	4.27 ^a	4.10 ^a	3.50 ^a	3.00 ^a
Interaction	ns	ns	*	ns	ns	ns	ns	ns	*

¹Means with common letter(s) in a column indicate no significant difference at P≤0.05 using LSD; * significant at P≤0.05; ns-not significant.

The days to initial occurrence of decay was defined as the number of days it took for the first sign of rotting to be observed from the day of harvest (DoD equal to 2). The alum treatment showed symptoms of disease at 6.6 days, while the negative control only took 2.7 days; similar to the guava leaf and mangosteen rind extract treatments (Table 4). As shown in Table 4, the alum treatment performed best. The symptoms of the disease were delayed by four days with the use of alum.

The action of alum in reducing decay led to better visual quality ratings of the butt-ends of the Chinese cabbage heads. At four days from treatment, the positive control (10% alum) gave the best visual quality at 2.3, while the negative control (distilled water) showed the poorest quality at 4.3 (Table 4).

The results of the study confirmed the efficacy of 10% alum in reducing soft rot incidence. Bayogan et al. (2009) reported a reduction of soft rot by 34.4% in transported cabbage using 10% alum. For other bacteria such as *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli* and *Klebsiella pneumonia*, the minimum inhibitory concentration of alum was at 20% (Bnyan et al., 2014). The mode of action of alum against various microorganisms is not well studied. It was reported that alum salts such as aluminium chloride caused severe damage on bacterial membrane (Mills et al., 2006). Acedo and Weinberger (2009) reported that alum acts both as an antimicrobial agent and as a moisture-withdrawing substance. The

desiccating effects of alum can be seen in the dried appearance of the alum-treated butt-end, in which bacteria were unable to proliferate.

Table 4. The visual quality at four days, time to occurrence of disease and shelf life of inoculated Chinese cabbages treated with alum and extracts from mature guava leaves and mangosteen rind¹.

Factor	Visual quality, 4 d	Number of days to occurrence of disease	Shelf-life (days)
Inoculation time (h from harvest)			
After 24 h	3.92 ^a	3.46 ^b	4.67 ^a
After 48 h	3.60 ^a	3.63 ^b	5.04 ^a
After 72 h	2.63 ^b	4.92 ^a	5.63 ^a
Treatment			
10% Alum	2.28 ^b	6.56 ^a	7.22 ^a
Water	4.30 ^a	2.67 ^b	3.61 ^c
Guava	3.50 ^a	3.50 ^b	4.83 ^b
Mangosteen	3.44 ^a	3.28 ^b	4.78 ^{bc}
Interaction	ns	ns	ns
Standard error	0.19	0.23	0.21

¹Per factor, means with common letter(s) in a column indicate no significant difference at $P \leq 0.05$ using LSD; ns-not significant.

Though not significant with mangosteen extract, the inhibitory effect of guava improved the shelf life of Chinese cabbage by one day compared to the negative control. However, this was not comparable to the efficacy of alum. Several active phytochemical components of guava extracts include saponins, flavonoids, tannins, eugenol and triterpenoids (Mohamed et al., 1994; Mailoa et al., 2014; Godstime et al., 2014; Bisht et al., 2016).

The concentration of secondary metabolites and the antibacterial inhibitory properties of plants differ among plant parts (Packialakshmi and Naziya, 2014). In mangosteen, Palakawong et al. (2013) reported that leaves had higher phenolics than the pericarp and bark at different temperatures. In addition to other factors, this could account for the lower activity of mangosteen rind extracts against soft rot. In the bioassay, mangosteen rind seems to have greater inhibition zone, but it had lesser action when applied in vivo to Chinese cabbage heads compared to guava leaf extracts. This is probably because the inhibition zone does not indicate the death of bacteria, thus it cannot identify the bactericidal and bacteriostatic effects of extracts (Balouiri et al., 2016). Disk-diffusion is still widely used in plant extracts as it is a simple and cheap method that can be used in a wide range of microorganisms and antimicrobial agents (Balouiri et al., 2016). Other significant properties of guava are its high content of antioxidant, ascorbic acid and phenolic acid (Iamjud et al., 2014). These properties may have positive effect on the butt-end of Chinese cabbage which probably contributed to the efficacy of guava leaf extracts when applied in vivo.

Soft rot progression was faster for Chinese cabbage inoculated with the *P. carotovorum* 24 h after trimming, followed by inoculation after 48 h, with the slowest being those inoculated after 72 h. The longer time before inoculation may allow wound healing in Chinese cabbage inoculated with *P. carotovorum* after 72 h. The butt-end of the Chinese cabbage heads inoculated with *P. carotovorum* after 24 h may not have completely healed relative to the 72 h inoculation.

The difference in the rates of decay progression may be attributed to the fact that the butt-ends of the Chinese cabbages were all cut at day 0. Open wounds provide more sites for bacteria to enter, thus increasing the rate of lesion development (Bruton, 1994; Barth et al., 2009). Crops however have the capability to self-heal wounds. According to Morris et al.

(1989) wound healing in plants is optimally performed under room temperature (25°C) and high relative humidity (98%). These conditions, particularly the temperature, were quite close to the storage conditions of this experiment (26.18°C and 84.75% relative humidity). The wound healing of Chinese cabbage is also associated with its resistance to various postharvest diseases including *P. carotovorum*, which only enters through wound injuries (Coates and Johnson, 1997; Bhat et al., 2010).

Shelf-life was terminated when the visual quality rating of the butt-end reached 4. Among treatments, the negative control exhibited the shortest shelf life (3.6 days), while the positive control had twice the shelf-life (7.2 days) (Table 4). The shelf-life of those treated with mangosteen rind extract was 4.78 days, which was similar to the negative control. Guava leaf extract, gave a slightly better shelf life than mangosteen rind extract that was not different from that shown by the negative control samples. The shelf-life of those inoculated 72 h after harvest was longer than those inoculated earlier at 24 and 48 h.

Trimming involves the removal of damaged and wilted outer leaves to improve the marketability of Chinese cabbage (Thongsavath et al., 2012). Trimming was performed on the head portion once the visual quality reached 3 to simulate the practice done in the market. However, the decline in the visual quality of the leaves was mostly due to the natural deterioration of the leaves through time and not due to the test microorganism. Therefore, no consistent trend among treatments nor among inoculation time points was observed (data not shown).

CONCLUSION

Chemical extraction (with methanol and ethanol) exhibited higher antibacterial properties in laboratory testing compared to the mechanically produced extracts. In the test using a bioassay, mangosteen rind extract exhibited a greater inhibition zone but had lesser action in vitro compared to mature guava leaf extracts. No significant differences were observed among the five concentrations used. These showed greater inhibition than the negative control (water).

Guava leaf extract (at 1000 µg mL⁻¹) maintained a low soft rot infection for a day longer than the distilled water control. On the other hand, shelf life of those treated with mangosteen rind extract (at 500 µg mL⁻¹) did not vary with the negative control. The 10% alum was the most effective treatment in reducing the soft rot organism in vitro and in vivo. Compared to the guava leaf and mangosteen rind extracts, 10% alum was able to significantly delay the initial occurrence of decay in the butt-end portion of the Chinese cabbage later by 3.9 d relative to the negative control.

The time of bacterial inoculation and application of extracts (24, 48 and 72 h) after harvest also impacted soft rot progression. Those inoculated and treated at 24 h after harvest were diseased earlier, while those inoculated and treated at 72 h after harvest were infected later. This variation in infection rate may be attributed to the cutting of butt-ends during day 0, which allowed wound healing in heads inoculated after 72 h. Those inoculated after 24 h were more susceptible to infection relative to the 72 h inoculation treatment.

For future studies, additional concentrations of the extracts and the solvent used for extraction can be tested further for inhibition.

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Physicochemical quality of sweet pepper (*Capsicum annuum* 'Smooth Cayenne') treated with 1-methylcyclopropene

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Abstract

Four concentrations (0, 0.1, 1 or 10 $\mu\text{L L}^{-1}$) of 1-methylcyclopropene (1-MCP) were applied to sweet pepper (*Capsicum annuum* L. 'Smooth Cayenne') fruit that was then stored for 10 days under ambient conditions ($27.4\pm 0.7^\circ\text{C}$, $82.0\pm 10.1\%$ RH). Treated sweet pepper consistently maintained better visual quality and reduced shriveling starting from 4 days after treatment (DAT). Ripening was delayed best by 10 $\mu\text{L L}^{-1}$ 1-MCP, followed by 1 $\mu\text{L L}^{-1}$ 1-MCP, as exhibited by lower a^* (greenness) and chroma (intensity) during the initial period of storage. Later, skin lightness (L^*) and b^* (yellowness) were higher in treated fruit. Vitamin C content and total soluble solids were only slightly affected at 4 and 6 DAT, respectively. Shriveling and decay were reduced in treated fruit. Weight loss did not vary with treatment. There was delayed ripening and better postharvest quality maintenance of sweet pepper under ambient conditions using 10 $\mu\text{L L}^{-1}$ 1-MCP for 12 h.

Keywords: *Capsicum annuum* L., visual quality, shriveling, color, vitamin C

INTRODUCTION

Sweet pepper is an economically important crop that is grown throughout tropical, subtropical and temperate regions by small, medium and large producers (Pickersgill, 1997). It is an important food ingredient, and it is consumed fresh or processed (Costa et al., 2009). The major postharvest factor limiting marketing of sweet pepper is its short shelf life (Ilić et al., 2011). Rapid deterioration due to poor handling and storage is the main cause of postharvest losses in sweet pepper (Nyanjage et al., 2005). Green sweet pepper can better withstand transport stresses because of its thick pericarp wall, and therefore has a longer postharvest life than fully ripe fruit (Cao et al., 2012; Ilić et al., 2011). After sweet peppers are detached from the plant, the fruit undergoes several postharvest processes that lead to its rapid decline in quality, such as rapid senescence, loss of nutritive components, water loss and pathological decay (Du et al., 2007). Thus, it has been a challenge to maintain the quality of sweet peppers in the fresh produce industry (Xie et al., 2004).

1-Methylcyclopropene (1-MCP) is widely used to extend the shelf life of many fruits and vegetables. 1-MCP is an ethylene blocker, preventing ethylene-dependent processes such as ripening, senescence, yellowing and softening in many crops (Li et al., 2011). 1-MCP is effective in delaying senescence and retaining the quality of sweet pepper by reducing ethylene production and maintaining firmness and acidity (Ilić et al., 2009, 2011; Li et al., 2011). In this study, the effect of 1-MCP in maintaining quality and delaying the ripening of 'Smooth Cayenne' sweet pepper was investigated.

MATERIALS AND METHODS

The experiment was conducted at the Postharvest Biology Laboratory of the University of the Philippines Mindanao. Three hundred and sixty newly harvested mature green ($L^*=43.79$; $a^*=1.41$; $b^*=18.51$) 'Smooth Cayenne' sweet peppers of uniform quality (mean weight 47.8 g) that were grown on a farm 54 km away were purchased from Bankerohan Public Market in Davao City, Philippines. From the market, samples were transported to the laboratory in an air-conditioned vehicle, washed briefly with tap water, soaked in 500 ppm bleach solution (5.25% NaOCl) for 2 min, laid on paper towels and air-



dried before treatment. The samples were treated with 0, 0.1, 1 and 10 $\mu\text{L L}^{-1}$ 1-MCP for 12 h and then stored under ambient room conditions (26.8-28.1°C, 71.95-92.1% RH) for 10 days.

The following data were gathered at 2-day intervals: weight loss; skin color (using a Minolta CR-400 Chromameter) by determining L^* (lightness; black = -100; white = 100), a^* (green or red; - = green; + = red), b^* (blue or yellow; - = blue; + = yellow), chroma and hue angle; visual quality (1, excellent; 3, good, limit of salability; 5, poor); shriveling (1, no shriveling; 3, moderate, 16-30% of the surface area shriveled; 5, extreme, 50% or more of the surface area shriveled); decay (1, no decay; 3, moderate, 6-10% of the surface area decayed; 5, extreme, 16% or more); total soluble solids (TSS) using an Atago Pocket Refractometer PAL-1; vitamin C (redox titration using iodine solution); and decay incidence (%). The experiment followed a completely randomized design (CRD) with three replications at six fruits per replicate. Data were analyzed using analysis of variance (ANOVA) and differences between treatment means were compared using least significant difference (LSD) at 5% level of significance.

RESULTS

Physical evaluation

1. Weight loss.

Weight loss in both control and treated fruit increased during storage, but it did not vary with 1-MCP concentration (Figure 1A). This is in contrast to 1-MCP at 450 nL L^{-1} , which reduced weight loss and decay incidence and inhibited color development in treated *Capsicum annuum* 'Silica' and 'H1530' sweet peppers (Ilić et al., 2009). Cultivar could have influenced this response to 1-MCP.

2. Skin color.

Color represents a major external characteristic of a fruit vegetable like sweet pepper, and is therefore a crucial consideration in determining its quality (Jiménez León et al., 2013). Color change as an indication of ripening was delayed by up to 4 days after treatment (DAT) in treated sweet pepper as depicted by lower a^* (greenness) (Table 1). At 2 DAT, samples treated with 10 $\mu\text{L L}^{-1}$ 1-MCP had the lowest a^* , or the most delayed ripening, followed by those treated with 1 $\mu\text{L L}^{-1}$ 1-MCP. In a study by Cao et al. (2012), 1-MCP at 1 $\mu\text{L L}^{-1}$ was most effective in delaying senescence of green bell pepper fruit. Lightness of sweet pepper skin color, L^* , was not affected by 1-MCP concentration up to 8 DAT; however, treated samples had lighter skin color (L^*) at 10 DAT. Yellowness, positive b^* , was not affected by 1-MCP at 2, 6 and 8 DAT, but was higher in samples treated with 1 and 10 $\mu\text{L L}^{-1}$ 1-MCP at 4 DAT. At 8 DAT, 1-MCP-treated sweet pepper also seemed to be more yellow than the control. Compared with the control, 1-MCP also prevented the increase in skin chroma (saturation) up to 4 DAT. The higher concentrations, 1 and 10 $\mu\text{L L}^{-1}$ 1-MCP, had the lowest chroma values, followed by 0.1 $\mu\text{L L}^{-1}$. According to Jiménez León et al. (2013), the green chromaticity value decreases because of the disappearance of chlorophyll and the gradual increase of polyphenol compounds. Hue did not vary among treatments.

1-MCP manifested an effect on delaying ripening processes, as shown by inhibited color development from mature green stage. Similar studies also reported a positive effect of 1-MCP on senescence and quality maintenance of green bell pepper fruit as evidenced by delayed color change and softening (Huang et al., 2003; Ilić et al., 2009, 2011). 1-MCP inhibits ripening by irreversibly occupying ethylene-binding sites, so that ethylene cannot bind and stimulate subsignal transduction and translation in the ripening process (Serek et al., 1994).

According to Cao et al. (2012), the mechanism of the delay of ripening process and senescence of green sweet pepper by 1-MCP is attributed to enhanced antioxidant enzyme activities that destroy reactive oxygen species (ROS), which cause damage to cellular macromolecules, and these lead to plant senescence. Further, polyamine contents that are known to be associated with cell division and growth and act as antisenesescence agents in

plant tissues are also enhanced by 1-MCP.

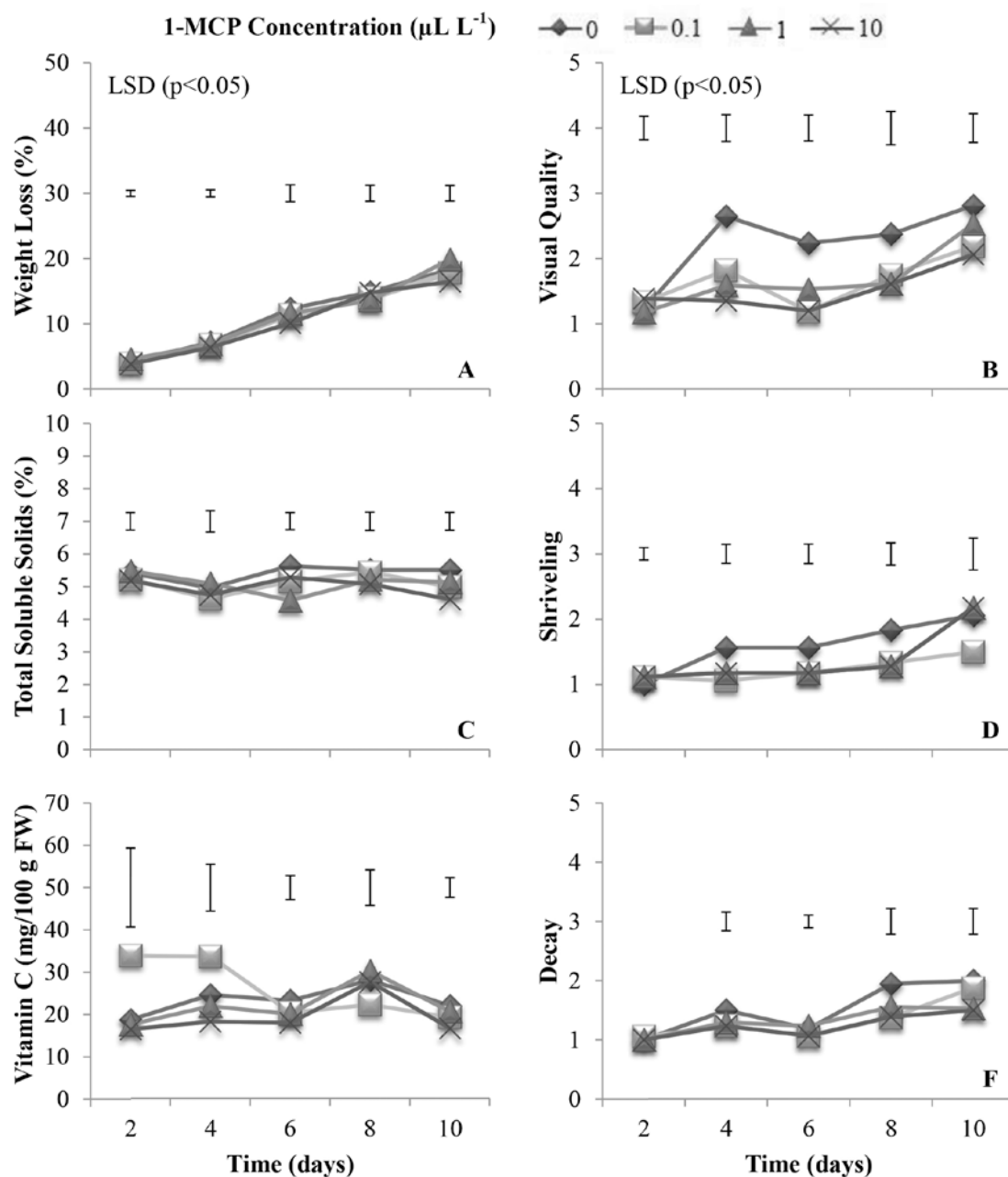


Figure 1. Weight loss (A), visual quality (B), total soluble solids (TSS) (C), shriveling (D), vitamin C (E) and decay (F) of sweet pepper held in ambient conditions ($27.4 \pm 0.7^\circ\text{C}$, $82.0 \pm 10.1\% \text{RH}$) as affected by 1-MCP concentration. Vertical bars indicate mean separation at $p < 0.05$.

3. Visual quality, shriveling and decay.

The visual quality of treated sweet pepper was consistently better, and shriveling was lesser than the control starting at 4 DAT (Figure 1B, D). 1-MCP better maintained visual quality of sweet pepper fruit at 4 DAT, with $10 \mu\text{L L}^{-1}$ as the most effective concentration. On the other hand, decay did not vary with 1-MCP up to 8 DAT; however, at 10 DAT, fruit treated with 1 and $10 \mu\text{L L}^{-1}$ 1-MCP showed less decay (Figure 1F). Ilić et al. (2009, 2011) reported that 1-MCP treatment had a pronounced effect on delaying ripening processes by

maintaining quality of green pepper fruit.

Table 1. Mean L*, a*, b*, chroma and hue of sweet pepper held under ambient conditions (27.40±0.7°C, 82.0±10.1%RH) as affected by 1-MCP concentration.

1-MCP concentration ($\mu\text{L L}^{-1}$)	Time (days)				
	2	4	6	8	10
			L* ¹		
0	42.80 ^a	38.74 ^a	37.80 ^a	36.83 ^a	35.62 ^b
0.1	42.28 ^a	39.89 ^a	39.44 ^a	38.18 ^a	37.26 ^a
1	42.25 ^a	40.85 ^a	38.46 ^a	37.26 ^a	37.11 ^a
10	44.97 ^a	41.35 ^a	40.83 ^a	37.88 ^a	36.60 ^{ab}
			a* ¹		
0	22.23 ^a	32.06 ^a	29.19 ^a	29.36 ^a	32.53 ^a
0.1	19.32 ^{ab}	22.14 ^b	26.07 ^a	30.95 ^a	33.13 ^a
1	13.41 ^b	27.32 ^{ab}	28.64 ^a	32.05 ^a	32.62 ^a
10	5.15 ^c	22.40 ^b	22.14 ^a	30.19 ^a	33.98 ^a
			b* ¹		
0	17.49 ^a	15.53 ^c	14.90 ^a	13.77 ^a	12.82 ^b
0.1	17.91 ^a	15.99 ^{bc}	15.75 ^a	14.84 ^a	14.30 ^a
1	16.97 ^a	17.05 ^{ab}	16.31 ^a	14.42 ^a	14.19 ^a
10	18.83 ^a	17.34 ^a	16.65 ^a	14.62 ^a	14.50 ^a
			Chroma ¹		
0	31.66 ^a	35.85 ^a	33.67 ^a	33.52 ^a	34.97 ^a
0.1	27.19 ^b	29.72 ^b	31.90 ^a	34.41 ^a	36.12 ^a
1	22.52 ^c	32.85 ^{ab}	34.10 ^a	35.16 ^a	35.59 ^a
10	21.16 ^c	30.86 ^b	32.52 ^a	34.04 ^a	36.95 ^a
			Hue ¹		
0	24.56 ^a	25.83 ^a	19.56 ^a	17.05 ^a	21.51 ^a
0.1	45.52 ^a	21.90 ^a	15.80 ^a	25.80 ^a	23.39 ^a
1	44.44 ^a	34.60 ^a	22.82 ^a	24.19 ^a	23.47 ^a
10	34.33 ^a	24.07 ^a	15.02 ^a	27.95 ^a	23.08 ^a

¹In each column per parameter, means with common letters are not significantly different using LSD at 5% level of significance.

Chemical evaluation

1. TSS.

TSS did not vary with 1-MCP concentration for the entire storage duration except at 6 DAT, where sweet pepper fruit treated with 1 $\mu\text{L L}^{-1}$ 1-MCP exhibited lower TSS than control and other concentrations (Figure 1C). The TSS of this sweet pepper was higher (4.9-5.3%) than that of *Capsicum frutescens* L. 'Longreen', which was between 4.5 and 4.7% (Du et al., 2007).

2. Vitamin C.

Sweet pepper is one of the most nutritive vegetables, containing a large amount of ascorbic acid (Li et al., 2011). Vitamin C gradually increased up to 8 DAT and decreased towards the later period of storage in chlorine dioxide-treated peppers held at 10°C for 40 days (Du et al., 2007). In the present study, vitamin C concentrations did not vary among treatments for the entire storage duration except at 4 DAT, when sweet pepper fruit treated with 0.1 $\mu\text{L L}^{-1}$ had higher vitamin C than those treated with 1 and 10 $\mu\text{L L}^{-1}$ (Figure 1E).

CONCLUSIONS

When 1-MCP (0, 0.1, 1 or 10 $\mu\text{L L}^{-1}$) was applied to sweet pepper for 12 h, treated fruit

consistently maintained better visual quality and reduced shriveling starting from 4 DAT. Further, 1-MCP treatment was effective in delaying ripening of sweet pepper up to 4 DAT by restraining color change, as indicated by higher skin lightness (L^*) and lower greenness (a^*) and chroma. Visual quality was best in the lot treated with $10 \mu\text{L L}^{-1}$ 1-MCP. Weight loss and decay incidence did not vary with 1-MCP concentration, while TSS and vitamin C were only slightly affected. These results suggest that delayed senescence by 1-MCP is associated with delayed color changes up to 4 DAT and better visual quality due to lesser shriveling up to 8 DAT. Ripening was delayed and postharvest quality of sweet pepper held under ambient conditions was better maintained using $10 \mu\text{L L}^{-1}$ 1-MCP applied for 12 h.

ACKNOWLEDGMENTS

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Quality changes in sweet pepper (*Capsicum annuum* L. 'Smooth Cayenne') under simulated retail conditions

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Abstract

The handling chain of 'Smooth Cayenne' sweet pepper produced in Digos City, southern Philippines, was tracked from harvest to wholesale. Quality was evaluated after transit at wholesale and at simulated retail for 15 days. Farmers harvested fruit at different maturities, but with more green fruit. There was an increase in light and full-red sweet peppers after a total of 25 h from harvest. Upon receipt at the retail level, losses at each section (i.e., top, middle, bottom) of the polypropylene sack were determined and quantified. Damage found at the bottom section included bruised (3.8%), cracked/split (48.1%), detached pedicel (23.1%), disease and rots (23.1%) and combination of damage (1.9%). The quality of sweet pepper during the simulated retail as affected by maturity (i.e., green, breaker, turning, orange and red) and container section was also evaluated. Those packed in the upper third of the sack had the fewest cracks and least weight loss, shriveling and decay relative to fruit from the middle and bottom portions. Further, fruit in the upper third also exhibited the slowest ripening and best visual quality. Mature green fruit had slower color change than the other maturity stages, as depicted by lower color index, a* value (green or red on surface) and chroma. Visual quality was better in turning and orange fruit until 9 d, while shriveling was less at 15 d. Overall, sweet pepper located in the upper portion of the polypropylene sack and green fruit had better quality after 15 d of simulated ambient retail conditions compared with fruit in lower portions of the container and later maturity stages, respectively.

Keywords: *Capsicum annuum*, harvest maturity, handling chain, postharvest loss

INTRODUCTION

Sweet pepper (*Capsicum annuum* L.) is an important food ingredients all over the world. It is an excellent source of ascorbic acid and a fair source of provitamin A carotenoids, which make it an object of attention for its potential in prevention of certain types of cancer and cardiovascular diseases (Rao et al., 2011; Simonne et al., 1997). The postharvest quality of fresh pepper fruit is influenced by physiological and pathological factors (Smith et al., 2006). Like any other crop, sweet pepper undergoes a wide range of environmental shocks along the supply chain that contribute to high losses upon reaching the wholesale and retail levels. Players in the supply chain, especially consumers, consider color, freshness, firmness, freedom from defects and diseases as well as shelf-life as important attributes during handling and storage (Nyanjage et al., 2005; Sigge et al., 2001). Further, most retailers keep their produce under ambient conditions, and could only reach up to 3-4 d shelf-life. This study determined and quantified the losses at each section (i.e., top, middle, bottom) of the package upon receipt at the retail level, and evaluated the quality of sweet pepper during simulated retail as affected by package section and maturity (i.e., green, breaker, turning, orange and red).

MATERIALS AND METHODS

Loss assessments in the supply chain

Freshly harvested 'Smooth Cayenne' sweet pepper fruit were obtained from a farm



(3107 m²) in Kapatagan, Digos City, in the southern Philippines. The harvest was sorted into marketable and non-marketable produce and then packed in unused polypropylene sacks, covered on top with a plastic mesh and sewed on the edge with polypropylene string. A Hobo temperature logger (UX100-001) was placed inside each sack after packing was completed, and left in until final transit. Three sacks of marketable produce (mean 46 kg) were tracked from harvest to the wholesale level. The sacks were transported by horse from the farm to the consolidation area (20 min, 25.9±0.7°C), remaining there before being carried onto a truck for transport (5 h, 25.8±0.4°C), transported to the wholesaler in Davao City (3 h, 26.2±0.1°C) and, lastly, to the laboratory in an air-conditioned vehicle (1 h, 26.5±0.3°C). After transport, each sack was opened and divided into three sections: top, middle and bottom. Fifty random samples from each section were assessed according to their maturity at harvest (i.e., green, breaker, turning, orange, light red and full red). Further, at the wholesale level, all rejects (those with defects and damage that materially detracted from acceptable quality) from each section of the three sample sacks and causes of rejection were assessed.

Simulated retail

Postharvest evaluation was conducted at the Postharvest Biology Laboratory of the University of the Philippines Mindanao. A total of 900 newly harvested sweet pepper fruit of uniform size and quality from three sections of each of the three sacks were used in the study. The samples were first wiped with a clean damp cloth and then wiped with a cloth moistened with 500 ppm bleach (5.25% NaOCl) and air-dried. Three replications with 25 samples each for different maturity stages (green, breaker, turning and orange) from different sections (top, middle and bottom) were held under ambient conditions (26.4±0.9°C, 83.96±5.7% RH). A Hobo logger (UX100-003) was used to monitor temperature and RH.

The following data were gathered every 3 days for 15 days: weight loss; skin color (using a Minolta CR-400 Chromameter) by determining a*, green or red (- = green; + = red) and chroma; color index [(no. of green samples × 1) + (no. of breaker stage samples × 2) + (no. of turning samples × 3) + (no. of orange samples × 4) + (no. of light red samples × 5) + (no. of full red samples × 6)/total no. of samples]; firmness (using a Fruit Pressure Tester model FT 327); visual quality (1, excellent; 3, good, limit of salability; 5, poor); shriveling (1, no shriveling; 3, moderate, 16-30% of the surface area shriveled; 5, extreme, ≥50% of the surface area shriveled); and decay (1, no decay; 3, moderate, 6-10% of the surface area decayed; 5, severe, ≥16% surface area decayed); and postharvest losses by determining the percentage of unusable fruit per replicate.

The study was laid in a completely randomized design (CRD) with section in packaging and maturity as the factors. Data were analyzed using analysis of variance (ANOVA) and differences between treatment means were detected using least significant difference (LSD) at 5% level of significance.

RESULTS AND DISCUSSION

Temperature changes during transport

Proper temperature management is the most efficient method for prolonging the shelf-life of fresh horticultural produce. In sweet pepper, the temperature increased by almost 2°C from packing to transport (Figure 1). This increase in temperature inside the sweet pepper container could also raise the rate of water loss, while further fluctuations in temperature could increase vapor pressure deficit between the tissue and its micro-environment air, leading to enhancement of transpiration and thereby causing weight loss (Nyanjage et al., 2005). After 14 h of transport, sweet pepper showed a weight loss of 0.93%. Cooling peppers as soon as possible after harvest has been shown to extend their shelf-life (Jiménez León et al., 2013).

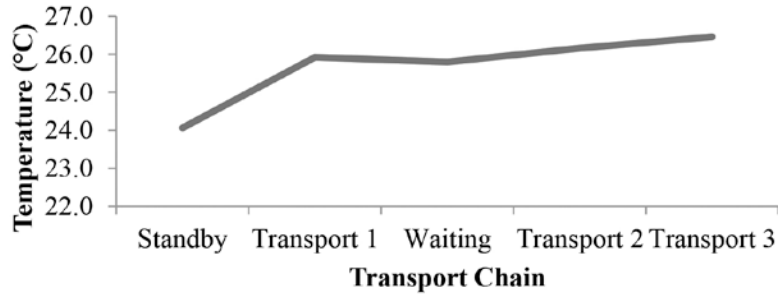


Figure 1. Temperature inside the polypropylene sack container of sweet pepper during 14 h of transport, starting from the farm.

Maturity and postharvest losses after transport

More mature green fruit (34.5%) were reckoned at harvest than other maturity stages, with more of these in the middle and bottom sections of the sack (Figure 2). After transit, with a total of 14 h, and an additional 11 h holding, an increase of 17.8% from 2.2% was observed in full-red sweet pepper, indicating rapid color development. In terms of postharvest loss after transport, all types of damage were found in the bottom section of the package, such as cracks/splits, bruised fruit, disease and rots, detached pedicel and a combination of these damage types (Figure 3). Of all the damage types, detached pedicel was highest in the top section (49.0%) while cracks/splits (48.1%) and disease and rots (23.1%) were highest at the bottom.

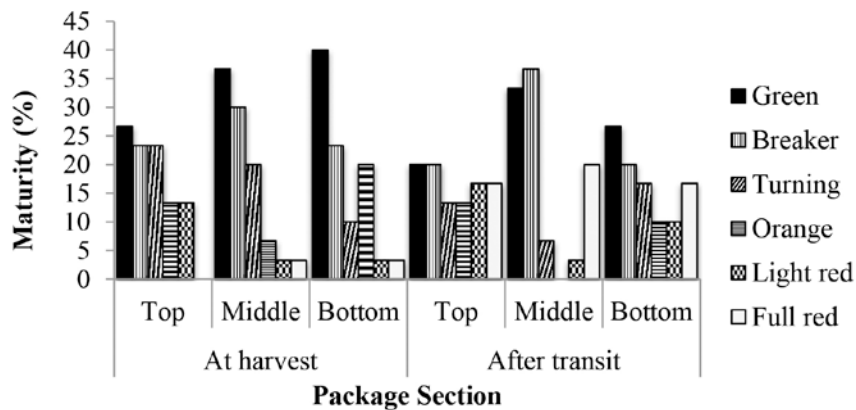


Figure 2. Sweet pepper maturity at harvest and after transit ($n=3$ sacks).

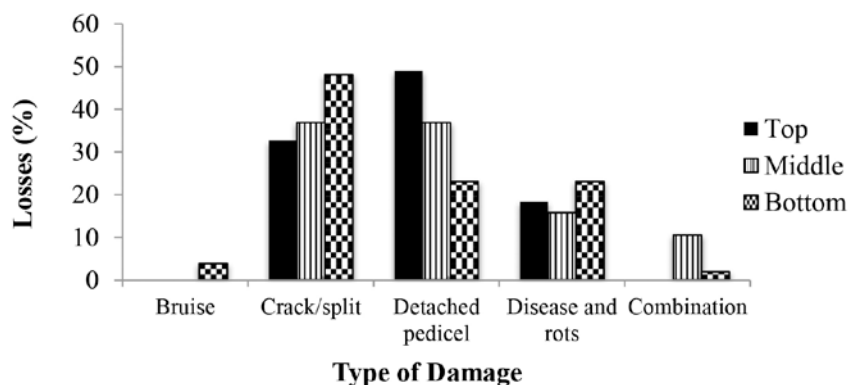


Figure 3. Types of damage and losses in sweet pepper after transport. Percentages are relative to all sweet pepper fruit with damage and defects ($n=3$ sacks).

Physical evaluation

1. Weight loss.

Weight loss corresponds to loss of marketable weight in horticultural produce (Nyanjage et al., 2005). Water loss accounts for most of the weight loss in the majority of crops. Further, excessive water loss results in softening and reduced shelf-life in sweet pepper (Díaz-Pérez et al., 2007). Water loss is one of the principal physiological defects during shipment, storage and marketing (Smith et al., 2006). In this study, weight loss was 5.6% higher in fruit located in the middle and lower portions of the sack (Figure 4A). This high weight loss in the bottom and middle portions of the package held under ambient conditions could be attributed to larger top weight during postharvest handling. While fruit didn't exhibit bruising, some internal damage was reflected as greater weight loss (Lipinski et al., 2013). Mature green fruit had slightly lower weight loss than the other maturity stages (Figure 5A). A study by Díaz-Pérez et al. (2007) showed decreased water loss with increasing fruit size and fruit maturity. They reported that the rate of water loss was highest in immature fruit, and showed no difference between mature green and red fruit.

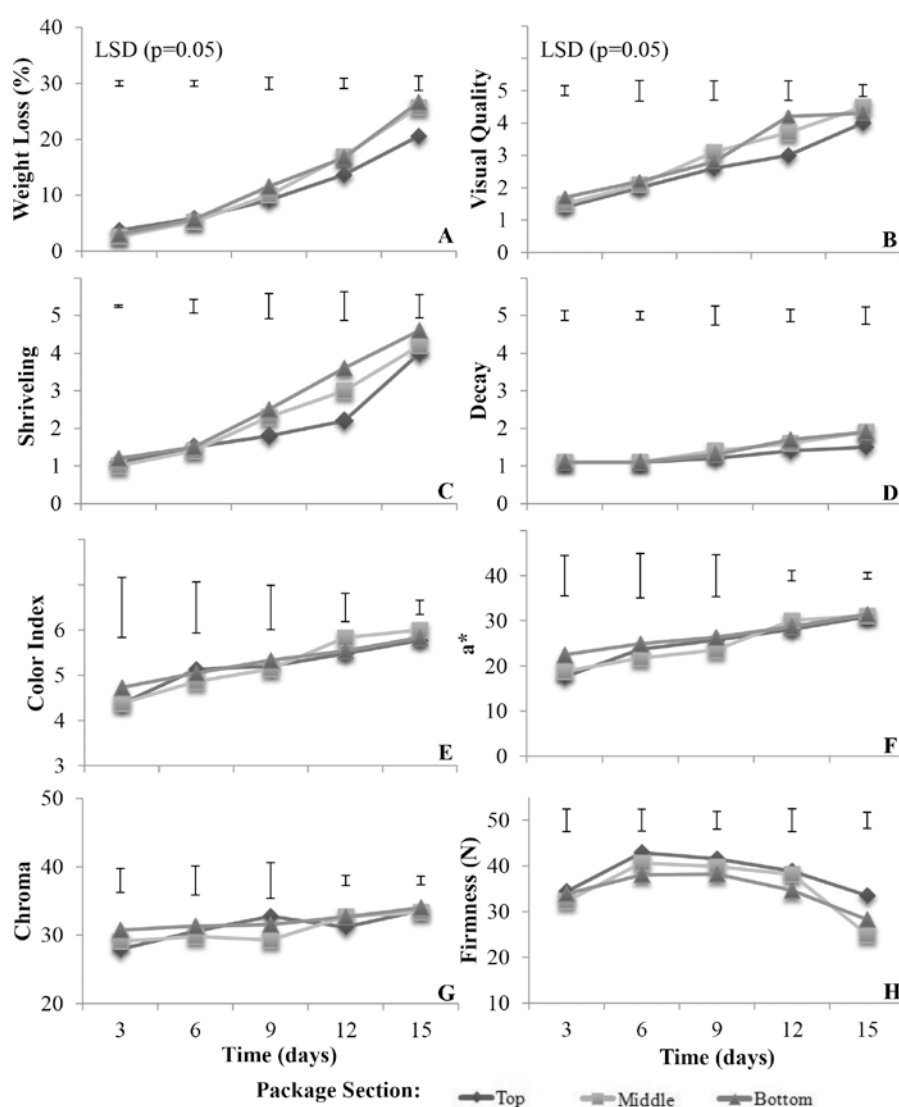


Figure 4. Weight loss (A), visual quality (B), shriveling (C), decay (D), color index (E), a* (F), chroma (G) and firmness (H) of sweet pepper as affected by package section under simulated retail conditions ($26.4 \pm 0.9^\circ\text{C}$, $84.0 \pm 5.7\%$ RH).

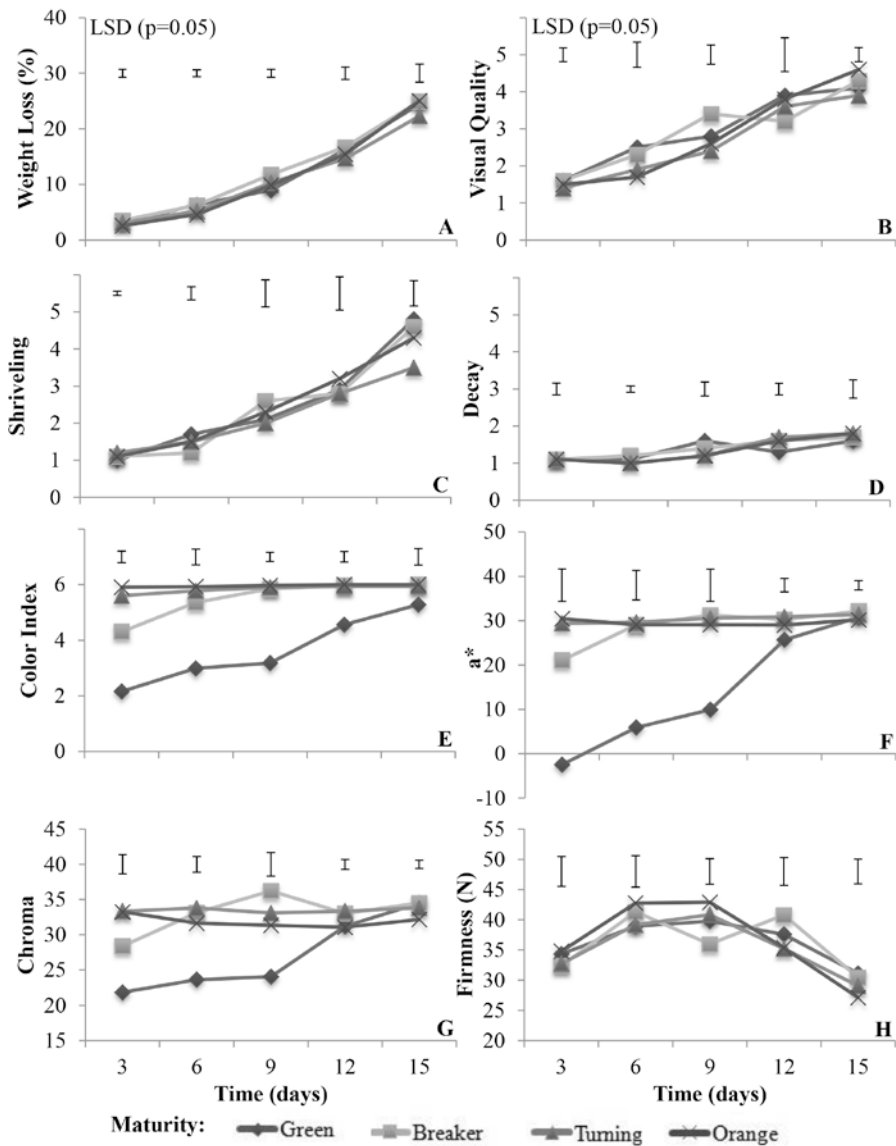


Figure 5. Weight loss (A), visual quality (B), shriveling (C), decay (D), color index (E), a^* (F), chroma (G) and firmness (H) of sweet pepper as affected by maturity under simulated retail conditions ($26.4 \pm 0.9^\circ\text{C}$, $84.0 \pm 5.7\%$ RH).

2. Visual quality and shriveling.

After harvest, quality becomes a more important aspect than quantity. For instance, sensory quality mostly affects the purchase decision (Jiménez León et al., 2013). Overall visual quality decreased over time (Figures 4B and 5B). At 12 and 15 d, sweet pepper fruit from the top section had better visual quality than those from the middle and bottom (Figure 4B). Fruit in these sections had greater top weight than those in the upper third of the container. Visual quality did not vary with fruit maturity (Figure 5B). In this study, sweet pepper fruit reached the limit of salability 12 d after harvest.

Fruit from the top section had the least shriveling, followed by those in the middle and bottom sections (Figure 4C). Fruit harvested at the turning stage had lesser shriveling at 15 d than the other maturities (Figure 5C). This result could be attributed to less mechanical impact incurred by fruit at the top during hauling and transport compared with those in the lower portions of the sack.

3. Decay.

Decay was high in fruit located at the middle and bottom of the package (Figure 4D). At 3 d, incidence of decay was higher at the bottom (17.4%) than in the upper portions (8.5%). Decay did not differ with fruit maturity (Figure 5D). However, fruit harvested at green (12.9%) and breaker (12.4%) stages showed higher decay incidence at 3 d than turning (2.7%) and orange (6.8%) stages. Decay could be due to high RH and water condensation around the produce in the packaging, which promote the development of postharvest decay at the retail level (Nyanjage et al., 2005).

4. Skin color index, a* and chroma.

Retention of green color is an important consideration in postharvest handling to ensure appealing and nutritious food to consumers. Packaging section did not affect the color index of the fruit (Figure 4E). Turning and orange fruit ripened the fastest, while green fruit were the slowest to ripen (Figure 5E). In terms of a* (red or green on surface), fruit from the top and middle sections were greener than those in the bottom section (Figure 4F). Green fruit at harvest maintained lower a* up to 12 d (Figure 5F). At 12 d, middle and bottom fruit had less yellowing on the skin surface. Chroma was lower for fruit located in the middle portion of the packaging at 9 d (Figure 4G). At 3 d, chroma was lowest in green fruit, and this was true until 15 d (Figure 5G). The loss of green color under ambient conditions is caused by increased breakdown of chlorophyll and synthesis of β -carotene and lycopene pigments, which occur during ripening (Nyanjage et al., 2005). These authors further showed that perforated and non-perforated packages did not significantly affect color retention; however, it was higher in sweet pepper fruit stored at 6.5°C for up to 25 d than under ambient conditions.

5. Firmness.

At 3-15 d, fruit located in the top portion were more firm than those located in the middle and bottom portions of the packaging (Figure 4H). Sweet pepper fruit harvested at the orange stage were more firm until 9 d, but firmness dropped as time progressed (Figure 5H). Firmness decreases with increasing fruit water loss during storage (Díaz-Pérez et al., 2007).

CONCLUSIONS

Color development in sweet pepper is rapid. At wholesale market, cracks/splits, diseases and detached pedicels reduced the volume of marketable fruit. At 12 d under ambient conditions and relative to fruit from the middle and bottom portions, those packed in the upper third of the polypropylene sack gave the fewest cracks, the least weight loss, shriveling, decay and the best visual quality. Fruit harvested at mature green stage had the slowest ripening at simulated retail storage under ambient conditions.

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SHELF LIFE OF TWO SWEET PEPPER (*Capsicum annuum*) CULTIVARS STORED AT AMBIENT AND EVAPORATIVE COOLING CONDITIONS

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ABSTRACT. *Sweet pepper (Capsicum annuum L.) is one of the most commercially important horticultural crops developed in temperate and tropical regions. This crop is highly perishable and high postharvest losses often result if handled poorly or stored in unfavorable conditions. An alternative non-refrigerated storage that can potentially maintain quality longer is the use of evaporative coolers. In this study, the effects of evaporative cooling (23.91±3.85°C, 93.84±9.33% RH, 1.79kPa VPD) on the shelf life and physico-chemical characteristics of two sweet pepper cultivars ('Smooth Cayenne' and 'Sultan') were evaluated and compared with those stored under ambient conditions (28.74±0.94°C, 65.68±7.43% RH, 13.62kPa VPD). Cultivar and storage conditions had significant effect ($P \leq 0.05$) on the shelf life of the sweet peppers. Storage of sweet pepper in the evaporative cooler (EC) resulted in reduced weight loss (9.65% and 28.86% for 'Sweet Cayenne' and 'Sultan', respectively), slower decline in moisture content and longer retention of acceptable visual quality and firmness due to lesser color change and shriveling, respectively, for both cultivars. There were rapid changes in total soluble solids and titratable acidity in both cultivars stored at ambient condition indicative of ripening. Decay can however reduce benefits from EC thus moisture control is essential. The shelf life of 'Smooth Cayenne' was at 9 and 18 days when stored in ambient and EC conditions, respectively, while this was at 7 and 15 days for 'Sultan'. The shelf life of these sweet peppers was prolonged by eight days in the present burlap-walled EC.*

KEY WORDS: *sweet pepper, 'Smooth Cayenne', 'Sultan', evaporative cooler, shelf life.*

INTRODUCTION

Sweet pepper (*Capsicum annuum* L.) is an economically important crop for both local and export market (Shehata et al. 2013). It is known for its antioxidant properties as it is a good source of vitamins A and C as well as phenolic compounds (Shotorbani et al. 2013). With its nutritional contribution, it is believed to prevent certain types of cardiovascular diseases, atherosclerosis, cancer, and haemorrhage (Marin et al. 2004).

Unlike dried grains and legumes, fresh fruits and vegetables such as peppers have an extremely low level of natural protection against biochemical and physiological deterioration in warm and humid places (FAO 1981). During prolonged storage, the main factors for the quality degradation of sweet pepper include poor external appearance, decay development, shriveling associated with water loss and its high susceptibility to chilling injury (Shehata et al. 2013).

Temperature management during storage period is the most effective tool in maintaining the quality and extending the shelf life of fresh horticultural crops such as sweet pepper (Leon et al. 2013). However, refrigeration requires high initial cost and power sources which cannot be afforded by most small-scale farmers, retailers and wholesalers in developing countries (Basediya et al. 2013) such as the Philippines. Evaporative cooling is a postharvest treatment that is usually done in rural areas. It is a physical process wherein evaporation of a liquid cools an object in contact with it. It is far less expensive than the usual refrigeration cooling. Bautista et al. (2007) explained that the heat of respiration of the produce evaporates the water that is applied to its immediate surroundings. When it is windy or when air movement is greater, there is also faster evaporation. Compared to the surroundings, it maintains a lower temperature and a higher relative humidity in the storage chamber (Dadhich et al. 2008).

Studies have shown the efficiency of evaporative coolers as a good postharvest treatment for fresh crops. Awole et al. (2011) conducted a study on the yield and storability of hot peppers harvested mature green and stored in two storage conditions (ambient and evaporative cooling). After 16 days of storage, nearly all pepper fruit stored at ambient condition were found to be unmarketable. On the other hand, those that were stored in the evaporative cooler chamber were kept up to 28 days. In another study conducted by Vanndy et al. (2008) using a brick-walled evaporative

cooler (EC), reddening of chili was hastened but weight loss was lower regardless of variety and initial ripeness stage. Weight loss reduction is one of the primary effects of EC as a result of the humid condition in the storage chamber (Acedo 1997; Acedo et al. 2009).

The shelf life and quality of different chili cultivars were examined by Acedo et al. (2009) using three storage conditions: (1) ambient, (2) polypropylene bag as modified atmosphere package (MAP), and (3) simple EC. Chili quality deteriorated rapidly at ambient due to weight loss and shriveling although inhibited in MAP and EC. Decay incidence was very high in MAP due to moisture condensation inside the plastic bag which favored rot development. EC created a more humid and cooler environment, causing reductions in weight loss and shriveling but at lower magnitude than MAP.

The effectivity of evaporative cooling was further validated in Nigeria by Olosunde et al. (2016) who developed a solar-powered evaporative cooler (SPECSS) to help smallholder rural farmers improve the shelf life of their produce. The mangoes, tomatoes, bananas and carrots that were stored in the SPECSS had shelf lives of 14, 21, 17, and 28 days, respectively, while those that were stored in ambient condition lasted for 5-8 days only. This present study assessed the effect of ambient and evaporative cooling on the shelf life and physico-chemical characteristics of two cultivars of sweet peppers.

MATERIALS AND METHODS

Sample preparation

Freshly harvested, mature green, uniformly-sized and blemish-free 'Smooth Cayenne' and 'Sultan' sweet pepper fruit were procured from Bangkerohan, Davao City, Philippines. Samples were wiped with soft cloth moistened with distilled water to remove dirt and then surface-sterilized by soaking in 200 mg·L⁻¹ sodium hypochlorite for 2 min. There were three replicates of 35 fruit samples each per storage condition per cultivar for a 21-day evaluation done at three day intervals.

Treatments

The sweet pepper samples were kept in two storage conditions: ambient and evaporative cooler (EC). The EC was covered with two layers of jute sack (burlap) as walls of the EC cabinet. The sack wall was constantly bathed with water from a

container placed on top of the EC cabinet to keep it moistened. The EC had three racks and a burlap door.

Temperature and relative humidity (RH)

The relative humidity (RH) and temperature of the ambient and EC conditions were measured daily using a digital data logger (HOBO UX 100-003) placed inside the EC and in the laboratory for the ambient conditions.

Postharvest evaluation

Weight loss. Five samples from each replicate were weighed at the beginning and regularly throughout the storage period. The difference between the initial and final weight of each sweet pepper was considered as total weight loss during each storage interval and computed as:

$$\% \text{ Weight Loss} = (\text{initial weight} - \text{final weight}) / \text{initial weight} * 100$$

Firmness. Firmness of two intact fruit samples per replicate was measured using a Wagner fruit penetrometer using a cylindrical stainless steel probe of 8 mm in diameter.

Visual assessment. The following parameters were evaluated visually using pertinent rating scales: visual quality rating (1=excellent, fresh appearance; 2=very good, slight defects; 3=good, limit of saleability, defects progressing; 4=fair, usable but not saleable; 5=poor), shriveling (1=no shriveling; 2= slight, 1-15% of surface area shriveled; 3= moderate, 16-30% of surface area shriveled; 4= severe, 31-49% of the surface area shriveled; 5=extreme, $\geq 50\%$ of the surface area shriveled), color index (1=mature green; 2=breaker; 3=turning; 4=orange; 5=light red; 6=dark red), degree of decay (1=no decay; 2=1-10% decay/slight; 3=11-25% decay/moderate; 4=26-50% decay/moderately severe; 5=more than 50% decay/severe). Samples with a visual quality rating exceeding 3 were considered as unmarketable and have reached the end of shelf life.

Moisture content. Moisture content was determined using 10 g samples from each replicate. Each fruit was cut into pieces and dried in a forced air circulation oven at 70°C to a constant weight as described by Antoniali et al. (2007).

Total soluble solid (TSS). Total soluble solids (TSS) was measured using an Atago PAL-1 digital refractometer by placing one or two drops of the juice on the prism surface.

Titrateable acidity (TA). Titrateable acidity was measured by titrating five mL of juice from three fruit samples which had been juiced using a juicer (Kyowa) against 0.1N NaOH (standardized titration solution). When the end point of titration was reached, the amount of NaOH used on the burette was read off and recorded to calculate TA using the following formula (Antoniali et al. 2007):

$$\%TA = (\text{Titer} * 0.1N \text{ NaOH} * 0.67 \text{ malic acid}) / 1000 * 100$$

Experimental design and statistical analysis

The study was laid out in 2x2 factorial experiment arranged in Completely Randomized Design replicated three times with cultivar and storage condition as factors. Data were analyzed using two-way Analysis of Variance (ANOVA) through DSAASTAT. LSD test at 5% level of significance was used for treatment mean comparison.

RESULTS

Temperature and Relative Humidity (RH)

During the storage period, the mean temperature and RH for ambient and evaporative cooler conditions were 28.76°C ; 66.42%, and 23.94°C; 93.92% (Fig 1) respectively with vapor pressure deficits (VPD) of 13.62kPa (ambient) and 1.79kPa (EC). A difference of 4.82°C, 26.78% RH, and 11.83 kPa VPD constituted the improvement in conditions in storing produce in the present EC design.

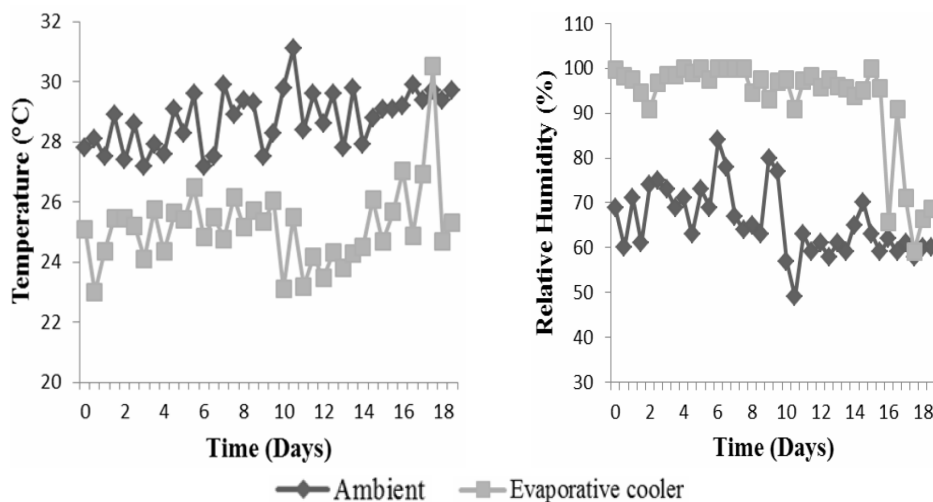


Figure 1. Temperature and relative humidity in ambient and evaporative cooling conditions.

Weight Loss

There was no significant difference in percentage weight loss between the two cultivars. However, in comparing the two storage conditions, all sweet peppers in ambient condition ended its shelf life on day 12 with a total

weight loss of 47.84%, while the lot in EC condition which was still marketable had a weight loss of 9.65%. At day 18, when no samples remained in the ambient condition lot, sweet peppers stored in EC condition had an accumulated weight loss of 24.32% (Fig 2).

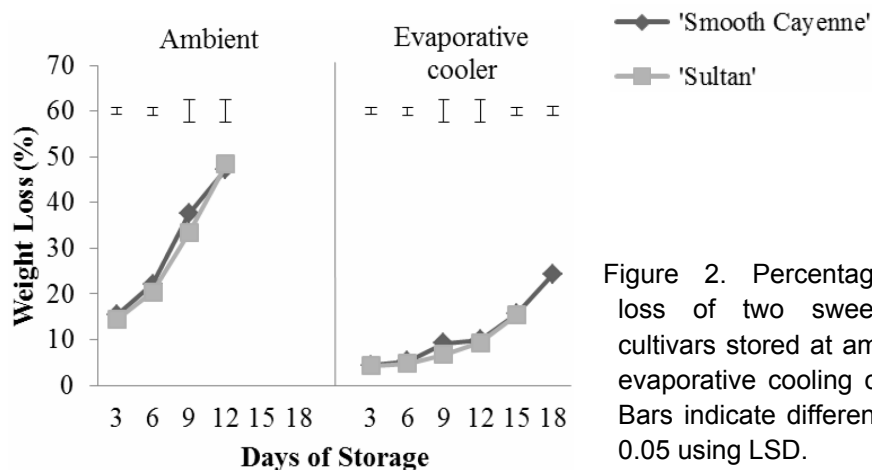


Figure 2. Percentage weight loss of two sweet pepper cultivars stored at ambient and evaporative cooling conditions. Bars indicate difference at $P \leq 0.05$ using LSD.

Fruit Firmness

The effects of storage condition was highly significant on the firmness of two cultivars of sweet peppers held under ambient and evaporative cooling conditions for 18 days. The maximum and minimum fruit firmness were recorded at full green (4.37 kgf) and at complete ripe (1.48 kgf) stages, respectively (Table 1). In both storage conditions, 'Smooth Cayenne' maintained acceptable firmness longer than 'Sultan' throughout the duration of storage (Fig 3).

Table 1. Fruit firmness (kgf) of sweet pepper stored under ambient and evaporative cooling conditions*

Storage condition	Storage period (days)						
	0	3	6	9	12	15	18
Ambient	4.37 ^a	3.23 ^b	2.48 ^b	2.54 ^b	1.48 ^b	-	-
Evaporative cooler	4.00 ^b	3.43 ^a	3.38 ^a	3.69 ^a	2.23 ^a	2.80	1.93

* Means within a column followed by the same letter are not significantly different at $P \leq 0.05$

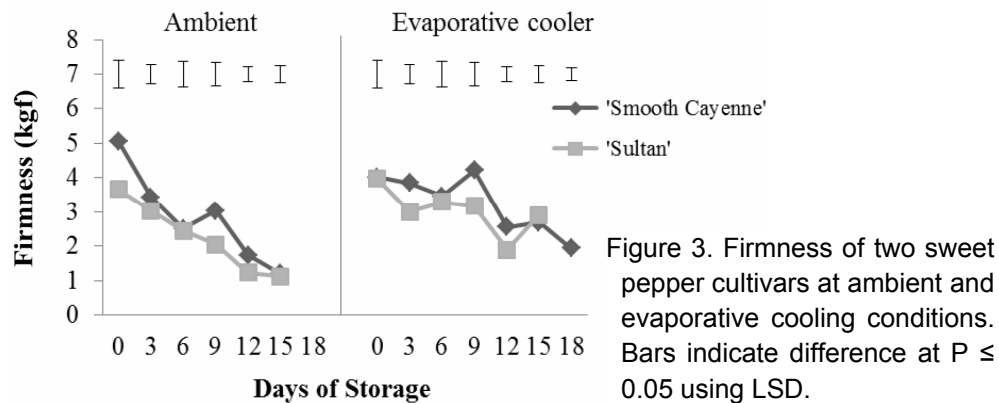


Figure 3. Firmness of two sweet pepper cultivars at ambient and evaporative cooling conditions. Bars indicate difference at $P \leq 0.05$ using LSD.

Shriveling

Sweet peppers held in ambient conditions shriveled faster compared with sweet peppers stored in evaporative cooling conditions. In both storage conditions, 'Sultan' shriveled faster than 'Smooth Cayenne' (Fig 4).

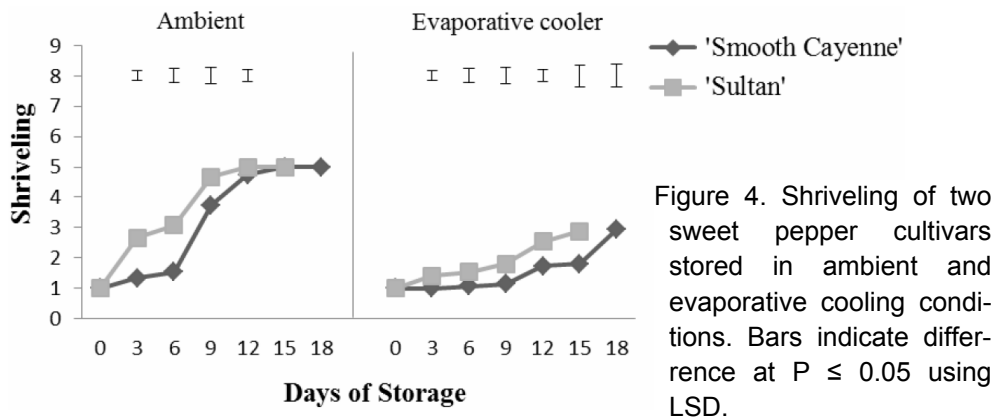


Figure 4. Shriveling of two sweet pepper cultivars stored in ambient and evaporative cooling conditions. Bars indicate difference at $P \leq 0.05$ using LSD.

Color

In both storage conditions, 'Smooth Cayenne' changed color more rapidly than 'Sultan' starting at days 9 (ambient) and 12 (EC). Furthermore, 'Smooth Cayenne' reached the color index rating of 6 (full red color) but not 'Sultan'. Both cultivars held at ambient conditions changed color faster than those stored under EC condition (Fig 5). This indicated faster ripening.

Visual Quality

Visual quality of the two sweet pepper cultivars was significantly affected by

cultivar and storage condition. An acceptable visual quality of 'Smooth Cayenne' was maintained longer than that of 'Sultan' (Fig 6). Sweet peppers stored in ambient condition reached its limit of shelf life at day 8 and continued to deteriorate thereafter. On the other hand, those that were stored in EC showed good quality characteristics until day 16.

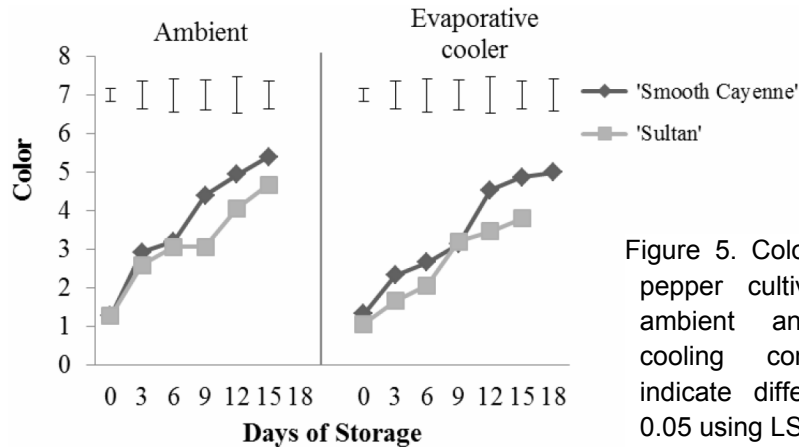


Figure 5. Color of two sweet pepper cultivars stored at ambient and evaporative cooling conditions. Bars indicate difference at $P \leq 0.05$ using LSD.

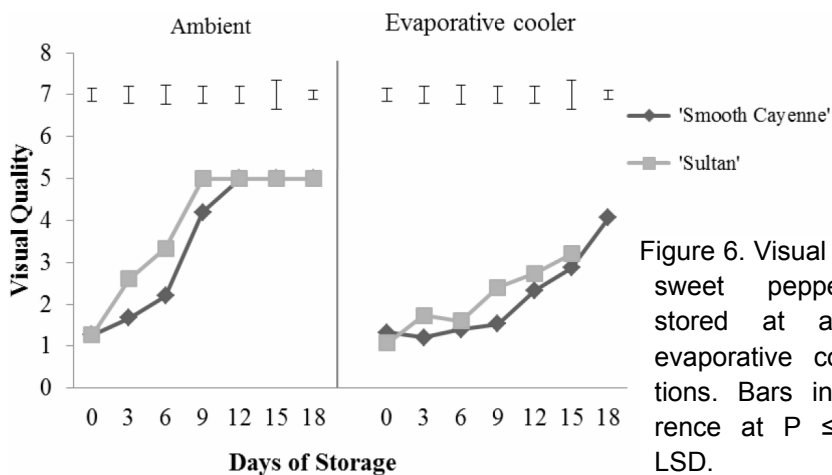


Figure 6. Visual quality of two sweet pepper cultivars stored at ambient and evaporative cooling conditions. Bars indicate difference at $P \leq 0.05$ using LSD.

Degree of Decay

The onset of decay was on day 9 for both storage conditions. Sweet peppers stored in EC conditions had a higher incidence of decay especially in 'Sultan' where soft rot development was observed. On the other hand, 'Sultan' sweet peppers stored in ambient did not show any further sign of

disease (Fig 7) and ended its shelf-life only when it had fully shriveled. White mold growth was observed in 'Smooth Cayenne' stored in the evaporative cooler. Furthermore, based on the visual symptoms, anthracnose was observed near the fruit base for 'Smooth Cayenne' in both ambient and EC conditions.

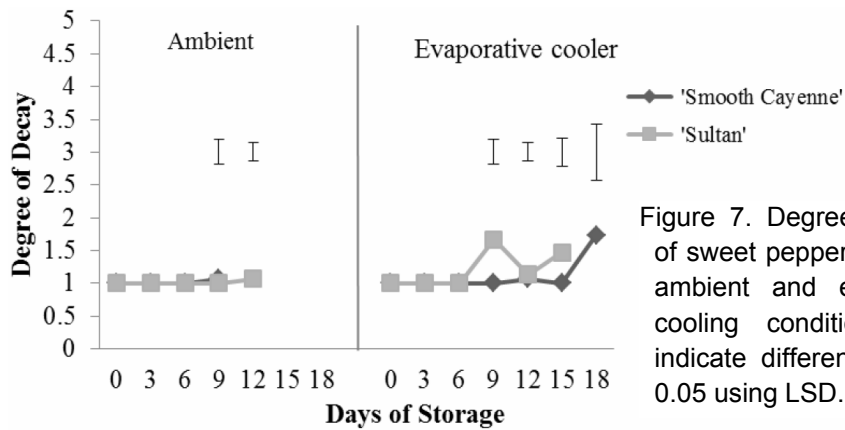


Figure 7. Degree of decay of sweet peppers stored at ambient and evaporative cooling conditions. Bars indicate difference at $P \leq 0.05$ using LSD.

Shelf Life

Sweet peppers held in EC conditions had a longer shelf life compared to sweet peppers stored in ambient conditions. At ambient conditions, 'Sultan' had a shelf life of 7 while it was 9 days for 'Smooth Cayenne'. On the other hand, 'Sultan' stored in EC conditions had a shelf life of 15 days and 'Smooth Cayenne', 18 days (Fig 8). For both storage conditions, 'Smooth Cayenne' exhibited a longer shelf life of about three days than 'Sultan' (Fig 8).

Moisture Content

Moisture content of the two sweet pepper varieties stored under the two storage conditions displayed a decreasing trend in significant variations ($P \leq 0.05$) throughout the storage period as shown in Fig 9. Retention of moisture content was greater in sweet peppers stored in evaporative cooler. Furthermore, between the two cultivars, 'Sultan' had greater moisture content than 'Smooth Cayenne'.

Total Soluble Solids (TSS)

At day 6, a drop from the increasing trend for total soluble solids was

observed for both cultivars and storage conditions. The TSS increased thereafter. Between the two sweet pepper cultivars, 'Smooth Cayenne' presented a higher TSS content compared with 'Sultan' almost throughout the storage period but a decrease was noted on day 15 (Fig 10).

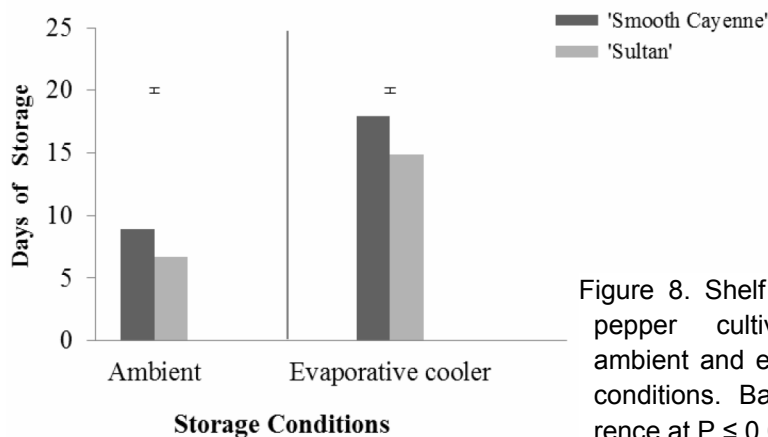


Figure 8. Shelf life of two sweet pepper cultivars stored at ambient and evaporative cooling conditions. Bars indicate difference at $P \leq 0.05$ using LSD.

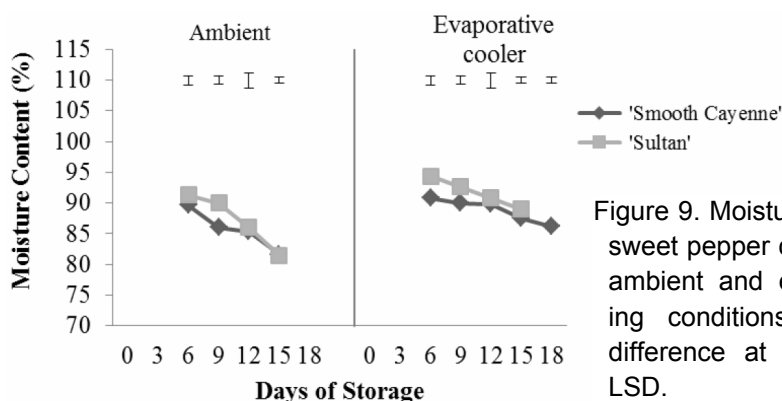


Figure 9. Moisture content of two sweet pepper cultivars stored at ambient and evaporative cooling conditions. Bars indicate difference at $P \leq 0.05$ using LSD.

Titrateable Acidity (TA)

There was an increasing trend for TA content of the sweet pepper cultivars and storage condition until day 9 with a drop starting at day 12. An increase in TA was exhibited in sweet peppers in EC. 'Smooth Cayenne' had greater TA than 'Sultan' in both storage conditions. Also, there was a distinct increase of TA in 'Smooth Cayenne' and almost stable TA for 'Sultan'. Sweet peppers in ambient condition resulted in faster increase in TA content until day 9 and a decline on day 12 (Table 2).

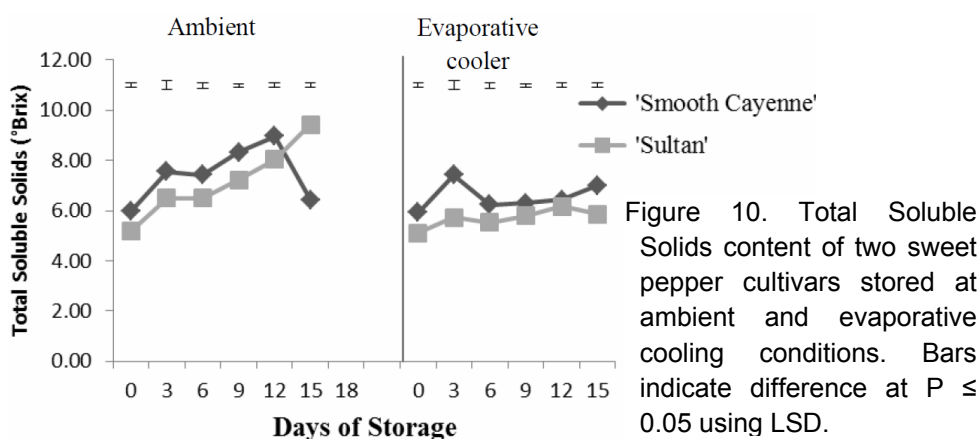


Table 2. Titratable acidity (%) of two sweet pepper cultivars stored under ambient and evaporative cooling conditions*

Factors	Storage period (days)						
	0	3	6	9	12	15	18
Cultivar							
Smooth Cayenne	.03 ^a	.04 ^a	.05 ^a	.05 ^a	.04 ^a	.03 ^a	.04
Sultan	.02 ^b	.03 ^b	.03 ^b	.03 ^b	.03 ^b	.02 ^b	-
Storage							
Ambient	.02 ^a	.04 ^a	.05 ^a	.05 ^a	.03 ^a	.02 ^a	-
Evaporative cooler	.03 ^b	.03 ^b	.03 ^b	.03 ^b	.04 ^b	.04 ^b	.04

*Per factor, means within a column followed by the same letter (s) are not significantly different at $P \leq 0.05$

DISCUSSION

Temperature has been established to be the most significant factor in maintaining product quality of fresh horticultural crops such as sweet pepper longer (Leon et al. 2013). Sweet peppers stored under ambient conditions could have an excessive amount of water loss leading to quality deterioration because of its very low RH. High vapor pressure deficit may also cause wilting since it hastens transpiration of water. By maintaining an average of $23.91 \pm 3.85^\circ\text{C}$, $93.84 \pm 9.33\%$ RH, 1.79kPa VPD for temperature, relative humidity and vapor pressure deficits, respectively, quality preservation of sweet peppers was achieved for a little over a week more

(16 days in EC, 8 days in ambient) by storing the samples in the burlap-walled evaporative cooler (EC).

Water loss is a primary physiological factor that impacts on sweet pepper fruit quality during shipment, storage and marketing (Dumville & Fry 2000). Sweet peppers stored under ambient conditions displayed a significantly higher percentage of weight losses compared to those stored under EC condition. The higher RH and reduced temperature and VPD values given by the EC relative to ambient conditions allowed the former to sustain reduced produce weight losses and at the same time slowed down the rate of shriveling. Furthermore, although there was a decline in firmness on the sweet peppers in both storage conditions, this physical characteristic was better maintained by storing the samples in the EC. The difference in average weight loss percentages between the two sweet pepper cultivars could be due to the varying morpho-anatomical characteristics, surface types and underlying tissues of the cultivar that affect the rate of water loss (Bondada & Keller 2012; Wills et al. 1998).

The ripening rates of peppers are highly variable especially among cultivars (Samira et al. 2013). This explains why the two cultivars in this study differ in their rate of color change. Furthermore, as ripening is often signaled by changes in fruit color, it can be concluded that storing sweet peppers in EC could slow down the rate of ripening. This allows farmers, retailers, and other handlers to store a fraction of their harvest for some time instead of exposing these crops to the harsh environment of the marketplace which poses a higher risk for contamination and mechanical damages.

One of the visual aspects that totally eliminate a crop from being accepted by the consumers is decay. Due to observed diseases on sweet peppers, it can be implied that the sanitizing agent (e.g. NaOCl) used may not have been sufficient to eradicate the decay-causing organism. In addition, the development of anthracnose on 'Sweet Cayenne' may have been favored by the environment inside the EC since *Colletotrichum* sp. thrives on warm wet weather at an optimum temperature and RH of 27°C and 80%, respectively (Ali et al. 2016). There was also surface moisture in the storage walls of the EC that sometimes flowed to the shelves where the trays were placed.

Sweet peppers stored in evaporative cooling condition showed greater moisture content than sweet peppers stored in ambient condition from days 6 to 18. Furthermore, reduced respiration might have taken place because

of the low temperature in the evaporative cooler which resulted in delayed fruit ripening and consequently reduced moisture loss (Atta-Aly & Brecht 1995). The percentage of decrease in moisture content was greater in sweet peppers stored at ambient condition. This was due to transpiration as well as ripening during the storage period. Ripening of sweet pepper makes variations in the permeability of cell membranes that makes them more subtle to water loss (Samira et al. 2013).

The increase in TSS content for all the sweet peppers throughout the storage in both EC and ambient conditions indicate continuous metabolic conversion of sugars from starch (Samira et al. 2013). The lower TSS content in evaporative cooling condition might be due to its high RH which reduces water loss; hence, slowing the conversion of sugars from starch which decreases the amount of soluble solids.

Change in TA content comes along with the changes in different organic acids like ascorbic acid (Salisbury & Ross 1994). Results indicate that TA in sweet peppers increases as these ripen. The decline of TA in ambient conditions might be due to high temperature which promoted high respiration that used up titratable acids as a substrate. On the other hand, the lower temperature in EC reduced the rate of respiration, decreasing the use of titratable acids (Getenit et al. 2008).

Shelf life was ended when the fruit exceeded a visual quality rating of 3 (good, limit of saleability, defects progressing). Sweet peppers stored under EC condition was kept up to 16 days of storage while those stored in ambient was 8 days.

CONCLUSIONS

This study has verified the efficiency of the evaporative cooler (EC) in prolonging the shelf life of a highly perishable crop like sweet pepper. As compared to ambient condition, the EC was able to provide a storage environment with lower temperature, lower vapor pressure deficit and a higher relative humidity. Weight loss was effectively reduced in EC as well as the extent and rate of moisture loss. Results also showed that physical characteristics of sweet pepper in terms of visual quality, color, firmness, and shriveling were maintained better when stored under EC condition. The rate of the chemical changes for total soluble solids and titratable acidity for sweet peppers stored in EC condition were slowed down contributing to its

extended shelf life. Furthermore, samples in EC condition exhibited a longer shelf life of a maximum of 9 more days than those stored in ambient. Decay, however, can reduce the benefits gained from the use of the EC. Controlling the amount of moisture may reduce disease occurrence in the produce. In addition, between the two cultivars, 'Smooth Cayenne' exhibited better visual quality characteristics and longer shelf life. Hence, selecting a good cultivar of sweet pepper paired with storage in EC could lengthen the shelf life of sweet peppers.

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Trimming losses in Chinese cabbage

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Abstract

Trimming in Chinese cabbage heads removes wrapper leaves and other parts that detract from a good appearance, thus improving marketability. Trimming losses were quantified in various assessments at the wholesale and retail (as influenced by sack position, cold storage and alum) levels. Trimming losses of 43.9% by weight were measured in Chinese cabbage for product grown in Kapatagan, Davao del Sur at the wholesale market in Bankerohan, Davao City. The trimming loss was less, 28.5% by weight, for Chinese cabbage grown in Lantapan, Bukidnon, at the Bulua, Cagayan de Oro City wholesale markets. Heads procured from Bankerohan market showed 11.1% more trimming losses in the drier conditions of cold storage (at 21.1±1.3°C and 49.8±9.8% relative humidity (RH)) after two days, compared with ambient storage (27.0±0.6°C and 69.6±6.1% relative humidity). However, the opposite occurred after six days of storage, due to accelerated decay at ambient temperatures. Within polypropylene sack sections (top, middle and bottom), trimming losses were similar in all positions. Under ambient conditions, where no control heads remained, all alum-treated heads were still useable at six days, but trimming loss reached 70%.

Keywords: alum, postharvest losses, wholesale market, wrapper leaves, leaf removal

INTRODUCTION

Chinese cabbage, a widely cultivated crop in Asia, is a hardy, shallow-rooted, cool-season crop with an optimal temperature range of 13-21°C. This leafy vegetable crop is grown in the Philippines, contributing about PhP 1.08 B to the local economy in 2006 (PCAARRD, 2006). In the southern Philippines, production areas of this crop include the provinces of Zamboanga del Norte, Bukidnon, Camiguin, Misamis Occidental, Misamis Oriental, Davao del Sur, Compostella Valley, North Cotabato, Sarangani, South Cotabato and Sultan Kudarat (CountrySTAT Philippines, 2015). Two main domestic markets are located in Davao City and Cagayan de Oro City – both big cities in Mindanao. Crops produced any distance from these domestic markets are potentially vulnerable to different kinds of mechanical stress during transport. Mechanical stresses are evident in damaged, crushed or torn leaves, which reduce its visual appeal and useable biomass. If the crop loses its quality, then its marketability is also reduced. Trimming is done in order to maintain the appearance of quality and increase marketability.

Trimming is practiced on farm during harvest and by wholesalers and retailers. This postharvest practice involves the removal of the outer (wrapper leaves), other leaves and parts of the butt-end of Chinese cabbage heads. This crop has the high surface area to volume ratio that is characteristic of a leafy vegetable. Wilting, yellowing and damage due to ethylene exposure, high temperature conditions or decay caused by soft rot bacteria (*Pectobacterium carotovorum*) are the main reasons for trimming Chinese cabbage (Acedo and Weinberger, 2009; Jiang and Pearce, 2005). Whilst trimming could maintain the quality of the crop, it could also reduce the marketable weight that can be sold in the market and delay the onset of soft rot. Thus, excessive trimming causes postharvest loss for farmers, traders, wholesalers and retailers.

An assessment of trimming losses in Chinese cabbage in the market was needed with the aim of improving postharvest handling. In this study, trimming losses were evaluated in the wholesale market; and the effects of position in the package, storage conditions and alum treatment were evaluated.



MATERIALS AND METHODS

Wholesale trimming loss assessment

Chinese cabbage trimming loss was evaluated at the Bankerohan market in Davao City as well as at Bulua, Westbound, Cagayan de Oro where both wholesale and retail transactions take place. A total of 51 sacks and 20 individual Chinese cabbage heads were assessed for trimming loss at the Bankerohan market. The first evaluation for trimming loss was done at Bankerohan market, wherein the overall weight of the trimmed leaves was deducted from the total weight of 51 sacks. The second evaluation for Bankerohan market involved the trimming loss of individual Chinese cabbage heads. For the trimming loss in Cagayan de Oro market, three carton boxes (75 kg box⁻¹) of Chinese cabbage were evaluated. The damaged wrapper and outer leaves of the Chinese cabbage heads were trimmed until an acceptable marketable quality was attained. Chinese cabbages assessed from the Bankerohan market were grown in the highland area of Kapatagan, Davao del Sur whereas those from Cagayan de Oro were produced in Lantapan, Bukidnon. The Kapatagan area is considered to be cooler and thus allow this temperate vegetable crop to thrive better than Lantapan. Trimming loss (%) was calculated by subtracting the weight after trimming from the weight before trimming/weight before trimming × 100 (Klieber et al., 2001).

Trimming loss at simulated retail conditions as affected by package section

Chinese cabbage transported from the farm to wet market was evaluated under laboratory conditions simulating retail. Trimming loss of the samples as affected by location within the sack (top, middle, and bottom) was assessed at the Postharvest Biology Laboratory. Three sacks of newly harvested Chinese cabbage were procured from Kapatagan, Davao del Sur. Upon arrival in the laboratory, the samples were grouped into three treatments based on their position (top, middle, and bottom) inside the polypropylene sacks and were trimmed to a marketable retail standard. Trimming knives were sanitized with 200 ppm sodium hypochlorite. The evaluation was carried out in a completely randomized design. Samples were then held for 15 days at ambient conditions (26.46±1.01°C, 84.23±7.18% RH) as Chinese cabbage heads would be retailed in local market stalls minus the use of electric fans by retailers. Trimming was done repeatedly during the evaluation period at three day intervals. Trimming loss was calculated as indicated earlier (Klieber et al., 2001) except that initial weight before trimming was the weight just before trimming at each evaluation period.

Trimming loss in two storage conditions

Chinese cabbage heads procured from Bankerohan Public Market, Davao City were stored unpacked in ambient (27.0±0.6°C, 69.6±6.1% RH) or cool storage (21.1±1.3°C, 49.8±9.8% RH) conditions. Trimming loss was evaluated after two and six days of storage.

Trimming loss in Chinese cabbage heads inoculated with *Pectobacterium carotovorum* followed by alum treatment

Newly-harvested Chinese cabbage heads from Kapatagan, Digos City were procured. Upon arrival at the postharvest laboratory, each of the heads were checked for damage or disease. Prior to treatment and to simulate retail conditions, three to five wrapper leaves were trimmed, including damaged leaves, while the butt-end was re-cut. The samples were inoculated with soft rot bacteria, *Pectobacterium carotovorum* within 24 h of harvest. The inoculation was done by pricking the butt end of the Chinese cabbage once to a depth of 1 mm and applying a single drop of inoculum with the concentration of 10⁻⁸ CFU mL⁻¹. One hour later, each sample was grouped according to treatment (distilled water and 10% alum solution) and each head sprayed until dripping wet. The Chinese cabbage heads were held in ambient storage (26.2±0.3°C, 84.8±2.0% RH) for 8 days and trimming was repeated as necessary. Trimming was done solely on the leaves and not on the butt-end of the heads.

RESULTS AND DISCUSSION

Postharvest practices in Chinese cabbage

Farmers harvest Chinese cabbage based on the fullness of the head and size of the plant. During harvest, they trim off the undesirable parts and retain 3-5 wrapper leaves to protect the fragile produce during handling. The ground lined with leaf trimmings are where the Chinese cabbage heads are stacked before they are packed in overpacked sacks (practiced in Kapatagan) or carton boxes (practiced in Bukidnon) (Figure 1). A practice in Kapatagan locally called “resekó” involves the deduction of 15-20% of the weight of the packed Chinese cabbage as an allowance for mechanical and physical damage during the handling of the produce (Concepcion and Montiflor, 2003). “Reseko” is thus used in calculating the weight of the commodity that is paid for. Trimming, packing, hauling, weighing and transport are normal procedures after harvest. Some or all of these practices are often repeated by the wholesaler or retailer until the crop reaches the consumer.



Figure 1. Packaging containers for Chinese cabbage: carton box (70-80 kg, left) and sack (50-75 kg, right).

Wholesale trimming loss assessment

The assessment of Chinese cabbage losses at two wholesale markets in two Mindanao cities showed that trimming losses of 43.9% (sacks: 43.0%, heads: 44.8%) were measured at Bankerohan, Davao City markets, whereas only 28.5% trimming losses were measured at Bulua, West bound, Cagayan de Oro City markets. Postharvest packaging of the crop was observed in the two trading areas. Chinese cabbage grown in Kapatagan, Davao del Sur was packed using polypropylene sacks. The sacks were overfilled with the container being extended using leaf sheaths of the banana pseudostem and fastened using a plastic twine (Concepcion and Montiflor, 2003) or plastic twine alone. On the other hand, produce from Bukidnon was packed using cardboard boxes. These had one end open (exposing the top layer of produce), however the Chinese cabbage heads were secured using packaging tape (Figure 1) and sometimes tied with plastic twine. It was also observed that the compactness of the heads varied. Those in Cagayan de Oro were more compact than those produced in Davao del Sur. Sozzi et al. (1981) reported that an extended storage life can be achieved if heads are harvested at a more compact head stage. The current packaging practices in the two areas contribute to high postharvest losses at the wholesale market level. This is unlike South Korean practice, where Chinese cabbages are packed in plastic crates, which provide better protection from physical or mechanical damage (Kim and Choi, 2014).

In Kapatagan, filling the sacks involves packing around 60 kg or more of the fragile produce into a 50 kg polypropylene sack, which results in overfilling (Rasco et al., 2005). Overfilling the sacks is one way that the farmers attempt to compensate for the losses along the handling chain, especially during transportation and hauling. Hence, when the sack

reaches market, a substantial amount of the crop still remains. Due to this practice, the quality of the harvested produce is compromised. The oversized carton box used in the Bulua wholesale market is slightly more rigid compared to the sack. The packaging material was not the only reason for a greater percentage trimming loss in Chinese cabbage in Bankerohan. Since the Chinese cabbage in the two markets is from different production sources, the cultivar used and on-farm quality could also be factors in the losses incurred. The losses may also depend on the perception of the market intermediary, since they do the trimming and selling of the crop in the market. Over-trimming is a cause of postharvest losses at the wholesale market.

In a study by Concepcion and Montiflor (2003), farmers perceived that mechanical injuries are not important, while the market intermediaries are concerned about it. The reason for greater trimming losses at Bankerohan wholesale market was not limited to one factor. Trimming losses could be due to the packaging material used, the handling practices of farmers or the perception of quality by market intermediaries as the produce goes through the handling chain (Concepcion and Montiflor, 2003; Manalili, 2003; Rasco et al., 2005).

Trimming loss at simulated retail condition as affected by location in package

The produce was handled as growers and traders would until the crop reached the market, thus simulating the handling conditions from the farm to market. Samples were further observed in the laboratory and showed that Chinese cabbage trimming losses for each location (top, middle, and bottom) within the sack was similar (Table 1). Kapatagan farmers tend to overfill the polypropylene sack when they pack Chinese cabbage heads. Furthermore, sacks are handled roughly as they are hauled and piled onto vehicles with other farm products for transport. Polypropylene sacks are non-rigid containers and do not afford adequate protection to the packed produce. Each section of the sack was subjected to the same degree of injury, such as compression, which led to similar percentages of trimming loss. The higher percentage of trimming loss on Day 3 can be attributed to removal of wrapper, torn, broken and wilted leaves, as well as insect damaged leaves.

Table 1. Mean trimming loss (%) of Chinese cabbage held at ambient conditions ($26.46 \pm 1.01^\circ\text{C}$, $84.23 \pm 7.18\%$ RH) as affected by location in the polypropylene sack.

Packaging section	Trimming loss ¹				
	Time (days)				
	3	6	9	12	15
Top	52.82 ^a	22.74 ^a	14.84 ^a	24.29 ^a	12.49 ^{ab}
Middle	52.46 ^a	21.87 ^a	14.30 ^a	21.48 ^a	11.09 ^b
Bottom	50.21 ^a	18.71 ^a	16.54 ^a	17.92 ^a	17.48 ^a

¹Means in a column with common letters are not significantly different using LSD at 5% level of significance.

Trimming loss in two storage conditions

After two days of storage in a cool room ($21.1 \pm 1.3^\circ\text{C}$, $49.8 \pm 9.8\%$ RH) Chinese cabbage had 11% more trimming loss than product in ambient conditions ($27.0 \pm 0.6^\circ\text{C}$, $69.6 \pm 6.1\%$ RH) (Figure 2). The higher trimming loss in the cool room was attributed to the drier conditions compared with ambient humidity. Water from the air and from the vegetables is drawn out by the air conditioner during defrost cycles, which results in lower percentage relative humidity (Government of Western Australia, 2016). This in turn caused more wilting of leaves, which contributed to a greater trimming loss. However, at six days Chinese cabbage heads stored at ambient conditions exhibited higher trimming loss than those in cold storage. Ambient conditions, particularly the higher temperature (a difference of 5.9°C), increased the incidence of decay. In lower temperature conditions in the cool room, the growth of microorganisms was delayed, reducing trimming loss. According to Kramchote et al. (2012), a temperature of 0°C and a relative humidity of 90% are ideal conditions to

maintain the quality of stored Chinese cabbage. However, van den Berg and Lentz (1977) reported that during refrigerated storage, relative humidities of 98-100% resulted in lower storage losses in Chinese cabbage cultivar 'Michihli' (20-40% by weight after three months storage) than 90-95% relative humidity (25-75% loss by weight). Losses were due to wilting, yellowing of leaves at low relative humidity, mold on leaf edges and black spots on leaf veins.

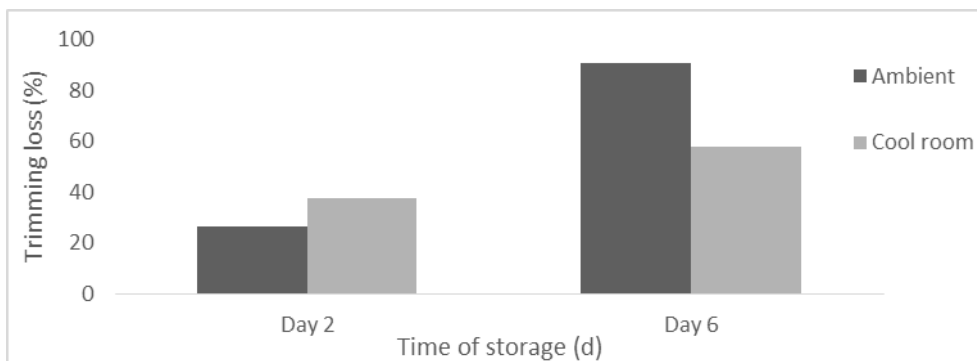


Figure 2. Percentage of trimming loss in Chinese cabbage heads stored in different storage conditions (ambient= 27.0±0.6°C, 69.6±6.1% RH and cool room = 21.1±1.3°C, 49.8±9.8% RH) for 2 and 6 days.

Trimming loss in alum-treated Chinese cabbage heads inoculated with *Pectobacterium carotovorum*

Alum is an antibacterial agent reported to reduce soft rot in cabbage heads transported from Northern Luzon to Southern Luzon (Bayogan et al., 2009). In the present study, newly-harvested Chinese cabbage heads inoculated with soft rot bacteria (*Pectobacterium carotovorum*), then treated with 10% alum within 24 h from harvest, lasted eight days in storage – albeit with large trimming losses of 79% at seven days and over 89% eight days after treatment (Figure 3). In wet markets and small retail stores in the Philippines, excessively trimmed Chinese cabbages are still offered for sale. In the Southern Philippines, retailers often resort to regular trimming of wilted, yellowed leaves or rotted portions to the extent that very small heads are retained – a practice that is unheard of in the cool, highland areas of the Cordillera Administrative Region in the Northern Philippines where most Chinese cabbages are produced. At six days, trimming losses reached 70% in alum-treated Chinese cabbages, while no control samples remained. After eight days of ambient storage, five out of six samples remained for alum-treated heads, whereas all of the control samples were unmarketable after six days of storage. Alum has the capacity to withdraw water, thus creating a dry surface area in the butt-end of the Chinese cabbage and making it an effective antibacterial agent that could prevent decay due to soft rot bacteria (Acedo, 2010). Since the control lot was not treated with alum, its butt-end area was exposed to the bacteria, which proliferated causing further rot. Hence, alum-treated Chinese cabbage heads had a longer shelf life than the controls.

CONCLUSION

Trimming losses in Chinese cabbage can be excessive at the wholesale and retail level. Excessive trimming constitutes a postharvest loss, therefore it is important to reduce trimming losses in Chinese cabbage. Trimming loss assessment in Chinese cabbage was done at the wholesale and simulated retail (location in the sack, cold storage, use of alum) levels. For the wholesale loss assessment, the packaging type and place of handling could affect trimming loss. In addition, preharvest quality of crops and market intermediaries' perception of quality of the produce could also affect the degree of trimming. Losses of 43.9 and 28.5% were observed in Chinese cabbages in the wholesale markets in Kapatagan, Davao del Sur and Bulua, Cagayan de Oro City, respectively. The arrangement of the produce

within the sack container did not affect trimming loss, since there was no difference among the top, middle and bottom sections and all heads were all equally exposed to stress. Cool room conditions are suitable for storing Chinese cabbage, but the optimum relative humidity should be achieved in order to prevent excessive wilting. At ambient conditions, trimming losses reached 70% at six days of storage in alum-treated Chinese cabbage heads, whereas no control samples remained. Future studies may explore effect of field hygiene and use of alum with cold storage and high relative humidity.

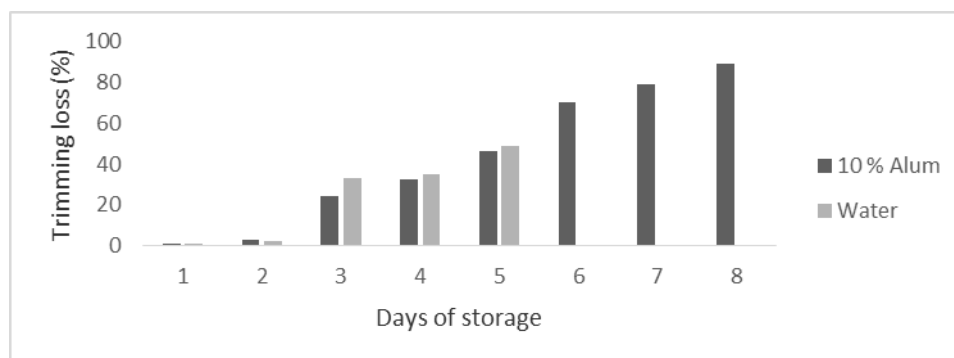


Figure 3. Trimming losses (% by weight) in Chinese cabbages inoculated with *P. carotovorum* and treated with 10% alum or water on the butt-end then stored in ambient conditions ($26.2\pm 0.3^{\circ}\text{C}$, $84.8\pm 2.0\%$ RH) for eight days.

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Postharvest Quality and Antioxidant Content of Organic Eggplant (*Solanum melongena* L.) in Modified Atmosphere Packaging

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Keywords: organic agriculture, vermicompost, total antioxidant content

Abstract

Organic farming is increasingly adopted to add value to vegetable production and marketing. This study was conducted to determine the effects of preharvest vermicompost application and postharvest modified atmosphere packaging (MAP) on the quality, shelf life and total antioxidant activity of eggplant fruits. Vermicompost was applied at 2.5 kg/plant at planting and compared with inorganic fertilizer application (farmer's practice) and without fertilizer applied. Harvested fruits were individually packed in 0.02 mm thick low density polyethylene (PE) bag or 0.05 mm thick polypropylene (PP) bag and stored at ambient; unpacked fruit served as control. Fertilizer application using vermicompost or inorganic material reduced weight loss and shrivelling of the fruit. Modified atmosphere packaging (MAP) was very effective in delaying shrivelling, reducing weight loss, improving visual quality, and extending shelflife of fruits. Fruit antioxidant activity was generally higher with vermicompost application than with chemical fertilizer application. Fruit browning, respiration rate and decay incidence did not differ with treatment after seven days of ambient storage.

INTRODUCTION

Eggplant (*Solanum melongena* L.) is a nightshade vegetable and known as purple pears which belong to the family *Solanaceae*. It is one of the most important vegetables nowadays due to many beneficial effects in human health and nutrition. It is a culturally important vegetable commonly consumed in South and South East Asia. It is grown for its fruits which are utilized as vegetables to contribute to the essential nutrients in our diet (Nafiu et al., 2011). While many fruits and vegetables are important for good health, eggplant appears to play a special role in the treatment and prevention of a number of very serious conditions. The nutritional value of this vegetable may not be high, however, it still contains some very essential nutrients and even some medicinal properties that are highly beneficial for the human body (<http://www.buzzle.com/article/eggplant-nutritional-value.html>). Eggplant is ranked as one of the top ten vegetables in terms of oxygen radical scavenging capacity due to the fruits phenolic constituents. Flavonoid is a large group of phytonutrients that are water-soluble pigments, considered to have antioxidant and anti-inflammatory properties. It protects the body against cardiovascular disease as they are able to reduce the oxidation of low density lipoprotein (Razak, 2012). For countless generations, anthocyanins, which belong to the parent class molecule of flavonoids, have also been consumed by man without an apparent ill effect to health. Anthocyanin can reduce coronary heart disease, improved visual activity; possess antioxidant and anticancer activities (Fuentes, 2002). Moreover, Ghosh and Konishi (2007) showed that high dietary intake of foods rich in flavonoids, including anthocyanin is associated with low prevalence of some diseases such as cancer and cardiovascular diseases as evidenced from epidemiological studies. Takamura et al. (1994), cited by Pino (2011), also reported that anthocyanin acts as a powerful antioxidant.

There is a research on the antioxidant activity carried out by a systematic examination on a phenolic acid content of the flesh fruit that is found on seven commercial cultivars of eggplant (Eun Ju Jung et al., 2011). A number of observations as

well as accumulated research findings had supported to claim that eggplant contains an array of health benefits (Sterling, 2001, cited by Racquel, 2006). Considering the medicinal benefits and the potential market demand of eggplant, techniques in management and production should be given much attention. Vermicompost constitutes a promising alternative to inorganic fertilizers in enhancing plant growth. Vermicompost is a microbiologically-active, nutrient-rich, peat-like material, which when added to plant growing media may influence plant growth directly or indirectly through different chemical, physical and biological mechanisms (Lazcano and Dominguez, 2011). Moreover, it provides better P nutrition from different organic waste (Ghosh et al., 1999). In addition, benefit of using vermicast is the presence of plant hormone compound which can enhance cell growth and plant metabolism. Studies on the production of important vegetable crop like eggplant have yielded good results by the use of vermicompost, but no scientific findings on postharvest quality and antioxidant content of organic eggplant (*Solanum melongena* L.) in modified atmosphere packaging.

MATERIALS AND METHODS

Newly harvested eggplant cultivar 'Morena F₁' at commercial maturity stage produced by a farmers' farm in Sta. Cruz, Baybay, Leyte and Eco-Farmi VSU, Baybay, Leyte was collected and brought to the Postharvest Technology Laboratory, Department of Horticulture, Visayas State University, Visca, Baybay, Leyte. On arrival, the fruits were sorted out and only those with more or less uniform size and no damage were used as samples. Nine were used for each treatment; three samples per treatment per replication were placed in cooled water to remove the field heat before placing in polyethylene bags. The experiment was laid out in 3×3 factorial experiments in Completely Randomized Design (CRD). The treatments were replicated three times: Factor A – fertilizer treatment: without fertilizer; inorganic fertilizer (farmers' practice); T₃ – vermicompost application (2.5 kg/per plant). Factor B – Modified atmospheric packaging: M₁ – open; M₂ – polyethylene bag (PE) (0.02 mm thick); M₃ – polypropylene bag (PP) (0.05 mm thick). Data gathered (weight loss, shriveling, visual quality rating, lightness, respiration rate and antioxidant content were subjected to analysis of variance using the Statistical Analysis System software. Significant differences ($P<0.05$) among treatments were identified by means of the least significant difference (LSD) test.

RESULTS AND DISCUSSION

Weight Loss and Shriveling

No significant interaction effect between organic fertilizer and modified atmosphere treatments was obtained for weight loss and shriveling. Weight loss in eggplant did not vary when eggplant fruits were stored from the first day of storage (Fig. 1). No differences were observed for all the fertilizer treatments and these values were significantly higher than the control. Among MAP, the open-stored fruits rapidly increased in weight loss and shriveling throughout storage. MAP using polymeric films might be considered to be beneficial to maintain high humidity essential for prevention of water loss. It creates a humid atmosphere by trapping the transpirational water within the package thereby decreasing the vapor pressure deficit which is driving force of water loss.

Visual Quality Rating and Shelf life

The visual quality of eggplant fruits was significantly affected by the interaction between the two types of modified atmosphere packages (PE and PP) where the fruits were stored and the fertilizer treatments (Fig. 2). Fruit in MAP had higher visual quality rating than in open-stored fruit. The fruit appeared fresh and defect free during the first 3-4 days of storage specifically in MAP; thereafter, visual quality decreased.

The shelf life in eggplant fruits was significantly affected by the interaction between the two types of modified atmosphere packages (PE and PP) and the fertilizer treatments (Fig. 2). Fruit held in PE and PP had longer shelf life than fruit held in open,

which had shorter shelf life. Rapid shriveling and loss of turgidity were the main factors to limit the shelf life of fruit in open condition. MAP treatments were rated to be more desirable and marketable which reached the limit of shelf life and also effective in inhibiting shriveling which consequently resulted to increased shelf life.

Browning incidence, lightness (L^* value) and respiration rate were not affected by the application of vermicompost and inorganic fertilizers and between the two types of packages (PE and PP) after seven days of storage (Table 1).

Total Antioxidant Content

The total antioxidant content in eggplant fruits in response to the vermicompost and inorganic fertilizer treatments and stored in modified atmosphere package under ambient condition were determined when the fruit sample reached to VQR 5. Based on the analysis of variance, there were significant interaction effects between fertilizer treatments and the two types of packages (MAP) where the eggplants were stored under ambient condition (Table 2). Based on the results, the level of antioxidants was generally higher in eggplant fruits fertilized with vermicompost compared with those applied with inorganic fertilizer. The antioxidant levels of eggplant from open-stored fertilized with vermicompost were at par with those obtained from the control (without fertilizer) stored in PP. Fruits gathered from plants applied with the inorganic fertilizer had the lowest amount of this compound. Findings of this study were in agreement with the recent researches that provide evidence for the relatively higher antioxidant contents of organically-grown crops than those treated with inorganic fertilizer. In basil plants raised from organic and bioorganic fertilization exhibited higher antioxidant activity (Taie et al., 2010). Similarly, one of the compounds of antioxidant content (vitamin C) of strawberry fruits (Mahadeen, 2009) and sweet pepper (Ghoname and Shafeek, 2005) was significantly increased with the application of organic fertilizer consisting of poultry manure and a combination of organic manure, mineral and Bio-N fertilizer, respectively.

CONCLUSIONS

The pre-harvest vermicompost application eggplant fruits effectively maintained the quality during storage. MAP (PE and PP) effectively delayed the shriveling, reduced weight loss and prolonged shelf life of fruits. Fruit antioxidant activity was generally higher with vermicompost application than with chemical fertilizer application.

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Tables

Table 1. Browning, lightness (L*) and respiration rate of eggplant fruits as affected by fertilizer treatments and modified atmosphere packaging (MAP) at ambient.

Treatments	Browning incidence (VQR 5)		Lightness L* value		Respiration rate (VQR 5)
	15 min cut	30 min cut	15 min cut	30 min cut	
Control					
Open	2.00	3.00	70.4	70.2	0.063
PE	1.00	2.00	74.93	70.96	0.08
PP	2.00	3.00	67.53	65.42	0.01
Inorganic					
Open	3.00	2.00	75.47	73.2	0.075
PE	2.00	2.00	76	71.85	0.311
PP	2.00	2.00	71.16	61.66	0.027
Vermicompost					
Open	3.00	3.00	68.61	65.56	0.074
PE	1.00	2.00	65.02	67.16	0.074
PP	2.00	2.00	62.81	59.8	0.051

Means within a column followed by the same letter and those without letter designation are not significantly different using LSD at 5% level.

Table 2. Total antioxidant content of eggplant fruits as affected by fertilizer treatments and modified atmosphere package under ambient condition.

Treatments	Total antioxidant content ($\mu\text{mol TE } 100 \text{ g}^{-1}$)
Control	
Open	343.48a
PE	317.56c
PP	319.76b
Inorganic	
Open	307.02d
PE	293.41d
PP	313.61c
Vermicompost	
Open	328.55ab
PE	285.06e
PP	311.85cd

Means within a column followed by the same letter are not significantly different using LSD at 5% level.

Figures

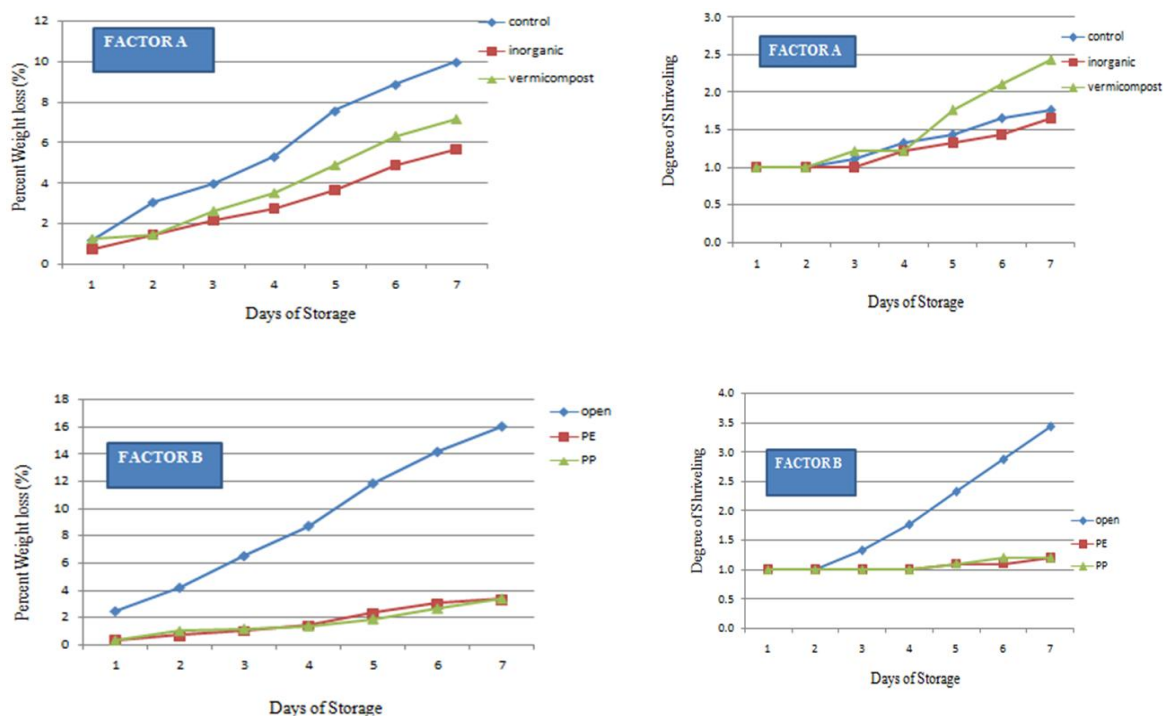


Fig. 1. Percent weight loss and shriveling of organic eggplant fruits (*Solanum melongena* L.) in modified atmosphere packaging.

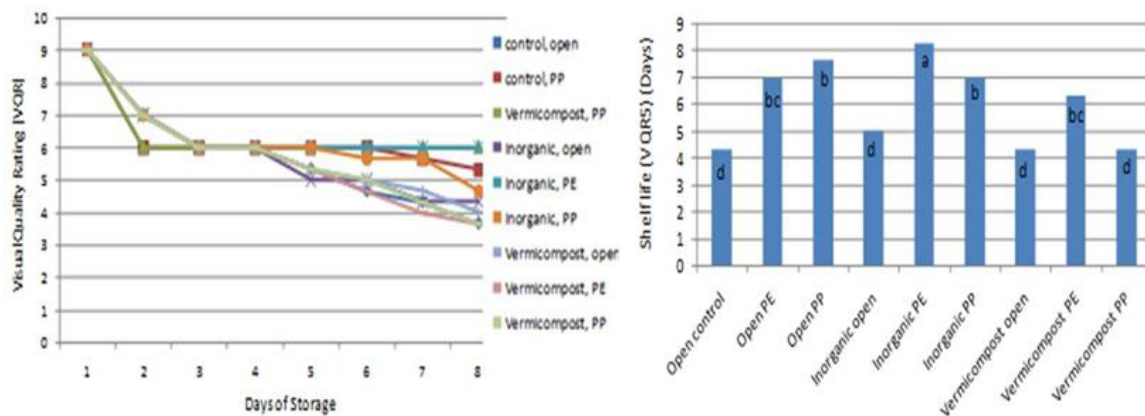


Fig. 2. Visual quality and shelf life of organic eggplant fruits (*Solanum melongena* L.) in modified atmosphere packaging.

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Theme: Global Food Safety and Security*Research Paper***Effects of Polyvinyl chloride film on bitter gourd quality under various storage conditions****Marilou Benitez¹, Aljay Valida¹, Fatima Rose Rivera¹, Michael Adonis Sudaria¹, Analita Salabao¹, Eutiquio Sudaria¹, Antonio Acedo Jr.², Emma Winley³ and Jenny Ekman³**¹Postharvest Technology Division, Department of Horticulture, Visayas State University, Leyte, Philippines²AVRDC-The World Vegetable Center, South Asia Regional Office, ICRISAT Campus, Patancheru 502 324, Hyderabad, India³Applied Horticultural Research, Suite 352, Biomedical Building, 1 Central Avenue, Australian Technology Park, Eveleigh NSW 2015, Australia**Corresponding author email addresses:**

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Abstract

Good packaging during transport and storage are important factors for bitter gourd because of their high perishability. The study evaluated the effects of polyvinyl chloride film and storage conditions to improve bitter gourd marketability. A 2 x 3 factorial experiment was laid out in Completely Randomized Design (CRD) with 6 treatments and 3 replications. Thirty (30) bitter gourd fruit samples per replication were used. The treatments were: Factor A- MAP (W₁- control, W₂- with polyvinyl chloride film) Factor B-Storage Conditions (S₁-Ambient room, S₂-Refrigerated, S₃-Evaporative Cooler). Polyvinyl chloride film was applied by wrapping the whole fruit. Ambient storage was done by keeping the fruits at 25°C, Evaporative storage using a box-type evaporative cooler while refrigerated storage was done using a chiller with temperature maintained at 7-10°C. Results demonstrated that bitter gourd treated with polyvinyl chloride film wrapping were similar in terms of weight loss, total soluble solids, titratable acidity and shelf-life relative to the control. Meanwhile, Bitter gourd under refrigerated storage prolonged the shelf life with the highest value of 12 days compared to 7.4 and 7.8 days for ambient and evaporative storage, respectively. Weight loss was higher for both ambient and evaporative storage. Soluble solid content and titratable acidity did not differ among storage treatments which ranged from 5.2-6.3°Brix and 0.18-0.24% citric acid, respectively. The prelim-

inary results could serve as basis for further intervention studies to improve bitter gourds marketability.

Keywords: *Momordica charantia*, plastic film, fruit quality, box-type evaporative cooler

1. Introduction

Good packaging during transport and storage are important factors for bitter gourd because of their high perishability. Bitter gourd shelf life is much shorter at tropical ambient conditions (25-32°C). The fruit also has high moisture content, a large surface area to volume ratio, and a relatively thin cuticle, which makes it very susceptible to moisture loss and physical injury. Undesirable changes including loss of colour, shriveling, and rotting will occur immediately after harvest. In addition, improper handling, packaging, storage and transportation could further result in huge losses (Mohammed, 2010). The use of Modified Atmosphere Packaging (MAP) such as polyvinyl chloride films could address damage during transport and prolong desirable bitter gourd quality. PVC is most commonly used for clear plastic wrapping because of its cheap cost and stretching capabilities as well as being easy to extrude into sheets (Pearson 1982). Modified-atmosphere packaging (MAP) of fresh fruits and vegetables refers to the technique of sealing actively respiring produce in polymeric film packages to modify the O₂ and CO₂ levels within the package atmosphere. It is often desirable to generate an atmosphere low in O₂ and/or high in CO₂ to influence the metabolism of the product being packaged or the activity of decay-causing organisms to increase storability and/or shelf life (Church et al., 1995). The study evaluated the effects of polyvinyl chloride film and storage conditions to improve bitter gourd marketability.

2. Materials and Methods

Bitter gourd fruits were harvested from a local farm located at Brgy. Curva, Ormoc City, Leyte. Samples uniform in size and free from damage were utilised. A 2 x 3 factorial experiment was laid out in Completely Randomized Design (CRD) with 6 treatments and 3 replications. Thirty (30) bitter gourd fruit samples per replication were used. The treatments were: Factor A- MAP (W₁= control, W₂= with polyvinyl chloride film) Factor B- Storage Conditions (S₁-Ambient room, S₂-Refrigerated, S₃-Evaporative Cooler). Polyvinyl chloride film was applied by wrapping the whole fruit. Ambient storage was done by keeping the samples under ordinary room conditions at 25°C, Evaporative storage using a box-type evaporative cooler while refrigerated storage was done using a chiller with temperature maintained at 7-10°C. Product quality was evaluated in terms of weight using a digital weighing scale, total soluble solids (TSS) using an Atago N1 Hand Refractometer, titratable acidity (% citric acid) by titration using standard 0.1 N NaOH and 1% phenolphthalein indicator, and the defects responsible for visual quality loss were noted. The number of days to reach VQR 5, using the visual quality rating: 9-Excellent, field fresh or no defect; 7-Good, defects minor; 5-Fair, defects moderate, limit of marketability; 3-Poor, defects serious, limit of edibility and 1-Non-edible under usual condition (Bautista et al., 2007), was taken as a measure of the potential shelf life of eggplant under ambient, evaporative, and refrigerated storage conditions. Statistical analysis of results was performed using the STAR program of the International Rice Research Institute (STAR, 2014).

3. Results and Discussion

Bitter gourd wrapped with polyvinyl chloride film was similar in terms of percent weight loss and total soluble solids relative to the control after 12 days storage (Fig. 1). Titratable acidity and shelf

life were also not significant among treatments which ranged from 0.19-0.23% citric acid and 11.4-12 days. The extent of benefits from MA use depends upon the commodity, cultivar, physiological age, initial quality, concentrations of CO₂ and O₂, temperature and duration of exposure to such conditions (Kader et al., 1989). For some commodities, susceptibility to low O₂ and/or high CO₂ stress is influenced by maturity stage which might have ensued in the present study. Ripe fruits often tolerate higher levels of CO₂ than mature green fruits. The effects of stress resulting from exposure to undesirable MA conditions can be additive to other stresses in accelerating the deterioration of the fresh produce (Kader et al., 1989). Meanwhile, Bitter gourd stored under refrigerated storage prolonged the shelf life of 12 days compared to 7.4 and 7.8 days for ambient and evaporative storage, respectively.

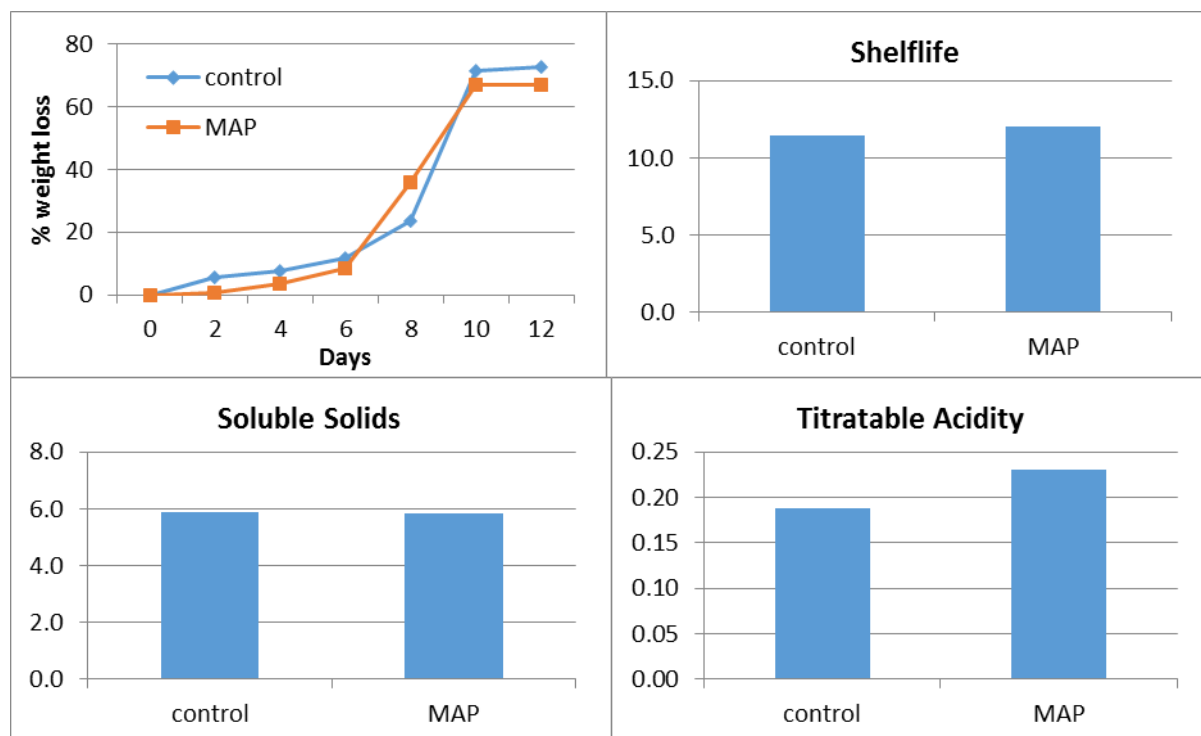


Figure 1. Effect of plastic overwrapping (MAP) on the weight loss, shelf life, soluble solids and titratable acidity of bitter gourd stored for up to 12 days. (Treatment means with a common letter are not significantly different at 0.05 level LSD).

Weight loss was higher for both evaporative and ambient storage (Fig. 2). The weight loss under ambient and evaporative condition can be attributed to high temperature and possibly uneven relative humidity particularly for evaporative condition. These conditions are favourable to moisture loss which is the major cause of losses in harvested vegetables. Among the storage conditions, cold storage has always been recommended due to its efficacy in extending commodity life over a long period of time, including the inhibition of decay development in fruits and vegetables with deep-seated infections which cannot be eradicated by postharvest treatment (Salunkhe et al., 1968). Soluble solid content and titratable acidity did not differ also among storage treatments which ranged from 5.2-6.3°Brix and 0.18-0.24% citric acid, respectively (Fig. 2). Soluble solids and titratable acidity are important fruit quality characteristics of bitter gourd. The changes in composition of sugars and organic acids and volatile compounds during ripening process play a key role in flavour development and can affect the chemical and sensory characteristics of fruit (Abeles et al., 1990).

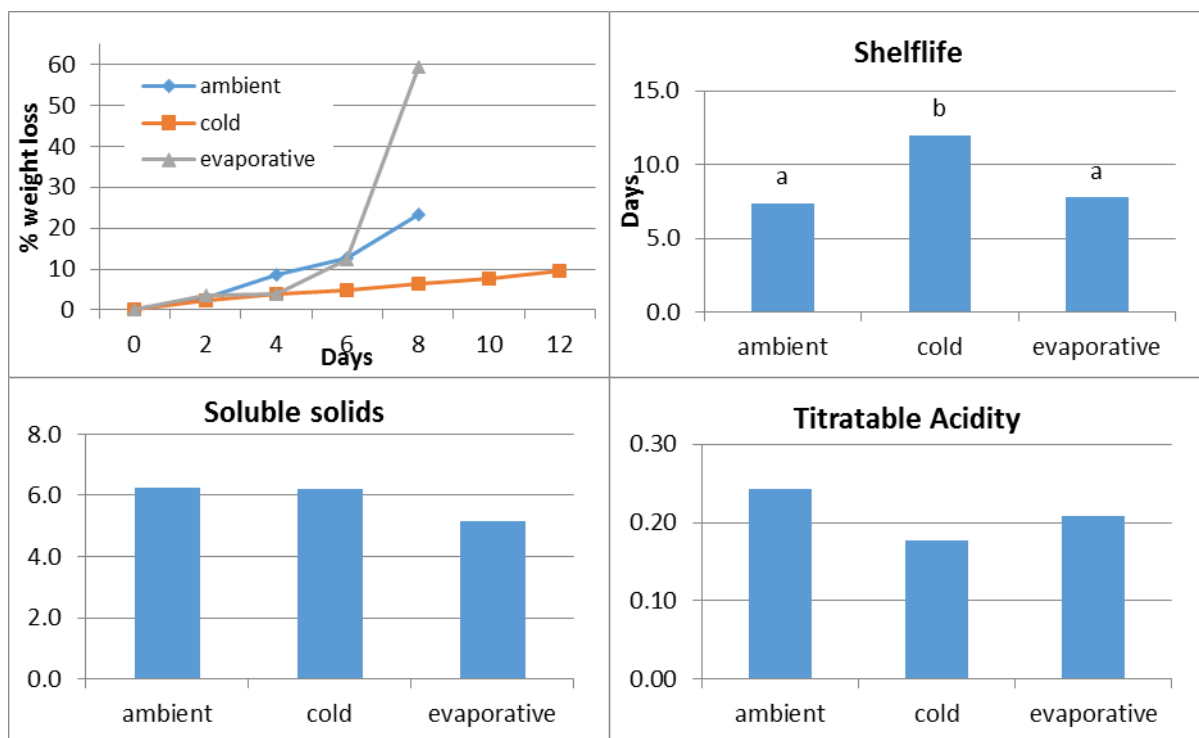


Figure 2. Effect of cooling method on weight loss, soluble solids, titratable acidity and shelf life of bitter gourd after 12 days storage. (Treatment means with a common letter are not significantly different at 0.05 level LSD).

4. Conclusion

Polyvinyl chloride film had no degenerative effect on bitter gourd quality and cold storage extended the shelf life. The preliminary results could serve as basis for further intervention studies to improve bitter gourds marketability.

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Postharvest treatment effects on 'B74' mango fruit lenticel discolouration after irradiation

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Abstract

Lenticel discolouration (LD) is a common disorder of mango fruit around the world. It results in poor appearance and disappointment of consumers. LD is exacerbated by treatment of mango fruit with gamma irradiation for insect disinfestation. The issue is problematic on the relatively new mango cultivar 'B74' and may represent an oxidative browning process. With a view to reducing irradiation-induced LD on 'B74', postharvest wax (one and three layers; 75% carnauba wax) and antioxidant (100 mM ascorbic acid, 100 mM calcium chloride, 10, 50 and 100 mM calcium ascorbate) dip treatments were investigated. Treatment of green mature fruit with three layers of wax prior to exposure to 557 Gy gamma irradiation reduced LD by 40% relative to the non-waxed control. However, the fruit failed to ripen properly as evidenced by delayed skin colour change, retarded softening and increased skin browning as compared to the controls and fruit coated with one layer of wax. Treatment with one layer of wax did not reduce LD. Mechanistically, the responses suggest that air exchange plays a pivotal role in LD. A lowered oxygen concentration in the lenticels may reduce the disorder after irradiation treatment. Postharvest treatments with the various antioxidants failed to reduce LD. Rather, all antioxidant treatments at the test concentrations, except calcium chloride, significantly increased skin browning.

Keywords: antioxidants, 75% carnauba wax, lenticel discolouration, skin colour, firmness, skin browning

INTRODUCTION

Lenticels are macroscopic pores in plant tissues that regulate gas exchange (Kader and Saltveit, 2003). Discolouration of lenticels on some fruit such as mango, apple and avocado is a cosmetic defect that reduces their appearance quality (Everett et al., 2008; Joyce et al., 2011; Grimm et al., 2012). This discolouration is typically manifested as red or brown 'halos' surrounding lenticels with/without a black or brown center (Hofman et al., 2010). Lenticel discolouration was reported to be associated with cork cambium and cells in the lenticels of 'Tommy Atkins' mango fruit (Bezuidenhout et al., 2005). LD has been suggested as a defence mechanism (Plooy et al., 2006). Exposure to rain, water during postharvest handling and hot water increase dark brown LD on mango fruit (Jacobi and Wong, 1989; O'Hare et al., 1996, Mitcham and Yahia, 2009). Exposure of mango fruit γ -irradiation as an insect disinfestation treatment also increases LD (Hofman et al., 2010).

'B74' (Calypso™) is a relatively new cultivar of mango developed in Australia (Whiley, 2000). This cultivar has an attractive appearance, fibre-free flesh and small seed to flesh ratio. It has been successfully commercialised in Australia and is exported to other countries; e.g., New Zealand, China (Dillon et al., 2013).

In order to eliminate insect infestation during storage and transport, phytosanitary treatment with γ -irradiation is applied to 'B74' mango fruit prior to export (Australian Mango Industry Association, 2014). However, 'B74' fruit are relatively sensitive to irradiation-induced LD and generally lowered skin appearance quality (Hofman et al., 2010). LD in mango may reflect an oxidative enzymatic tissue browning process.

In the present study we tested the efficacy of coating 'B74' mango fruit with carnauba



wax prior to irradiation treatment to reduce LD by restricting fruit gas exchange (O₂, CO₂). We also evaluated the capacity of pre-treating fruit with solutions of various antioxidants to reduce the disorder by directly inhibiting oxidative enzyme activity.

MATERIALS AND METHODS

Plant material

'B74' mango (*Mangifera indica*) fruit was grown under standard commercial conditions at an orchard near Childers (25°17' S, 152°17' E) in Queensland, Australia. Green mature fruit were harvested in January 2013 with a dry matter content of 13.6±0.21% (*n*=10). They were de-sapped in a commercial mango wash solution. The fruit were passed through a commercial packing line wherein they were washed, treated with a fungicide and insecticide, brushed and graded for uniformity. They were then packed into single layer tray cardboard trays and transported by car to the Maroochy Research Facility near Nambour, Queensland within 4 h. The fruit were maintained at 20°C and randomly allocated to treatments on the following day.

Chemicals

Carnauba wax (Natural Shine™ TFC 210, Pace international Pty Ltd, Australia) was dissolved in distilled water to a final concentration of 75% (v/v). Stock solutions of ascorbic acid (BDH Australia Pty Ltd, Australia), calcium chloride (BDH Australia Pty Ltd, Australia) and calcium ascorbate (Melrose Laboratories Pty Ltd, Australia) were also prepared in distilled water.

Experiment 1

Entire fruit were dipped into 75% carnauba wax solution for 10 s and then air-dried at room temperature (ca. 26°C) for 1 h. Additional fruit were dipped in the wax solution three times to increase the wax coating thickness. Fruit dipped into distilled water (DW) were controls. The fruit were then exposed to γ -irradiation treatment as described below.

Experiment 2

Entire fruit were dipped into solutions of either of 100 mM ascorbic acid (AA), 100 mM calcium chloride (CC) or 10, 50, 100 mM calcium ascorbate (CA) for 10 min. They were then air-dried as per experiment 1. Additional control fruit were dipped in DW. The fruit were then exposed to γ -irradiation as described below.

Irradiation treatment

Fruit were packed into cardboard trays in a completely randomized fashion and transported to a commercial irradiation facility (Steritec, Narangba, Queensland, Australia) within 1 h. The fruit were exposed to an average dose of 557 Gy (min-max 524-587 Gy of γ -irradiation from a cobalt-60 source. The fruit were then transported back to the Research Facility and maintained at 20°C and 90-100% relative humidity in the absence of exogenous ethylene until ripe. Fifteen individual replicate fruit were used per treatment.

Quality assessments

Fruit were assessed for various quality parameters on days 0, 4, 8, 11 and 14 of the experiments. LD was assessed using the scale (Hofman et al., 2010): 0 = no lenticel spotting; 1 = light spots on less than 25% or dense, pronounced spots on less than 5% of skin, not star-shaped or cracked; 2 = light spots on less than 50% or dense, pronounced spots on more than 5% and less than 10% of skin, not star-shaped or cracked; 3 = scattered pronounced spots on less than 50% or dense, pronounced spots on more than 10% and less than 25% of skin, not star-shaped or cracked; 4 = dense, pronounced spots on more than 25% and less than 50% of skin, star-shaped or cracked; 5 = dense, pronounced spots on more than 50% of skin, star-shaped or cracked. Skin colour assessment was based on the proportion of the non-blushed area with yellow skin colour using the scale: 1 = 0-10%

yellow, 2 = 10-30% yellow, 3 = 30-50% yellow, 4 = 50-70% yellow, 5 = 70-90% yellow, and 6 = 90-100% yellow. Firmness was assessed by hand using the scale: 0 = hard (no 'give' in the fruit), 1 = rubbery (slight 'give' in the fruit with strong thumb pressure), 2 = sprung (flesh deforms by 2-3 mm with moderate thumb pressure), 3 = firm soft (whole fruit deforms with moderate hand pressure), and 4 = soft (whole fruit deforms with slight hand pressure). Skin browning was assessed as the area on each fruit using the scale: 0 = no browning, 1 = less than 1 cm², 2 = 1-3 cm² (3%), 3 = 3-12 cm² (10%), 4 = 12 cm² (10%) to 25%, and 5 = more than 25%.

Experiment design and statistical analysis

Fruit were arranged in a completely randomised design in both experiments. LD, skin colour, firmness and skin browning data over time were analysed as factorial ANOVAs using the repeated measurement procedure of GenStat (Version 14). The significance between treatment means was tested with the unprotected Fisher's test at the 5% level.

RESULTS AND DISCUSSION

Experiment 1

1. Lenticel discolouration.

There was a significant interaction of irradiation, layers of wax and time on LD development in 'B74' fruit (Figure 1). Exposure to γ -irradiation significantly increased the incidence and severity of LD within four days of treatment as compared to non-irradiated fruit (Figure 1). γ -Irradiation treatment possibly increased total phenolics (Oufedjikh et al., 1999; Benoît et al., 2000) and/or polyphenol oxidase (PPO)/peroxidase (POD) activities in the skin leading to the increase in LD (Thomas and Janave, 1973). Treating fruit with three layers of carnauba wax significantly reduced γ -irradiation-induced LD by 40% relative to non-waxed fruit (Figure 1). However, coating fruit with three layers of wax impaired fruit ripening. Thick waxing presumably reduced oxygen entering into lenticels to limit enzymatic browning (Amarante and Banks, 2001; Baldwin, 2004) and lessened LD. Coating fruit with one layer of wax did not reduce LD (Figure 1).

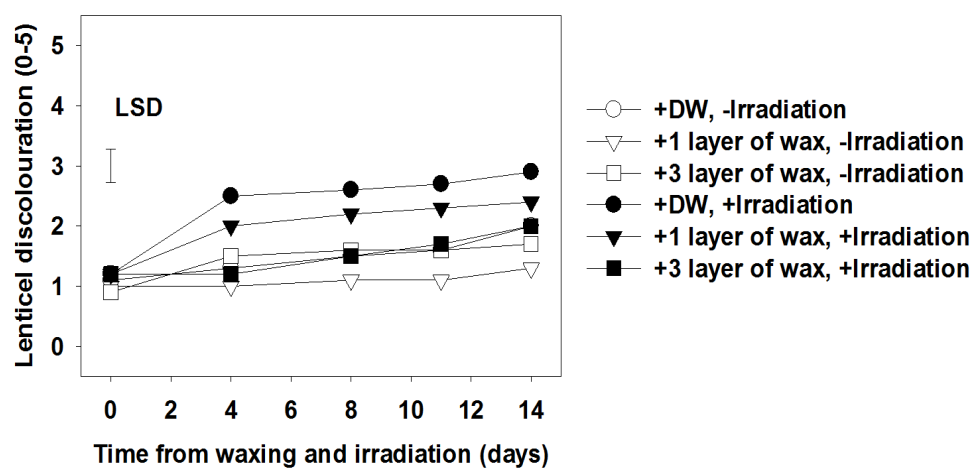


Figure 1. Effects of coating 'B74' fruit with one and three layers of 75% carnauba wax on lenticel discolouration after their subsequent exposure to 0 or 557 Gy γ -irradiation. Fruit dipped in distilled water (DW) were used as control ($n=15$). The LSD ($P=0.05$) bar is for factorials effects.

2. Skin colour.

There was a significant interaction of irradiation, wax coating and time on fruit skin colour development (Figure 2). The ripening-related loss of green skin colour typically

increased (Figure 2) as a function of chlorophyll degradation and carotenoids accumulation (Medlicott et al., 1986). Exposure of fruit to γ -irradiation delayed skin de-greening compared to non-irradiated fruit (Figure 2). This finding is consistent with other's observations on 'Kensington' mango fruit treated with γ -irradiation (Boag et al., 1990). Pre-treatment of fruit with three layers of carnauba wax significantly reduced the loss of green skin colour by 29 to 36%, in the latter case for fruit exposed to γ -irradiation (Figure 2). Thick waxing may have slowed chlorophyll degradation by higher concentrations of carbon dioxide in the fruit tissues (Holcroft and Kader, 1999; Maftoonazad and Ramaswamy, 2005).

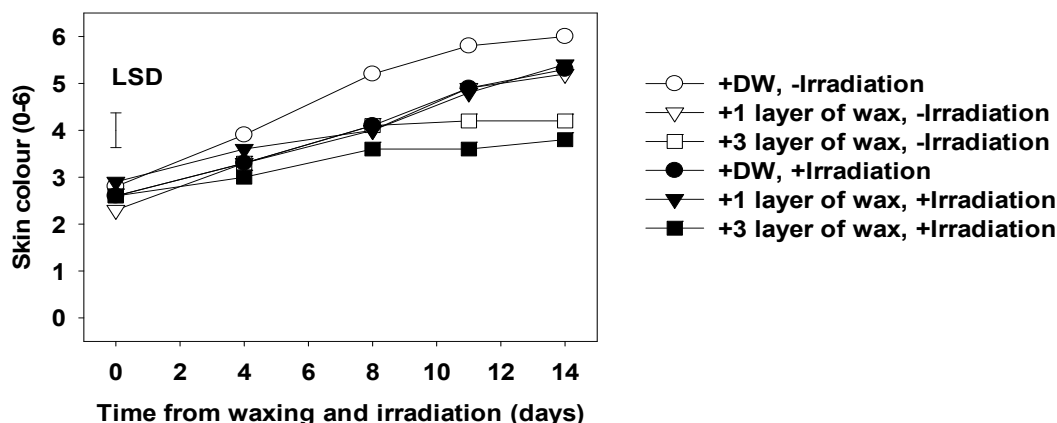


Figure 2. Effects of coating 'B74' fruit with one and three layers of 75% carnauba wax on skin colour after their subsequent exposure to 0 or 557 Gy γ -irradiation. Fruit dipped in distilled water (DW) were used as controls ($n=15$). The LSD ($P=0.05$) bar is for factorial effects.

3. Firmness.

There was a significant interaction of coating with wax and changes in fruit firmness over time (Figure 3). Irradiation did not influence firmness significantly (data not shown). Coating with three layers of wax significantly delayed and reduced fruit firmness (Figure 3). This is similar to other's observations on mango, guava and tomato (McGuire, 1997; Park et al., 1994; Dang et al., 2008). Heavy waxing may have impaired enzymes associated with cell wall digestion (e.g., pectinesterase, polygalacturonase) by limiting available oxygen (Hoa et al., 2002; Barman et al., 2011).

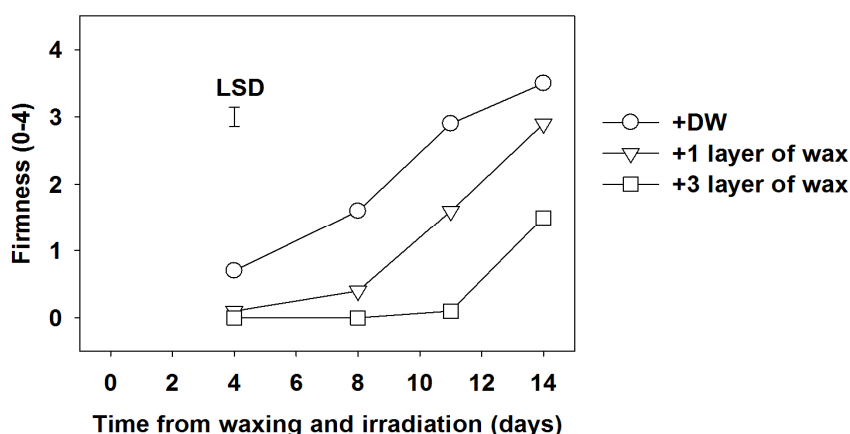


Figure 3. Effects of coating 'B74' fruit with one and three layers of 75% carnauba wax on firmness after their subsequent exposure to 0 or 557 Gy γ -irradiation. Fruit dipped in distilled water (DW) were used as controls ($n=30$). The LSD ($P=0.05$) bar is for factorials effects.

Experiment 2

1. Skin browning.

Irradiation and different concentrations of antioxidants was found induce skin browning (Figure 4). Exposure of fruit to γ - irradiation caused more skin browning than fruit not treated with irradiation (Figure 4). Similar results have been reported for banana and mushroom (Thomas and Nair, 1971; Beaulieu et al., 2002). Irradiation may damage cellular compartmentation integrity, thereby allowing phenolic substrates to come into contact oxidative enzymes (Kovács and Keresztes, 2002). Calcium ascorbate and ascorbic acid antioxidants have been shown to reduce browning on fresh-cut fruit and vegetables (Rico et al., 2007). However, treatment with 10, 50 and 100 mM calcium ascorbate (CA) and with 100 mM ascorbic acid (AA) in the present study induced greater skin browning on γ -irradiated mango than on non-irradiated controls (Figure 4). It is possible that ascorbic acid (AA) and calcium ascorbate (CA) had been oxidized completely after irradiation and enhance skin browning (Walker, 1995; Guerrero- Beltrán et al., 2005).

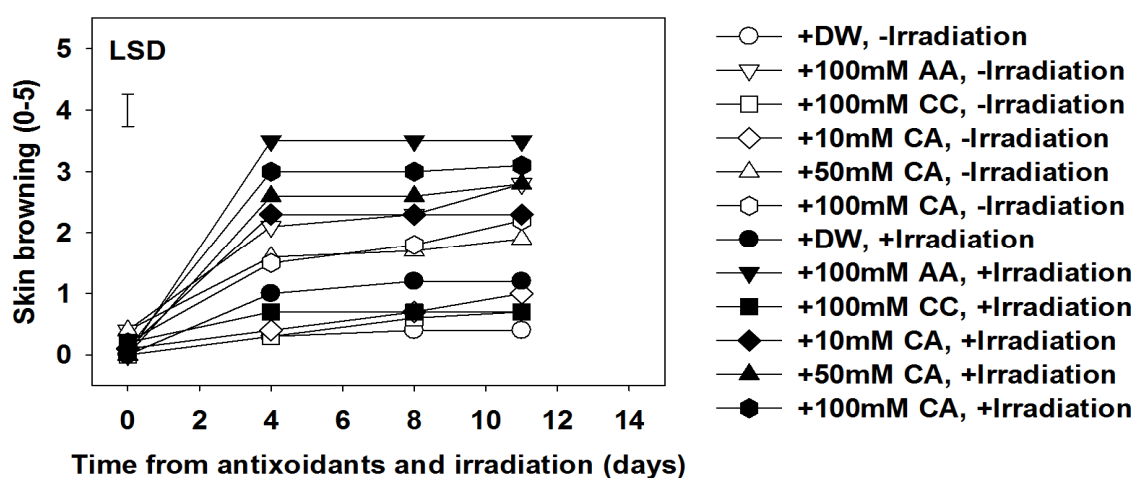


Figure 4. Effects of dipping 'B74' fruit with 100 mM ascorbic acid (AA) or calcium chloride (CC), or 10 mM or 50 mM or 100 mM calcium ascorbate (CA) on skin browning after their subsequent exposure to 0 or 557 Gy γ -irradiation. Fruit dipped in distilled water were used as controls ($n=15$). The LSD ($P=0.05$) bar is for factorials effects.

CONCLUSION

Treating 'B74' mango fruit with three layers of carnauba wax reduced irradiation-induced lenticel discolouration. However, this pre-treatment also delayed and impaired fruit ripening. Treating 'B74' mango fruit with different concentrations of antioxidants fail to reduce irradiation induced lenticel discolouration (data not shown), but this pre-treatment increased skin browning significantly.

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In-transit ripening of mango fruit: concepts and considerations

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Abstract

Refrigerated containers are used to transport mangoes over long distances. In Australia, mangoes grown in northern tropical production areas for the domestic market are transported by road and/or rail for up to 4000 km to southern ripening centres. There is no other viable option for growers to ripen their fruit. A current project is investigating sustained ethylene release systems for ripening mango fruit in road transport containers over an in-transit period of up to 4 days. This approach could potentially provide an alternative cost-effective ripening strategy for the mango industry. Studies have been and will be performed using shipments of 'B74' and 'Honey Gold' mango fruit, respectively, in refrigerated road containers. In-container environment monitoring, fruit shelf life and quality evaluation at outturn are carried out. Corresponding laboratory experiments that simulate transit are also to be conducted with a view to modelling mango fruit responses to in-transit conditions.

Keywords: mango, temperature, ethylene, carbon dioxide

INTRODUCTION

Mango production within Australia occurs in the Northern Territory, Queensland, Western Australia, New South Wales, Victoria and South Australia. The mango cultivars grown for domestic and export markets include 'Kensington Pride', 'B74', 'R2E2', 'Honey Gold' and 'Keitt' (AMIA, n.d.). Mango production in Australia for the last 5 years has been ~53,000 tonnes, with a gross farm gate value of ~AUD140 million per annum (AMIA, n.d.).

About half of Australian mangoes are produced in tropical areas (Figure 1). The climacteric fruit are harvested at mature firm green. They are generally pre-cooled to 12°C within 48 h of harvest. Such practices prolong their storage life and maximise ripe fruit quality, including final fruit skin colour development (Meurant et al., 1999).

In Australia, refrigerated containers are used to transport fruit by road and/or rail to ripening facilities located up to 3000-4000 km away in metropolitan centres. Controlled ripening techniques applied in Australia involve exposing mangoes to ethylene for 2-3 days (Ledger et al., 2014). The set concentrations are 10 $\mu\text{L L}^{-1}$ continuous ethylene in trickle systems and 100 $\mu\text{L L}^{-1}$ ethylene every 8-12 h in shot systems (Ledger et al., 2014). After gas ripening, most mangoes are sold through wholesale agents and/or supermarket chains.

Transporting fresh mango fruit within Australia poses challenges in association with maintaining uniform product with high end quality from farm gate to consumer. The many causes of postharvest loss can be classified into the two categories (Wills et al., 2007) of physical loss and quality loss. Physical loss can be from mechanical, pest and disease damage and also from transpiration leading to loss in saleable weight. Loss of quality can be from physiological and compositional changes that alter the appearance, texture and/or taste, making produce less desirable aesthetically for consumers. Normal senescence processes and abnormal events like chilling injury are other effectors of quality loss (Wills et al., 2007).

To minimise or avoid physical and quality losses when transporting mangoes by refrigerated containers, it is important to establish and maintain desirable environmental

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conditions in terms of temperature, relative humidity, ethylene concentration, and carbon dioxide concentration. These conditions should determine the success or failure of the in-transit ripening strategy.

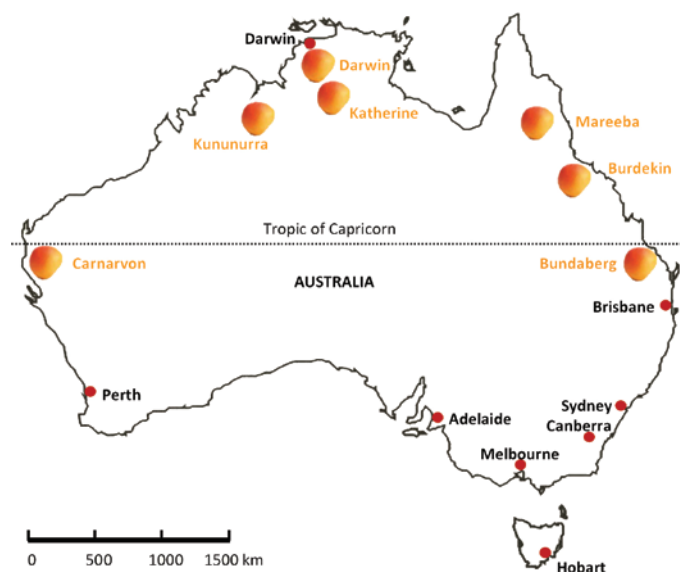


Figure 1. Map of Australia highlighting the main mango production areas and major city markets. Harvested mango fruit are transported up to 4000 km from farm to consumer.

THE CONCEPT

Logistically, ripening in-transit represents a currently unrealised opportunity for the Australian mango industry. It has potential to reduce the time that mango fruit spend in the postharvest system prior to their sale. It should prove a more cost-effective option for fruit ripening by decreasing requirements for expensive infrastructure for cooling, storage and ripening on-farm and/or in-market.

CONSIDERATIONS

Temperature

Maintaining optimum ripening temperature and relative humidity at recommended 18°C and 90-95% (Ledger et al., 2014), respectively, is important during postharvest for mangoes. Temperature is the single most important environmental factor controlling physiological changes associated with optimum ripening (Crisosto and Mitcham, 2015). Refrigerated containers are insulated boxes fitted with modular mechanical refrigeration units driven by diesel or electric motors (Wills et al., 2007). They are designed to maintain the product at a desired temperature. Rarely do the refrigerated containers have capacity to uniformly reduce the temperature of the mass of cargo to a desired level in a timely way (Anonymous, 1989). Pre-cooling on farm is typically the first step in the cool chain process. Rapidly removing field heat from crops is essential (Burdon, 1997). Meurant et al. (1999) recommend that close stacked pallets of mango fruit in trays be pre-cooled to 18-22°C in a forced air system. Pre-cooling and transporting mangoes at a maintained storage or holding temperature of 18-22°C is intended to avoid premature ripening (Meurant et al., 1999) and attendant loss of quality and shelf life (Brecht et al., 2009). Failure to do so can lead to an increase in water loss and pathogen development (Burdon, 1997). In-transit, fruit temperature is determined by relationships between the container set temperature, refrigeration capacity, airflow around and over the product, heat influx from outside, and product heat of respiration, which is greater at higher temperatures.

In monitoring refrigerated commercial mango consignments of 18-20 pallet road and rail shipments, Hofman et al. (2014a, b) found that air temperatures within the stow can be well above recommended levels. Marked temperature variation was recorded within a container with a set temperature of 18°C (Figure 2). The temperature had increased to ~35°C in the course of the 3-day journey. The temperature near the rear of the truck was 20°C at loading. Throughout the 3-day journey it fell slightly and then increased to ~22°C. The increase in temperatures in the face of ambient and product heat sources was attributed to poor air circulation throughout the stow. In another 3-day interstate truck shipment, higher temperatures were recorded to the rear of the truck and lower temperatures to the front (Figure 3). The fruit temperature at the front was ~14°C at loading; it increased steadily over the 3 days of transport to ~19°C. The logger toward the rear recorded higher temperatures of ~25°C at loading, decreasing to 22°C before increasing again to ~25°C.

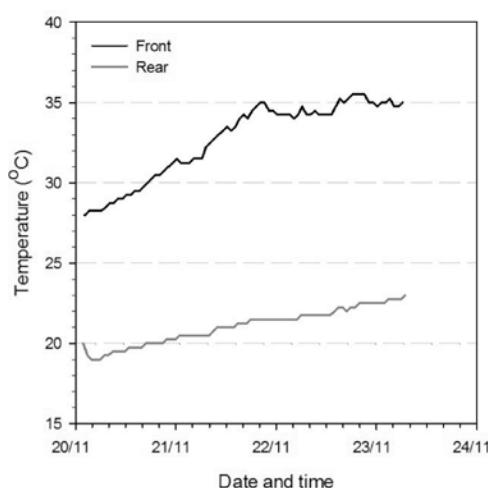


Figure 2. Air temperatures during a 3-day shipment as measured at two locations throughout the stow. The first logger was placed centrally in the second row of pallets from the front of the truck, four trays from the top of the pallet. The second logger was placed centrally in the second row from the rear of the truck, four trays from the top of the pallet. The temperature at the front of the truck was 28°C at outturn. The truck container was set at 18°C and contained 'B74' mango fruit. The temperature in the front of the truck exceeded 35°C (from Hofman et al., 2014a, b).

Elevated and variable temperature deviations from recommended industry standards can lead to poor and uneven ripening throughout a load. In turn, irregular ripening can reduce product quality, increase waste, and decrease profits.

Ethylene release

Ethylene (C₂H₄) concentration in a container is determined by its release from abiotic sources, such as the Ethylene Release Canister™ (ERC™) used to condition pallets of pears (Sharrock et al., 2010), from biotic sources, particularly its production by the product, and by container leakage rates. Ethylene production by mango fruit ripened at 20°C is typically moderate, peaking at 1-3 μL kg⁻¹ h⁻¹ (Brecht and Yahia, 2009). In regard to concentration effects, Nguyen et al. (2002) found there was little differential effect on ripening and quality of 'Kensington Pride' mango fruit with between 10-100 μL L⁻¹ ethylene.

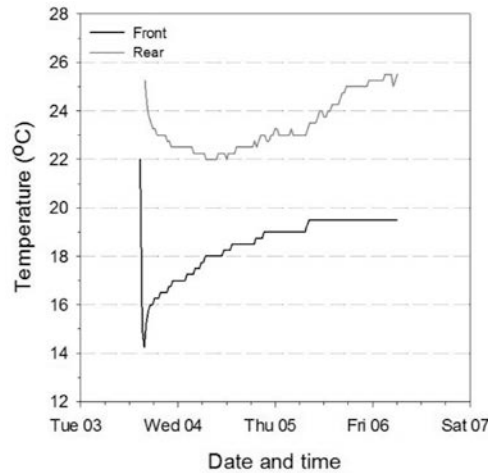


Figure 3. Air temperatures at the front and rear of another truck container of 'B74' mango fruit. The first logger was placed centrally in the second row of pallets from the front of the truck, four trays from the top of the pallet. The second logger was placed centrally in the second row from the rear of the truck, four trays from the top of the pallet. The temperature at the rear of the truck reached 25°C despite a set point of 18°C (Hofman et al., 2014a, b).

Initial trialling of ethylene release from an ethylene powder formulation, Ripestuff™, (Ho et al., 2013) (Figure 4) and from a semi-permeable plastic sleeve membrane (Hofman et al., 2014a, b) (Figure 5) established their potential to maintain ethylene in container atmospheres over 2-3 days. However, undesirably faster release can occur in sub-optimal circumstances such as higher permeability of plastic sleeve release systems. Ethylene leakage rates from transport containers are a major factor in maintaining the required ethylene concentrations in-transit.

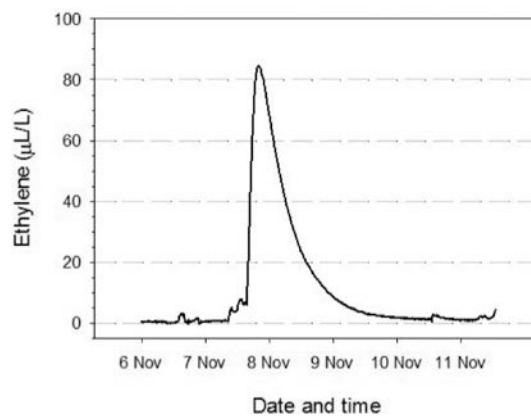


Figure 4. Rapid release of ethylene from bags of Ripestuff™ into a road container containing 'B74' mangoes in-transit for 5 days. This method is similar to the shot ethylene treatment method, but without re-injection of ethylene into the system. The ethylene concentration peaked over 80 µL L⁻¹ (Hofman et al., 2014a, b).

Carbon dioxide (CO₂)

Mangoes are climacteric fruit that produce 70-160 mg CO₂ kg⁻¹ h⁻¹ at 20-22°C (Thompson, 2015). Elevated CO₂ can suppress the effect of ethylene in initiating and coordinating ripening processes (Thompson, 2015). Keeping CO₂ levels below 0.5% assists in maintaining ripening times (Thompson, 2015). It is also important to limit worker exposure to CO₂ by adhering to Occupational Health and Safety guidelines (Thompson, 2015).

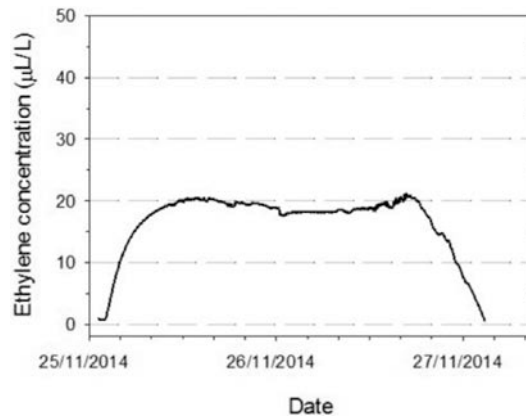


Figure 5. Slow release of ethylene from ethylene-in-nitrogen (4%)-filled semi-permeable plastic sleeves of ~60 cm circumference, and two thicknesses of 50 and 75 μm into a truck container of 'B74' mangoes in-transit for 3 days. The ethylene concentration remained steady at $\sim 20 \mu\text{L L}^{-1}$ (Hofman et al., 2014a, b).

The container CO_2 concentration is determined by product respiration in and leakage rates from the container. Controlled ventilation is currently not technically possible in-transit. Absorption of CO_2 can be achieved by using hydrated lime powder scrubbers.

Monitoring CO_2 production from fruit and utilising CO_2 absorption methods in-transit will minimise exposure risks to fruit in-transit and humans at outturn.

Air circulation

Efficient airflow within the truck container is critical in managing temperature and ethylene distribution when transporting fruit. Factors such as pallet stacking pattern and placement within the truck, void space around the refrigeration unit and bulkhead, air circulation aids like air delivery roof chutes and floor channels, and the placement and type of any load stabilising boards can all affect air flow (Figure 6). Currently there are marked differences between refrigerated trucking containers in terms of their fan capacity, air circulation aids, engineering design, stow dimensions, and general condition and age (Brecht et al., 2009). There are also differences between transportation mode and whether the container is suitable for road, rail, or a combination of both. The majority of mango consignments in Australia are transported by road; however, a combination of road and rail transport can be used, where generic containers are suitable for both forms of transport.

CONCLUSION

Initiating and managing mango fruit ripening in-transit would reduce the capacity demand for cold rooms on farm and the attendant cooling costs. At market, holding and ripening infrastructure costs and their attendant operating costs would be reduced by mangoes being ripened in-transit. Controlled ripening must be done well in order to realise good fruit quality, reduced product losses, increased sales, and greater profits (Crisosto and Mitcham, 2015). In this context, in-transit ripening should provide a useful alternative method for ripening mangoes without the need for specialised facilities. Moreover, it may provide for greater flexibility in managing bottle necks in the supply chain. Thereby, producers, wholesalers, retailers and consumers in the supply chain might all stand to benefit from in-transit ripening.

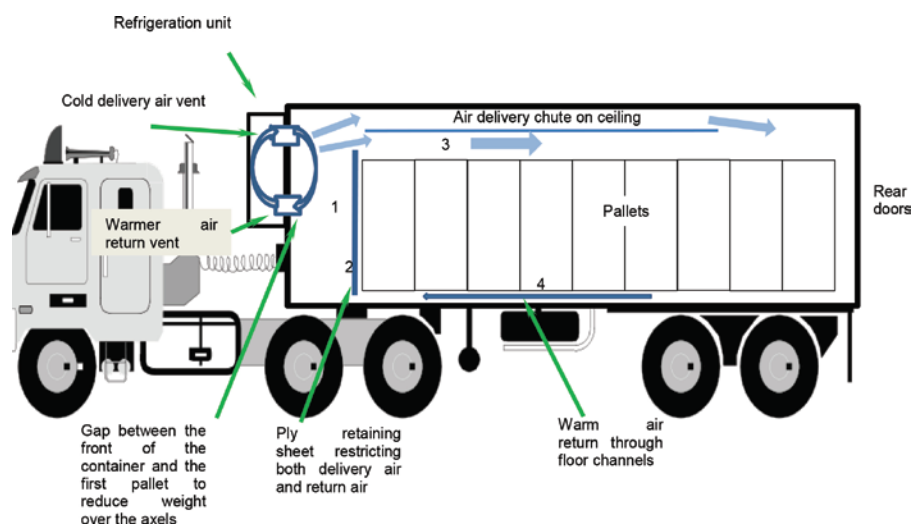


Figure 6. Schematic diagram of the stowage in a road shipment container (after Hofman et al., 2014a, p.10). The factors impacting adversely on air circulation throughout the stow include: 1, Gap where cold air is short circuiting directly to the return vent, with no cold air going to the rest of the container; 2, ply sheet obstructing cold air delivery to front pallets and obstructing return of warm air to return vent; 3, restricted cold air to front of pallets with interference from ceiling delivery chute; and 4, restricted warm return air through pallet boards and/or floor vents.

Research and development is continuing on cost-effective sustained ethylene release systems for up to 4-day in-transit treatment of mango fruit. Additionally, the modelling of 'Honey Gold' fruit quality and shelf life under a range of ethylene and CO₂ concentrations at several temperatures is also underway.

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Effects of Wood Vinegar on Tomato Fruit Quality and Shelf Life at Ambient and Low Temperatures

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Keywords: *Solanum lycopersicum* Mill., pyroligneous acid, postharvest dip

Abstract

Wood vinegar (pyroligneous acid or pyrolysis oil), a liquid produced through natural carbonization of plant refuse, has several potential benefits to agriculture and human health and has been reported to improve harvest quality and shelf life of fruits and vegetables but may also promote ethylene production. In this study, we used wood vinegar derived from citrus tree refuse and corn stover and applied on breaker tomato 'Diamante' at dilutions with distilled water of 1:10 or 1:5 (10 and 20%) as postharvest dip for 3 min before storage at ambient (25-30°C) or refrigerated condition (8-10°C) for 2 weeks. No remarkable effects of wood vinegar on quality changes were noted except in terms of weight loss and reddening (a^* values). Fruit stored at ambient and pretreated with 10-20% wood vinegar from corn stover or 20% wood vinegar from citrus refuse had lower weight loss than the control (water dip). Fruit reddening was favored at low temperature than at ambient. Sensory quality, soluble solids content, acidity and fruit decay incidence were not affected.

INTRODUCTION

Revival of wood vinegar (pyroligneous acid) application in agriculture and human health was prompted by the drive to develop organic and environment-friendly production systems and natural medicines. Pyroligneous acid has been used for ages as sterilizing agent, deodorizer, fertilizer and antimicrobial (Souza et al., 2012). The strong antimicrobial activity of pyroligneous acid was correlated to its high contents of organic acids and phenolic substances. Pyroligneous acid also exhibits antioxidant activity and was found to have superior free radical scavenging activity (Loo et al., 2007). The strong antioxidant activity is due to its high content of phenols. Wood vinegar consists of more than 200 water soluble compounds comprising of organic acids, phenolic, alkane, alcohol and ester compounds (Wei et al., 2010). Wood vinegar has many uses; in agriculture it is used in pest and disease control, improving yield and quality, increasing fruit size, weight and sweetness, and extending shelf life of fruit and vegetables (Mohan et al., 2006; Oramahi and Yoshimura, 2013; Tuntika et al., 2013; Zulkarami et al., 2011). This study explored the use of wood vinegar from citrus tree refuse and corn stover on the quality and shelf life of tomato fruit.

MATERIALS AND METHODS

Freshly harvested, uniformly sized and defect-free breaker tomatoes 'Diamante' (East-West Seed Co.) were treated with wood vinegar derived from slow pyrolysis of citrus tree refuse and corn stover at dilutions of 1:10 or 1:5 (10 and 20%) as 3 min dip. Fruit dipped in distilled water for 3 min served as control. The fruits were then stored at ambient (25-30°C) or refrigerated condition (8-10°C) for 2 weeks. Peel color reddening was measured as a^* values using a Minolta colorimeter CR-13; weight loss as percent of the initial weight; total soluble solids (TSS) using Atago handheld refractometer, percent acidity by the titrimetric method, and sensory appearance by 15 trained panelists using a rating scale of 9-1 in which 9 – excellent, defect-free, 7 – good, defects minor, 5 – fair, defects moderate, 3 – poor, defects serious, limit of marketability, 2 – limit of edibility,

and 1 – inedible under usual condition. The experiment was conducted in a completely randomized design using three replicates with 2 kg fruits per replicate. Results were analyzed using SAS software (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Fruit reddening measured as a^* values was not significantly affected by wood vinegar treatment both at ambient and refrigerated temperatures (Fig. 1). Reddening was more intense at low storage temperature than at ambient. Similarly, wood vinegar had no marked effect on TSS, acidity and visual quality (Table 1) as well as on fruit rot incidence (results not shown). However, weight loss at ambient was significantly reduced using wood vinegar from corn stover while the wood vinegar from citrus tree refuse had comparable effect as the control (Fig. 2). At refrigerated temperature, wood vinegar had opposite effect as it increased weight loss.

The results did not confirm earlier reports that wood vinegar improved quality and shelf life of fruit and that it has strong antimicrobial activity (Mohan et al., 2006; Souza et al., 2012; Zulkarami et al., 2011). A semblance of its beneficial effect on shelf life was the reduction of weight loss during ambient storage but using only the wood vinegar from corn stover.

CONCLUSION

Wood vinegar had little effect on quality and shelf life of tomatoes harvested and stored at the breaker stage. Other treatment conditions (e.g., wood vinegar concentration, dipping time, and infiltration method) need to be examined in follow up studies. Chemical components of the wood vinegar should also be determined to elucidate the different effects of raw material used.

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Tables

Table 1. Some quality attributes of wood vinegar-treated and untreated tomatoes after 2 weeks at ambient and refrigerated temperatures.

Treatments	TSS (°B)	% Acidity	Sensory visual quality
Ambient			
0, control	2.08	0.02	6.3
10% citrus	2.70	0.02	6.4
20% citrus	3.04	0.01	5.4
10% corn	2.80	0.02	6.0
20% corn	2.23	0.02	6.7
Refrigerated			
0, control	2.38	0.02	5.9
10% citrus	2.17	0.02	6.0
20% citrus	2.12	0.02	6.0
10% corn	2.61	0.03	6.0
20% corn	2.00	0.02	5.8

No significant treatment differences were obtained.

Figures

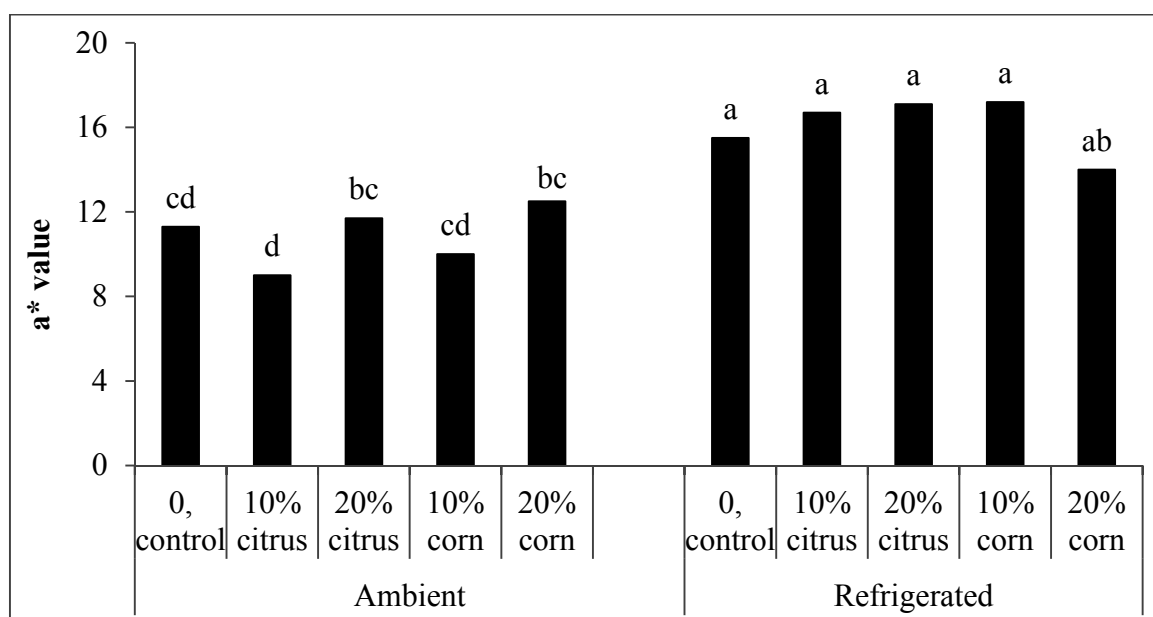


Fig. 1. a* values of wood vinegar-treated and untreated tomatoes after one week storage at ambient and refrigerated temperatures. Mean separation by LSD, 5%.

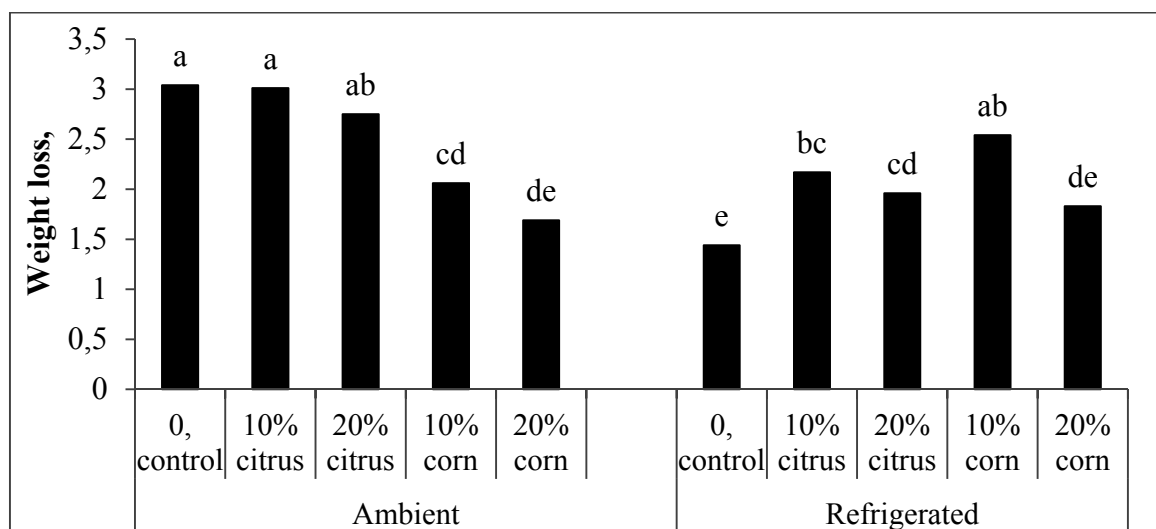


Fig. 2. Weight loss of wood vinegar-treated and untreated tomatoes after one week storage at ambient or refrigerated conditions. Mean separation by LSD, 5%.

γ -Irradiation effects on appearance and aroma of 'Kensington Pride' mango fruit

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Abstract

γ -Irradiation doses of 0.5 (target) and 1.0 (high) kGy were applied as insect disinfestation treatments to 'Kensington Pride' mango fruit. The effects of these treatments on fruit physicochemical properties and aroma volatile production were investigated and compared to non-irradiated controls. There were no significant effects of the irradiation treatments on flesh total soluble solids content. However, the loss of green skin colour usually associated with fruit ripening was inhibited by irradiation at both 0.5 and 1.0 kGy by approximately 32 and 52%, respectively, relative to non-irradiated fruit. Fruit exposed to 0.5 and 1.0 kGy exhibited a 58 and 80% reduction in emission of α -terpinolene volatiles, respectively. Thus, γ -irradiation at 0.5 and 1.0 kGy can have an adverse effect on 'Kensington Pride' mango fruit aroma volatile production and skin colouration.

Keywords: lenticel discolouration, *Mangifera indica*, skin colour, volatile production

INTRODUCTION

Mango (*Mangifera indica* L.) is considered "The King of Fruits" and is one of the main fruit crops in the tropics and subtropics. Mango fruit are highly nutritious and a rich source of amino acids, carbohydrates, fatty acids, minerals, organic acids, proteins, vitamins and antioxidants. Mango fruit are susceptible to various insect pests of quarantine significance; e.g., fruit fly and mango seed weevil. Susceptible fruit require appropriate preventative measures and postharvest disinfestation treatments to minimise the risk of pest incursion into importing countries. Exposure to sources of ionising radiation, such as gamma (γ) rays, is an effective postharvest quarantine/phytosanitary treatment to minimise quarantine pest presence in traded foods (Bustos et al., 2004).

Disinfestation treatments, and particularly physical treatments, often represent a fine balance between delivering adequate insect mortality and not damaging the product. γ -Irradiation of mango fruit can provide quarantine security. However, it can also reduce ripe fruit skin quality by increasing lenticel discolouration and decreasing the rate of ripening-related green colour loss (McLauchlan et al., 1989). In addition to affecting fruit skin pigmentation, treatment with γ -irradiation may potentially affect the production of aroma volatiles. For example, exposure of 'Chok Anan' mango fruit to 0.3 kGy γ -irradiation significantly reduced rates of terpene production as compared to non-treated samples (Laohakunjit et al., 2006).

In the present study, we investigated the effects of γ -irradiation on the physicochemical properties of 'Kensington Pride' mango fruit, a commercially important cultivar in Australia. An experiment was completed with a view to improving understanding of mango skin de-greening and aroma synthesis responses.

MATERIALS AND METHODS

Material

'Kensington Pride' mango fruit were harvested at commercial maturity from an orchard near Childers, Queensland, Australia (-25.237° S, 152.278° E). The fruit were destemmed in Mango Wash®, treated with a postharvest fungicide and insecticide and graded



for uniform quality as per commercial procedures. The fruit were then packed into cardboard trays and transported in an air-conditioned (ca. 22°C) vehicle to the Maroochy Research Facility postharvest laboratory near Nambour, Queensland within 1.5 day of harvest.

Treatments

Following arrival at the laboratory, the fruit were randomly assigned to treatment lots and labelled with a treatment number. The fruit in each treatment were randomly packed into six trays that each held about 10 kg of fruit. One dosimeter was placed into each tray. The fruit were transported in an air conditioned car to a commercial irradiation facility (Steritech, Narangba, Queensland) within 1 h. Fruit in trays were exposed to γ -irradiation from a Cobalt 60 source at doses of 0 kGy (control), the ideal commercial dose of ~0.5 kGy (actual dose of 0.52 kGy; min-max of 0.493-0.577 kGy), and twice the commercial dose at ~1.0 kGy (actual dose of 1.043 kGy; min-max of 1.003-1.079 kGy). The fruit were transported back to the laboratory immediately after irradiation. They were then treated with 10 $\mu\text{L L}^{-1}$ ethylene for two days at 20°C as per commercial practice. Thereafter, fruit were maintained at 20°C until ripe.

Quality assessments

1. Fruit firmness.

Fruit firmness was assessed on day 0 and then on every 1-2 days of the experiment using the following subjective hand firmness rating scale: 0 = hard, 1 = rubbery, 2 = sprung, 3 = soft and 4 = very soft. 'Kensington Pride' fruit are considered to reach eating ripe at a hand firmness score of 4, which corresponds to a Sinclair IQ firmness reading of 12. Fruit firmness was also assessed objectively on day 0 and then on every 1-2 days with a Sinclair firmness tester.

2. Skin colour development.

Skin colour was subjectively rated on day 0 and every 1-2 days of the experiment using the following scale 1 = 0-10% of the non-blush skin with yellow colour, 2 = 11-30% yellow, 3 = 31-50% yellow, 4 = 51-70% yellow, 5 = 71-90% yellow and 6 = 90-100% yellow .

3. Total soluble solids and titratable acidity.

When the fruit had reached the eating soft stage, one cheek of each fruit was removed using a knife. A vertical section of the flesh from stem end to base was chopped into small pieces and then frozen at -20°C. Within two months of processing, the flesh sample was equilibrated to room temperature (25-28°C) and immediately homogenised with an Ultraturrax stick blender. The Brix value (TSS) of the homogenate was determined using a PAL-1 Atago digital hand held "Pocket" refractometer. A 10 g sample of the homogenised pulp was also used to determine the titratable acidity with an automated Metrohm titrator using 0.1 M NaOH as the titrant. TA was expressed as the citric acid equivalent (%) in each sample.

4. Aroma volatiles production.

Aroma volatile production by fruit was determined using a modified headspace solid-phase micro extraction (SPME) GC-MS method (Siebert et al., 2005). Frozen flesh samples were prepared as described above and stored in glass bottles at -20°C for 4-6 weeks until analysis. The samples were thawed and blended as described above. A 2.5 g sample of homogenised flesh was then added to a 20 mL HS-SPME vial containing 2.5 mL of saturated sodium chloride (NaCl) solution and a magnetic stir flea (5 × 2 mm). Volatiles were measured by SPME GC-MS and data were collected using Agilent MSD Statistical analysis ChemStation (USA) software.

Statistical analysis

Data were subjected to analysis of variance or ANOVA with repeated measures using Genstat 16th edition software (VSN international Ltd, UK). One way analysis of variance was used for the three irradiation treatments comparisons. Fifteen single fruit biological replicates were used for external appearance assessments (i.e., hand firmness, lenticel discolouration and skin colour), five fruit replicates for Sinclair firmness and five fruit replicates for aroma volatiles plus three laboratory replicates per fruit. Differences between means were tested using Fisher's protected LSD ($P < 0.05$).

RESULTS AND DISCUSSION

Firmness

Exposure of 'Kensington Pride' mango fruit to 0.5 and 1.0 kGy γ -irradiation delayed initial rates of fruit softening as compared to non-irradiated mangoes (Figure 1). However, after seven and nine days of shelf life there was no significant difference in firmness between irradiated and non-irradiated fruit. Thus, the differential irradiation on firmness effect diminished as fruit progressively ripened.

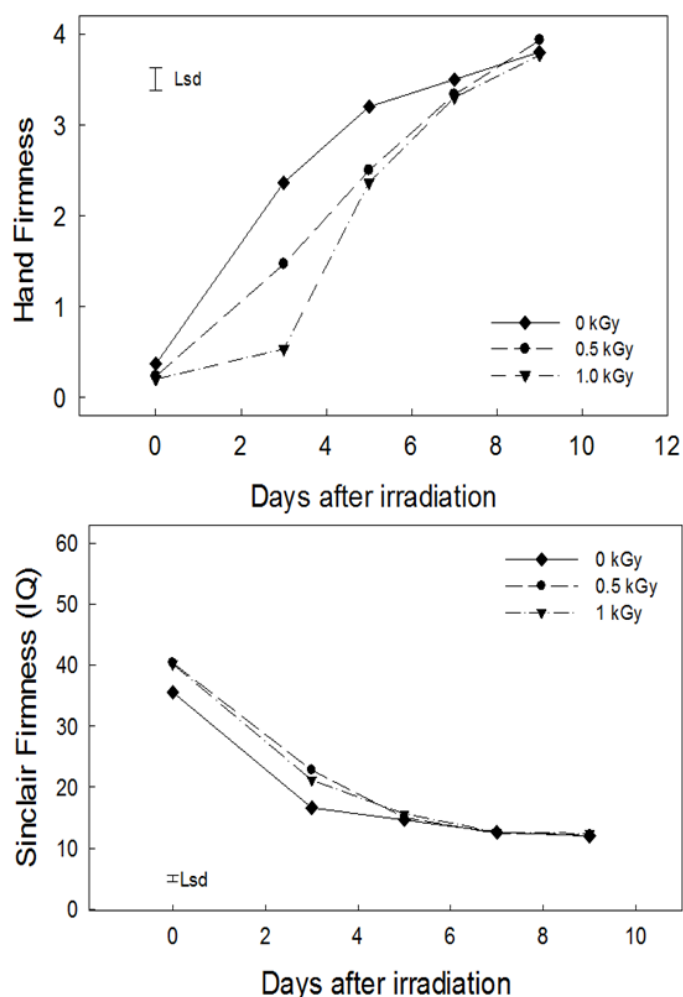


Figure 1. Effects of γ -irradiation at 0, 0.5 and 1.0 kGy on hand firmness (top) and Sinclair firmness (bottom) readings for ripening 'Kensington Pride' mango fruit. The LSD ($P = 0.05$) bar is for factorial effect (the factorial is the highest order interaction of dose and time).

Skin colour

In all treatments, the skin colour changed from green to yellow (higher colour scores) as the fruit ripened (Figure 2). In general, exposing fruit to γ -irradiation of either 0.5 or 1.0 kGy significantly reduced the loss of green skin colour during ripening. For example, irradiation with 0.5 and 1.0 kGy reduced the development of yellow skin colouration by ~32 and 52%, respectively, at 9-11 days of shelf life as compared to control fruit not exposed to irradiation.

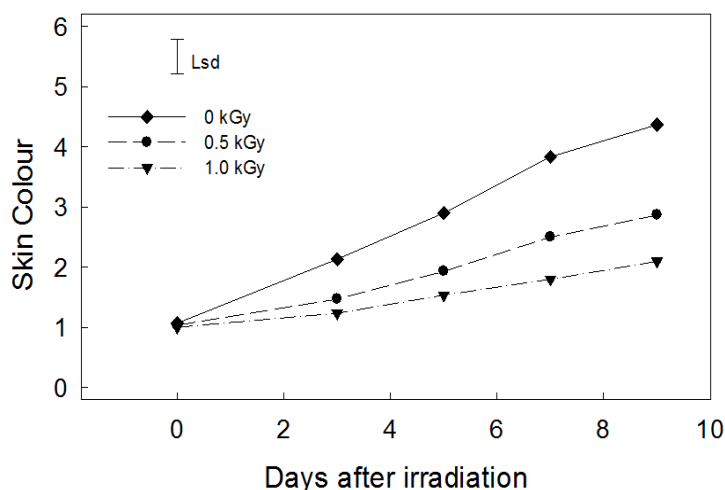


Figure 2. Effects of γ -irradiation at 0, 0.5 and 1.0 kGy on change of skin colour for ripening 'Kensington Pride' mango fruit. The LSD ($P=0.05$) bar is for factorial effect (the factorial is the highest order interaction of dose and time).

Flesh TSS and TA

There was no significant effect of γ -irradiation treatment on fruit TSS at eating soft. Treatment with γ -irradiation promoted a 2-fold increase in fruit acidity (TA) at eating ripe (Table 1). El-Samahy et al. (2000) found that the TA of 'Zebda' mango decreased more during ripening in non-irradiated fruit compared with irradiated fruit.

Table 1. Effects of γ -irradiation at 0, 0.5 and 1.0 kGy on the total soluble solids content ($^{\circ}$ Brix) and titratable acidity (citric acid % equivalent) in 'Kensington Pride' mango fruit at eating ripe. Means with the same letters in the same row are not significantly different at $P=0.05$ as tested by LSD.

	Irradiation dose (kGy)			LSD
	0	0.5	1.0	
TSS ($^{\circ}$ Brix)	12.4	13.1	12.4	n.s.
Acidity (%)	0.209a	0.677b	0.599b	0.153

Aroma volatiles in 'Kensington Pride' mangoes

Five volatile compounds produced in relatively major quantities by non-irradiated 'Kensington Pride' fruit tissues were 3-carene, β -myrcene, α -terpinene, D-limonene and α -terpinolene (Table 2). Among these compounds, α -terpinolene was the most abundant on the basis of chromatogram % peak area in the control. α -Terpinolene was the dominant volatile compound produced by 'Kensington Pride' mango pulp (Lalel et al., 2003). 3-Carene and D-limonene were also relatively highly abundant in 'Kensington Pride' (Table 2). Lalel et al. (2003) found that 3-carene was the second major volatile component produced by 'Kensington Pride' fruit. α -Terpinene production reached a maximum at the climacteric stage (day 4). Limonene, myrcene and p-cymene production were highest on day 1.

Table 2. Relative abundance of major volatile compounds from non-irradiated ripened 'Kensington Pride' mango fruit.

Peak	Compound	Retention time (min)	% max.
1	3-Carene	6.74	5.18%
2	β -Myrcene	7.07	3.92%
3	α -Terpinene	7.35	5.27%
4	D-Limonene	7.74	2.69%
5	α -Terpinolene	9.76	100.00%

Fruit exposed to 0.5 and 1.0 kGy γ -irradiation produced significantly lower concentrations of α -terpinolene as compared to the control samples (Figure 3). The pronounced reduction suggests that irradiation has a significant effect of suppressing the flavour profile of 'Kensington Pride' mango.

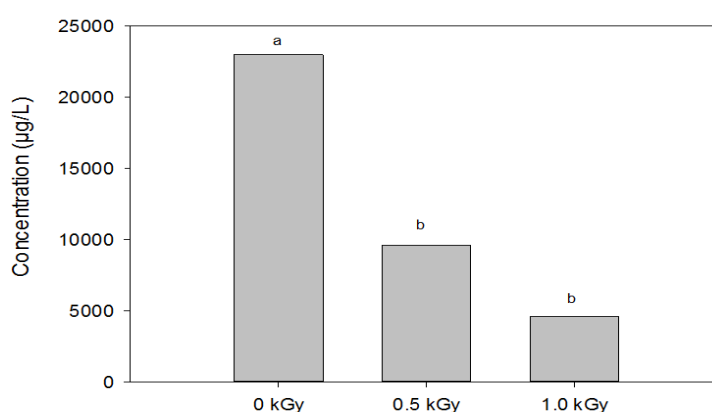


Figure 3. Effects of γ -irradiation at 0, 0.5 and 1.0 kGy on α -terpinolene volatile production by ripened 'Kensington Pride' mango fruit. Means with the same letters are not significantly different at $P=0.05$ as tested by LSD.

CONCLUSIONS

Our study confirms that exposure of green mature 'Kensington Pride' mango fruit to typical commercial γ -irradiation disinfestation doses of 0.5 and 1.0 kGy can significantly reduce the external appearance quality. Loss of green skin colour during ripening is retarded even at 0.5 kGy, resulting in undesirable more green skin colour at eating soft. In general, fruit firmness changes were not significantly affected by irradiation. Nonetheless, there may initially be unwanted slightly slower firmness loss. γ -Irradiation also reduced volatiles production by the ripened fruit. In concert with a relative increase in TA of 'Kensington Pride' in association with irradiation, a reduction in volatiles indicates an unacceptable effect of irradiation on fruit flavour. 'Kensington Pride' mangoes are susceptible to irradiation-related damage at commercial doses of 0.5-1.0 kGy. While lower doses of 0.15 kGy might kill fruit fly and not damage the fruit as much, the reality is that the higher doses (e.g., 0.4+ kGy) are required to kill other insect pests and meet market requirements. It suggests that the formulation of postharvest practices should be studied to optimise the quality of irradiated fruit.

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Anatomy of skin disorders afflicting Australian mangoes

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Abstract

Lenticel discoloration (LD), under-skin browning (USB) and resin canal disorder (RCD) are three important fruit skin disorders of concern to the Australian mango industry. They result in downgrading of fruit quality and loss of market confidence. LD is confined to the discoloration of lenticels on the fruit skin. USB is a sub-surface spreading grey-brown lesion that may only become evident in the marketplace. RCD is a dark brown discoloration of the finely branched pattern of ramifying sub-surface resin canals. These three browning disorders were compared and contrasted at the cellular level by light microscopy after free-hand sectioning. Discoloured lenticels on the surface of 'Honey Gold' mango fruit typically appeared as small round spots comprised of a dark brown centre surrounded by a white corona. Non-coloured lenticels were visible as small round spots surrounded by a white corona. In cross-section, there was browning of cells surrounding the cavity of discoloured lenticels. Nonetheless, there was no such browning in non-coloured lenticels. USB symptoms in 'Honey Gold' fruit were evident as dark-brown cells in the tissue surrounding the resin ducts. In contrast, RCD, which was evident in 'Kensington Pride' mango fruit, was characterised by browning of the lumen of resin ducts. Accordingly, LD, USB and RCD involve different browning biology processes at the cell and tissue levels. A greater understanding of symptom development and expression could aid in informed management of these physiological disorders.

Keywords: *Mangifera indica*, lenticel discoloration, resin canal disorder, under-skin browning

INTRODUCTION

Skin physiological disorders are of major concern to the Australian mango industry. They lead to downgrading of fruit quality and to loss of market confidence (Rymbai et al., 2012). Lenticel discoloration (LD), under-skin browning (USB) and resin canal disorder (RCD) have emerged in recent years as serious economic issues.

Lenticels are macroscopic openings on the fruit skin. They contribute to gaseous exchange and transpiration. Bally (1999) found that lenticel formation in 'Kensington Pride' fruit was due to rupturing of stomata. Rymbai et al. (2012) confirmed that lenticels in mango derive from stomata and enlarge during fruit growth, largely due to stretching of the fruit surface. Several studies on mechanisms of mango LD have been conducted. Wang et al. (2012) observed that discoloration occurred in the form of light purple areas due to pigment accumulation in the vacuoles of sub-lenticel cells. Thus, LD involves vacuolar deposition of phenolics in cells surrounding the lenticel cavity. It is associated with the increased reddening and eventual brown to black darkening of lenticels (Du Plooy et al., 2009).

USB mainly affects 'Honey Gold' mango fruit. It is manifest under the epidermis as a diffuse grey-brown discoloration with no damage to the flesh (Hofman et al., 2010). The

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defect is not usually obvious at harvest. Rather, the visual symptoms become evident as the fruit ripen. The mechanism(s) and management of USB are somewhat elusive. Delayed or slow cooling of fruit before or after packing can reduce USB incidence (Hofman et al., 2010). USB often appears around visible physical injury sites on fruit, such as those that result from prolonged transport (Marques et al., 2012).

RCD is associated with discoloration of the finely branched network of sub-cuticle resin canals (Macnish et al., 2014). RCD is visible as ramifying black traces within the skin of affected fruit. It is also associated with discoloration of resin canals in the outer layers of the pulp. Factors contributing to the development of RCD are as yet unidentified. Mechanisms regulating symptom expression are not understood.

In the present study, these three skin-browning disorders were compared and contrasted at the cellular level. The aim was to develop a better understanding of these skin disorders at the anatomical level. Knowledge about the anatomy of these disorders is expected to inform postharvest practices to optimise the quality of Australian mangoes.

MATERIALS AND METHODS

Materials

'Honey Gold' and 'Kensington Pride' mango (*Mangifera indica* L.) fruit were harvested at commercial maturity from orchards near Katherine and Darwin, respectively, in the Northern Territory, Australia. 'Honey Gold' fruit were de-stemmed in a solution containing 2.5 g L⁻¹ Mango Wash® (Septone, ITW AAMTech, Australia) for 2 min and graded for uniform quality as per commercial procedures. 'Kensington Pride' fruit were picked from the tree without standard commercial harvest practices. All fruit were packed into cardboard trays and air-freighted from the Northern Territory to Brisbane, Queensland within 1.5 d of harvest. They were then transported in an air-conditioned (~22°C) vehicle to the nearby Ecosciences Precinct laboratory.

Treatments

Upon arrival at the laboratory, trays containing the 'Honey Gold' fruit were placed on a vibration table in a cold room at 12°C. The intent was to simulate commercial refrigerated truck transport. They were exposed to 12-Hz vibration for 12 h. They were then removed from the table and held at 13°C for 6 d. This step was to simulate post-transport time in the cold chain. Thereafter, the fruit were maintained at 20°C until ripe. Skin tissues displaying USB symptoms were excised for anatomical study. Additional 'Honey Gold' fruit used to study LD were maintained at 20°C until ripe without vibration treatment. 'Kensington Pride' fruit were maintained at 20°C. Skin tissues from fruit exhibiting RCD symptoms were collected at firm and nearly ripe stages for anatomical study.

Microscopy

Fruit tissues ($n=5$) with and without skin browning symptoms were processed for light microscopy. USB and lenticels on 'Honey Gold' fruit and RCD on 'Kensington Pride' fruit were compared and contrasted at the cellular level. Free-hand sections were made from the excised tissues according to the method of Ruzin (1999). Tissue sections were transferred onto a drop of distilled water on a glass microscope slide and covered with a glass coverslip. The hand sections were viewed and photographed with an Olympus BX61 light microscope equipped with a DP 70 camera (Olympus, Japan).

RESULTS AND DISCUSSION

Anatomy of LD

Discoloured lenticels on the surface of mango fruit appear as small round spots comprised of a dark brown centre surrounded by a white corona. The discoloration did not affect the cuticle and epidermis. Cross-sections through brown or darkened lenticels on 'Honey Gold' fruit showed that browning occurred beneath the epidermis around the

cavities of lenticels (Figure 1B). There was no browning of the cells surrounding the cavity of non-coloured lenticels (Figure 1A). Tamjinda et al. (1992) found that polyphenols were associated with discoloured cells. The vacuolar deposition of phenolic compounds resulted in increased reddening and eventual darkening of the lenticels of ‘Tommy Atkins’ mango fruit (Du Plooy et al., 2009). Wang et al. (2012) observed that LD was due to pigment accumulation in the vacuoles of sub-lenticular cells.

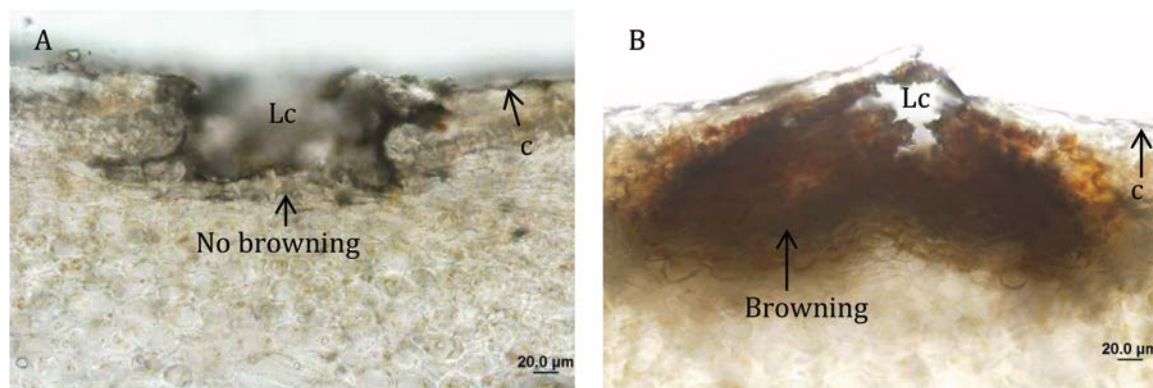


Figure 1. Light microscope images of lenticels on ‘Honey Gold’ mango fruit skin. (A) Transverse section showing no browning in a non-coloured lenticel. (B) TS showing browning in a discoloured lenticel. c, Cuticle; Lc, lenticel cavity. Scale bars, 20 µm.

Anatomy of USB

In transverse cross-section (TS), USB symptoms were localised to tissues under the epidermal layer and were not associated with the cuticle and epidermis (Figure 2A). USB-affected areas beneath the epidermal layer were dark brown to black. They spread along resin ducts. A TS through USB-affected tissues showed dark brown cells surrounding the epithelial cells that lined resin ducts (Figure 2B). Accordingly, USB may be related to discoloration immediately around latex vessels which spreads further to the surrounding cells (Hofman et al., 2010).

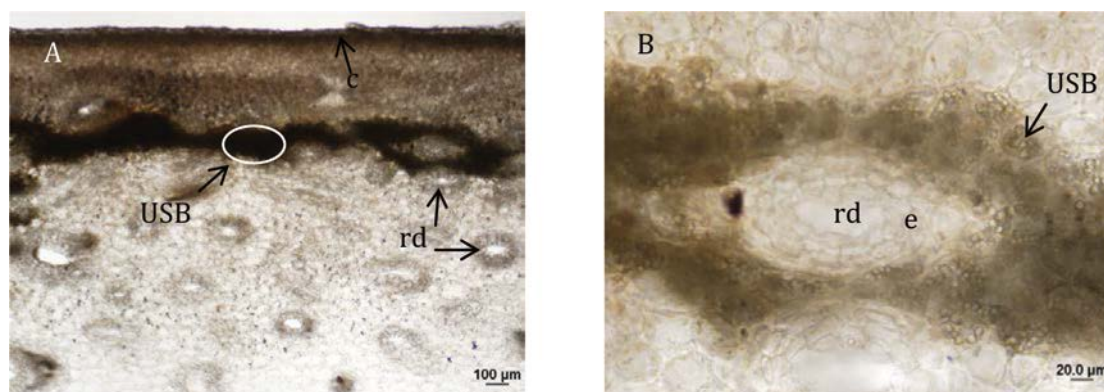


Figure 2. Light microscope images of USB in ‘Honey Gold’ mango fruit skin. (A) TS through USB-affected skin tissue showing USB beneath the epidermis. (B) TS through the dark-brown cells surrounding resin ducts. c, Cuticle; rd, resin duct; e, epithelial cells; USB, under-skin browning. Scale bars, 100 µm (A), 20 µm (B).

Anatomy of RCD

In sections through RCD-affected tissue, there was no browning of the cuticle (Figure 3A). TS revealed that RCD-affected areas were located beneath the skin. Affected areas visible through the skin extended over much of the fruit surface. Under the epidermis, many

branched browned resin canals were visible in RCD-affected regions. The browning was confined to within resin duct lumens (Figure 3B). Asymptomatic control fruit showed no such discoloration. Relative to LD and USB, even less is known about the causes of and controls for RCD. It is likely attributable to a combination of production and postharvest factors that increase fruit susceptibility and contribute to symptom expression, respectively (Macnish et al., 2014).

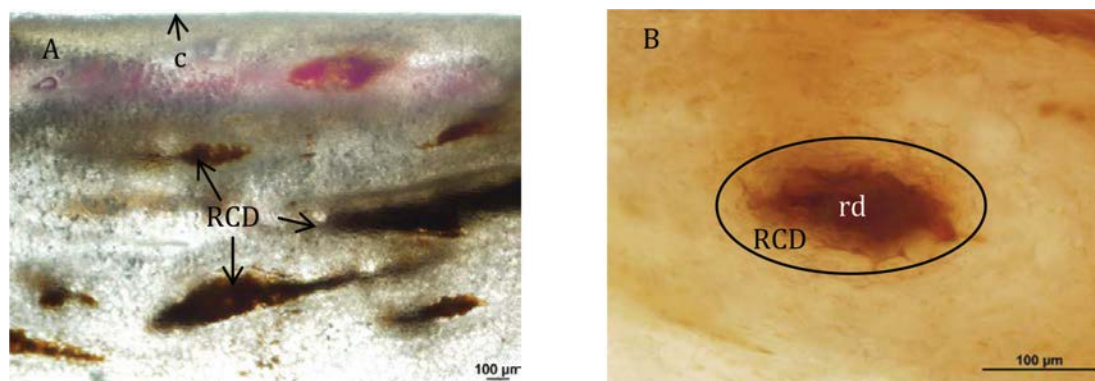


Figure 3. Light microscope images of RCD on 'Kensington Pride' mango fruit skin. (A) TS through affected tissue showing RCD beneath the skin. (B) TS through RCD-affected tissues showing localised browning inside the lumen of resin ducts. c, Cuticle; rd, resin duct; RCD, resin canal disorder. Scale bars, 100 µm.

CONCLUSION

LD was evident as browning of cells surrounding the lenticel cavity. No such browning of cells was observed around non-coloured lenticels. At the anatomical level, USB was characterised by dark-brown cells predominantly in tissues surrounding the epithelial cells of resin ducts. In contrast, RCD was distinguished by localised browning inside the lumen of resin ducts. Accordingly, USB and RCD evidently involve quite different cellular processes. Through gaining a fuller understanding of the comparative biochemistry of LD, USB and RCD symptom expression at cell and tissue levels, the formulation of practices to manage these physiological disorders can be better informed. Effective pre- and/or postharvest practices to lessen or ideally eliminate these physiological disorders would benefit all Australian mango fruit supply-chain players from farmers to consumers.

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THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

**LENTICEL DISCOLOURATION, UNDER-SKIN
BROWNING AND RESIN CANAL DISORDER IN
AUSTRALIAN MANGO FRUIT CULTIVARS**

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B.Sc Biotechnology

M.Sc Genetics

A thesis submitted for the degree of Doctor of Philosophy at

The University of Queensland in 2017

School of Agriculture and Food Sciences

Abstract

Lenticel discolouration (LD), under-skin browning (USB), and resin canal disorder (RCD) are three fruit skin disorders of concern to the Australian mango industry. LD is confined to lenticels on the fruit skin. It was suspected that irradiation may differentially induce LD on cvs. 'B74', 'Honey Gold', 'Kensington Pride', and 'R2E2' fruit. Irradiation at 0.5 kGy significantly ($P \leq 0.05$) increased LD and delayed the loss of green skin colour of all four cultivars. There was generally no significant differences ($P > 0.05$) between 0.5 ('commercial' dose) and 1.0 kGy ('upper limit' dose). The least pronounced effect was on cv. 'Honey Gold'. Irradiation also diminished aroma volatiles production by cvs. 'Kensington Pride' and 'R2E2'. Examination of the morphology of LD showed it in tissue sections as browning of sub-lenticellular cells around lenticel cavities. No such browning was evident in sub-lenticellular cells around non-coloured lenticel cavities. Coloured polymerised phenolic compounds appeared accumulate in the cell wall and in the cytoplasm of sub-lenticellular cells around the cavities of discoloured lenticels.

USB is a physiological disorder evident as spreading grey-brown sub-surface lesions in cv. 'Honey Gold' mango fruit. In contrast, RCD in cv. 'Kensington Pride' mango fruit is evident as ramifying dark brown sub-surface resin canals. These two largely cv. specific browning disorders were compared and contrasted at the cellular level. USB was characterised by dark brown surrounding parenchyma cells around the epithelial cells that line resin ducts. This disorder involves starch retention as well as deposition of phenolic compounds in parenchyma cells surrounding the epithelial cells of resin ducts. In contrast, RCD was distinguished by localised browning inside resin duct lumens. The browning process evidently involved accumulation of polymerised phenolics. Polyphenol oxidase and peroxidase enzymes were determined by tissue printing to be associated with both USB and RCD browning.

The effect of harvest time over the diurnal cycle was investigated with regard to the propensity of cv. 'Honey Gold' fruit to develop USB. USB expressed in postharvest is related to the cumulative interaction of physical (viz., vibration) and physiological (viz., chilling) stresses. Fruit harvested during the day were relatively more susceptible to developing USB than were those picked at night. The higher USB incidence in the afternoon harvest was related to temporally variable sap phytotoxicity. An important contributory factor to greater afternoon sap phytotoxicity was concomitant increases in the concentrations of volatiles in the sap and, especially, in the non-aqueous sap phase. Changing from day to night and early morning harvesting afforded reduced incidence and severity of USB on cv. 'Honey Gold' fruit.

Declaration by author

This thesis is composed of my original work and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Publications during candidature

Conferences

San, A.T., Smyth, H.E., Joyce, D.C., Webb, R., Li, G.Q., Hofman, P.J., Macnish, A.J., 2014. Mango quality and skin disorders. *In* 'QAAFI Annual Research Meeting', Brisbane, Australia. (Poster presentation).

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2. Heather Smyth (Thesis Advisor; UQ Senior Research Fellow): Advising on GC-MS analysis and critically revising drafts of aroma volatile results.
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5. Andrew Macnish (Thesis Advisor; Horticultural Scientist - Postharvest Queensland Department of Agriculture, Fisheries and Forestry): Advising on experiments and critically revising drafts of the work so as to contribute to interpretation.
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LIST OF ABBREVIATIONS

°	Degree
%	Percent
±	Plus or minus
®	Registered trademark
γ	Gamma
AAT	Alcohol acyl transferase
Acetyl CoA	Acetyl coenzyme A
ACS	Aminocyclopropane-1-carboxylic acid synthase
ACO	Aminocyclopropane-1-carboxylic acid oxidase
ADH	Alcohol dehydrogenase
ANOVA	Analysis of variance
AVG	Aminoethoxyvinylglycine
B	Browning
bg	Bright granules
C	Cuticle
Ca	Calcium
CCD	Carotenoid cleavage dioxygenase
Chls	Total chlorophyll
Chl a	Chlorophyll a
Chl b	Chlorophyll b
C	Chroma
Co	Cobalt
CV	Coefficient of variation
cw	Cell wall
Cyt	Cytoplasm
d	Day
DCL	Discoloured cell layer
de sta	Degraded starch
dg	Dark granules
di	Discoloured lenticel
DM	Dry matter
DMF	N,N-dimethylformamide
DVB/CAR/PDMS	Divinylbenzene/carboxen/polymethylsiloxane

e	Epithelial cells
ED	Electron-dense material
e.g.	For example
Ep	Epidermis
et al.	And others
FAA solution	Solution comprised of 50 ml 95% ethanol, 5 ml glacial acetic acid, 10 ml 37-40% formalin formaldehyde and 35 ml distilled water
j	Junction between two epithelial cells
h°	Hue angle
GC	Stomatal guard cells
GCMS	Gas-chromatography mass spectrometry
Gra	Granules
Gy	Gray
h	Hour
HS	Headspace
HPL	Hydroperoxide lyase
IM	Impenetrable materials
kg	Kilogram
kGy	Kilo Gray
kV	Kilovolts
Gy	Gray
l	Litre
<i>L</i>	Lightness
Lc	Lenticel cavity
LD	Lenticel discolouration
Li	Lipid
LM	Light microscopy
LOX	Lipoxygenase
LOD	Limit of detection
LOQ	Limit of quantification
LSD	Least significant difference
Max	Maximum
MB	Mild skin browning
MBTH	3-Methyl-2-benzo-thiazolinone hydrazone
MTS	Monoterpene synthase

1-MCP	1-Methylcyclopropane
ml	Millilitre
mm	Millimetre
MS	Mass spectrometry
mg	Milligram
min	Minute
Min	Minimum
mM	mmol/l
n	Non-coloured lenticel
N	Nitrogen
n.a	Not available
NaCl	Sodium chloride
NB	No browning
n.d.	Not detected
NMR	Nuclear magnetic resonance
NQ	North Queensland
n.s	No significant difference (P>0.05)
OAV	Odour activity value
P	Phenolics
P1	First type of phenolics
P2	Second type of phenolics
P3	Third type of phenolics
PDC	Pyruvate decarboxylase
POD	Peroxidase
PPD	Polymerised phenolic deposition
PPO	Polyphenol oxidase
PPARs	Peroxisome proliferator-activated receptor isoforms
PTV	Programmable Temperature Vaporizing
R ²	Determination coefficients
RC	Resin canal
RCD	Resin canal disorder
rd	Resin duct
RH	Relative humidity
RSD	Relative standard deviation
s	Second

SB	Severe skin browning
SBF-SEM	Serial block-face scanning electron microscopy
S.D.	Standard deviation
SEM	Scanning electron microscopy
SEQ	South east Queensland
Sta	Starch granules
Si	Silver paint layer
SIDA	Stable isotope dilution analysis
SIM	Selective ion monitoring
SL	Starch layer
SLC	Sub-lenticellular cells.
SPME	Solid-phase microextraction
SPS	Sucrose phosphate synthase
TA	Titrateable acidity
TEM	Transmission electron microscopy
THMF	3-Ketoacyl-CoA thiolase
t_R^a	Retention time
TS	Transverse section
TSS	Total soluble solids
TPS	Terpene synthase
USB	Under-skin browning
UHQ	Ultra-high quality water
Viz.	Namely
β	Beta
μ	Micro
μg	Microgram
μl	Microliter

CHAPTER 1. GENERAL INTRODUCTION

1.1 Research background

Mango (*Mangifera indica* L.) is one of the world's most popular tropical fruits (Tharanathan et al., 2006). World mango production increased from 25 million tonnes in 2000 (Galán Saúco, 2004) to 43 million tonnes in 2013 (Statista, 2016). Australia produces *ca.* 50,000 tonnes of mango fruit for domestic and export markets annually (AMIA, 2016). 'B74' (traded as Calypso™), 'Honey Gold', 'Kensington Pride' and 'R2E2' are the main cultivars grown commercially in Australia (Dillon et al., 2013). Although these cultivars are not widely grown overseas, they are in high demand both domestically and as premium products in export markets. Other cultivars such as 'Keitt', 'Kent', 'Palmer', 'Pearl' and 'Brooks' are also cultivated in relatively small volumes (AMIA, 2016).

'Kensington Pride' mango is the most widely planted cultivar in Australia, consisting of 65% of production (AMIA, 2016; Bally et al., 1999b). The parentage of 'Kensington Pride' is not clear. Johnson (2000) suggested that 'Kensington Pride' originated from an Indian cultivar and a South East Asian parent. 'Kensington Pride' is desirable due to its unique flavour (MacLeod et al., 1988). However, inconstant fruit bearing as well as unfavourable colour and size are the disadvantages of this cultivar (Johnson and Parr, 2000). Bally et al. (1999a) studied cross-breeding of 'Kensington Pride' with other cultivars to expand genetic diversity and enhance fruit quality attributes. Many hybrids of 'Kensington Pride' and other maternal parents including 'Delta R2E2', 'A67', 'B74', 'Honey Gold', 'NMBP 1243', 'NMBP 1201', and 'NMBP 4069' have been introduced in the past few years (Dillon et al., 2013).

R2E2 has been cultivated commercially since 1991 (Anonymous, 2014). 'R2E2' is a seedling from 'Kensington Pride' and 'Kent' parents (Dillon et al., 2013). 'R2E2' showed 20% dissimilarity RAPD molecular analysis compared to 'Kensington Pride' parents (Bally et al., 1996). 'R2E2' is characterised by large-sized (>600 g) highly-coloured fruit and accounts for 6 % of production in Australia (AMIA, 2016; Bally et al., 1999b).

Increased market share is being gained by 'B74' (20%) and Honey Gold (4%) (AMIA, 2016). 'B74' (Calypso™) was released in 2000 and is a hybrid of 'Kensington Pride' and 'Sensation' (Whiley and Dorrian, 2007). 'B74' has a small seed and fibreless flesh. 'Honey Gold' is a relatively newly released cultivar in 2002 that originates from 'Kensington Pride' and an unknown parent tree (Dillon et al., 2013). 'Honey Gold' is attractive with a juicy, fiber-free flesh and favourable essence (Marques et al., 2012).

Mango fruit are appreciated by consumers for their external appearance, unique flavour and other health-contributing qualities such as vitamin C, β -carotene and minerals (Tharanathan et al., 2006). External appearance, including size, shape, skin colour and blemishes, is critical to consumer intent to purchase, especially for first-time buyers. Skin blemishes can result from field marks (e.g. rubbing against branches and twigs), insect damage (e.g. russetting and fruit fly), pre- and postharvest diseases, harvest and postharvest physical damage, and physiological disorders (Sivakumar et al., 2011). Skin physiological disorders are of real concern to the Australian mango industry (Macnish et al., 2014; Marques et al., 2012). They lead to downgrading of fruit quality attributes and to loss of market confidence (Hofman et al., 2010; Rymbai et al., 2012). Lenticel discolouration (LD), 'under-skin browning' (USB) and 'resin canal damage' (RCD) (Figure 1.1) have emerged in recent years as serious problems in terms of poor fruit appearance that reduce the economic value of fruit.

LD has an adverse effect on fruit appearance and economic value and consequently is a major concern to the mango industry (Hofman et al., 2010). LD affects many mango cultivars around the world (Bezuidenhout and Robbertse, 2005; Self et al., 2006), including Australian genotypes (Hofman et al., 2010; Li et al., 2016; Marques et al., 2016). LD is confined to lenticels on the fruit skin and is characterised by red or black darkening associated with pigment accumulation in the vacuoles of sub-lenticel cells (Bezuidenhout et al., 2005; Self et al., 2006). LD is exacerbated by exposure to quarantine or phytosanitary treatments, such as irradiation. For example, irradiation of 'Kensington Pride' mango with 300 or 600 Gy delayed the ripening process and increased levels of LD (McLauchlan et al., 1989). Hofman et al. (2010) reported that irradiation increased the incidence of LD on 'B74' mango. Marques et al. (2016) showed that either irradiation at 441 - 610 Gy or harvesting and postharvest handling increased LD on 'B74' mango fruit.

USB is one of important skin browning disorders of 'Honey Gold' mango fruit (Hofman et al., 2010; Holmes, 1999) due to the resulting downgrading of fruit quality and loss of market confidence. USB visible on the surface of 'Honey Gold' fruit predominantly affects this cultivar (Marques et al., 2012). It manifests under the epidermis as a superficial bruise-like injury with no damage to the flesh (Hofman et al., 2010). The defect is usually not obvious at harvest. Rather, visual symptoms become pronounced at around 4 days after harvest. Limited published information is available on the mechanism and control of USB development. Hofman et al. (2010) showed that delayed or slow cooling of fruit before or after packing was effective in diminishing USB incidence. Marques et al. (2012) reported that, in combination with holding of 'Honey Gold' fruit at less than 14 - 16 °C, USB is associated with physical damage after harvest.

RCD is a quality defect that reduces the marketability of mango fruit (Holmes, 1999). The symptoms are visible through the skin as dark traces of finely branched network of sub-cuticle resin canals (Holmes, 1999). The occurrence of this disorder is seemingly highest in ‘Kensington Pride’ mango fruit (Macnish et al., 2014). Factors contributing to the development of this disorder are largely unknown (Macnish et al., 2014). Mechanisms regulating symptom expression are also poorly understood.

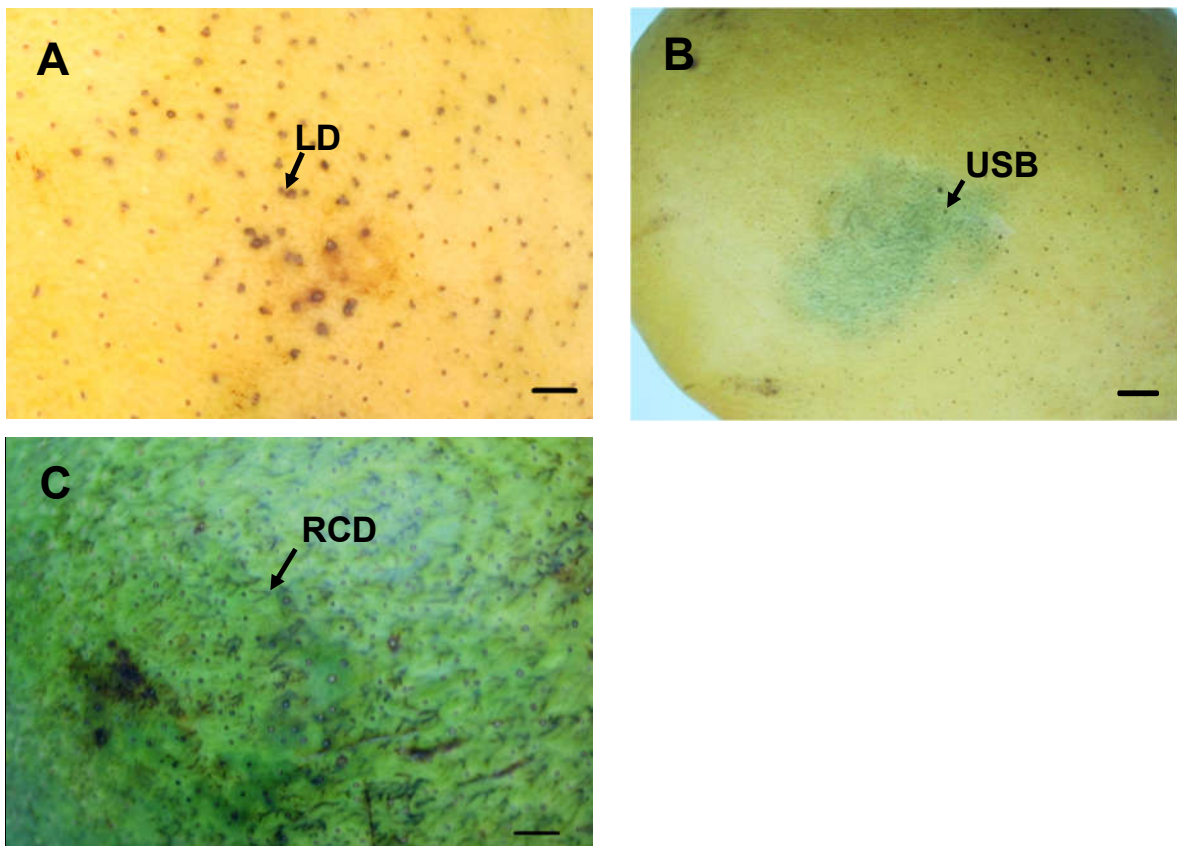


Figure 1.1 Typical symptoms of LD on ‘B74’ (A), USB (B) on ‘Honey Gold’ and RCD on ‘Kensington Pride’ (C) mango fruit. Arrows point to affected regions. Scale bars = A, B, C, 5 mm.

1.2 Research approach

The objectives of this PhD study were:

Part A: Towards understanding the mechanism of LD on the skin of mango fruit

Irradiation is effective post-harvest quarantine treatment to prevent insect pests, especially fruit fly and mango seed weevil (Bustos et al., 2004). Nonetheless, irradiation caused detrimental effects on mango quality attributes (Cruz et al., 2012; Moreno et al., 2006). The susceptibility of mango to LD depends on the cultivar (du Plooy et al., 2009; Oosthuysen, 1999). It was hypothesised that

irradiation would differentially induce LD on different Australian mango cultivars. In addition, it was proposed that irradiation would inhibit skin degreening in mango. Furthermore, it was proposed that other ripening parameters, including aroma volatile production and fruit eating quality, would be adversely affected.

LD was characterised as light purple areas due to pigment accumulation in the vacuoles of sub-lenticellular cells (Bezuidenhout et al., 2005). Polyphenols were also related to discoloured lenticels (Tamjinda et al., 1992). LM or SEM imaging revealed parts of lenticel structure.

Specific aims were:

(1) To investigate the effects of γ -irradiation on LD and other physical chemical properties, especially aroma volatiles, in the Australian mango cultivars including 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' mangoes.

(2) To investigate the general morphology of LD in these cultivars in terms of histological features and the overall structure of non-coloured and discoloured lenticels in 'B74' mango fruit.

Part B: Towards addressing 'under-skin browning' (USB) on 'Honey Gold' and 'resin canal damage' (RCD) on 'Kensington Pride' mango fruit

USB on Honey Gold mango and RCD of Kensington Pride mango are two important fruit skin-browning disorders concerning the Australian mango industry in recent years. However, there is limited published information about the mechanisms and anatomies of these disorders. Increased understanding of these disorders should help in their improved management. Symptomology suggest that USB and RCD involve different cell biology processes.

The specific aims were:

(1) To inform better understanding of these skin disorders at the anatomical level. Therefore, USB and RCD were compared and contrasted at the cellular level using light microscopy and transmission electron microscopy.

(2) To understand at the cellular level the potential roles of polyphenol oxidase (PPO) and peroxidase (POD) in tissue browning associated with of USB and RCD.

It was envisaged that, through gaining a fuller understanding of the comparative biochemistry of USB and RCD symptom expression at cell and tissue levels, future development and testing of treatments to manage these physiological disorders would be better informed.

Also examined was the hypothesis that USB incidence is higher in fruit harvested in the afternoon, particularly in combination with physical. A diurnal effect was proposed to be associated with varying sap components. It is likely that USB expression is a defence system against stress. Putative differences in USB incidence at different harvest times were considered to likely be due to temporal differences in sensitivity of mango fruit skin tissue to the concentrations of aroma volatiles in sap. In this regard, additional experiments focused on understanding the mechanism underpinning USB symptom expression. In general, it was envisaged that better understanding the USB mechanism would offer informed management of the problem.

The specific aims were:

(1) To investigate the postulated effect of harvest time over the diurnal cycle with regard to the varying temporal propensity of 'Honey Gold' fruit to develop USB.

(2) To characterise the putative effects of sap components and changes in the volatile composition on USB induction in 'Honey Gold' fruit.

1.3 Thesis structure

The current chapter (Chapter 1) provides a general introduction to the research focus and objectives. Chapter 2 reviews the literature on mango fruit quality attributes and mango skin disorders. Chapter 3 reports the development of a high throughput precise analytical method to quantify key volatiles produced by Australian mango fruit and sap. The novel method utilises a combination of Stable Isotope Dilution Analysis (SIDA) and Head-space Solid phase microextraction (HS-SPME) in combination with gas chromatography mass spectrometry (-GC-MS). Chapter 4 examines the effects of γ -irradiation on LD, physical-chemical properties and aroma volatiles in four Australian mango cultivars. Chapter 5 presents the anatomical features of LD with special emphasis on an integrated ultrastructural visualisation of non-coloured and discoloured lenticels to deliver a better understanding of LD. Chapter 6 reports on the effects of diurnal harvest time on USB susceptibility and possible relationships to the chemical composition of fruit sap. Chapter 7 describes anatomical and histological disparities between USB and RCD and investigates the potential spatial involvement of PPO and POD activities in these disorders. Chapter 8 presents the final discussion

and conclusions for the research chapters described above and proffers future directions to inform better management of these disorders.

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CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

The attractive appearance of fruit is critical to a consumer's decision to purchase, especially for first-time buyers. In addition to size, the main external characteristics of fruit typically include shape, colour and blemishes. Consumers often equate blemishes to poor quality, including off flavours and limited useable flesh. Accordingly, they can be intolerant of blemishes. Skin blemishes can result from field marks (e.g. wind rub), harvest and postharvest physical damage, insect damage, pre- and postharvest diseases, and physiological disorders. Skin physiological disorders are of major concerns to the Australian mango industry due to the resultant downgrading of the fruit appearance and quality attributes (Hofman et al., 2010; Marques et al., 2016). Pre-harvest conditions are important factors affecting mango skin disorders (Bally, 2007). Besides pre-harvest factors, post-harvest treatment, especially irradiation, should be emphasized (Marques et al., 2016).

In this chapter, the general background of mango quality attributes and their metabolic and compositional changes during ripening are presented to provide a better overview and understanding of the potential contribution to metabolism and nutrition. The mechanisms and factors associated with skin browning disorders including 'lenticel discolouration' (LD), 'under-skin browning' (USB) and 'resin canal damage' (RCD) are reviewed. In addition, pre-harvest management and postharvest factors such as irradiation affecting skin browning disorders are also discussed. Finally, general prospects and hypothesis for the development of these disorders should be given due consideration.

2.2 Quality of mango fruit

2.2.1 External quality

Consumer acceptance is dependent on fruit quality attributes, including appearance, blemish marks, colour, texture, eating experience, ripeness levels, etc. Appearance is the first factor easily perceived by the consumer in the process of making a decision to purchase a particular type of fruit or its products, with colour being the vital appearance attribute (Akhtar et al., 2009). Consumers prioritize colour as it is perceived best in providing information on ripeness level. Consumer acceptance is higher for mangoes free from external damage, including bruises, latex or sap injury and decay, along with uniform weight, colour and shape (Sivakumar et al., 2011).

2.2.2 Internal quality

Valente et al. (2011) reported that texture is a good indicator for establishing the eating quality and the freshness of fruit, making it a major index in determining product acceptability. Internal attributes such as pulp fibrousness, sweetness, and aroma are important for a full perception of fruit quality (Araújo and Garcia, 2012).

2.2.3 Nutritional contributions

Mangoes play a role in human nutrition and health as they provide a rich source of antioxidants including ascorbic acid, carotenoids and phenolic compounds (Brecht and Yahia, 2009). Mangoes are also regarded as a source of bioactive compounds with human health-promoting properties. Wilkinson et al. (2008) reported that mango components and metabolites may contribute to health because activations of transcription factors in human diseases, such as peroxisome proliferator-activated receptor isoforms (PPARs), are inhibited by quercetin and norathyriol.

Different values of vitamin C content were shown among mango cultivars, maturity stages and postharvest handling. For example, ascorbic acid contents in the fruit of nine Indian cultivars were different from 14 mg/100 g to 57 mg/100 g (Kohli et al., 1987). Total ascorbic acid concentrations in 'Tommy Atkins' and 'Ubá' were 10 and 78 mg/100 g, respectively. Vitamin C concentration in the flesh decreased substantially from 88 to 22 mg/100 g between 5 weeks after fruit set up to maturity in 'Amini', 'Mullgoa', 'Pico', and 'Turpentine' mangoes (Spencer et al., 1956). 'Haden', 'Irwin' and 'Keitt' showed a general loss in vitamin C content from 16 to 23% during ripening at different storage temperatures, whereas 'Kent' showed a steady increase (Vazquez-Salinas and Lakshminarayana, 1985). Postharvest disease control treatments and nitric oxide fumigation did not significantly influence the concentration of ascorbic acid in 'Kensington Pride' mangoes (Dang et al., 2008; Zaharah and Singh, 2011).

In addition, mango fruit contain many valuable antioxidant compounds, such as carotenoids and phenolics (Ornelas-Paz *et al.*, 2007; Rocha-Ribeiro *et al.*, 2007). Main phenolics in mango are leucocyanidin, catechin, epicatechin, chlorogenic acid, quercitrin and quercetin (Berardini *et al.*, 2005; Schieber *et al.*, 2000). Some carotenoids function as provitamin A as expressed as Retinol Activity Equivalents (RAE) (Rocha Ribeiro et al., 2007). RAE values were 74; 51; 55 and 185 µg/100 g respectively in 'Haden', 'Tommy Atkins', 'Palmer' and 'Ubá' cultivars.

2.3 Mango ripening physiology

2.3.1 Respiration

Mango fruit show a climacteric pattern in which the respiration increased during the ripening (Aina and Oladunjoye, 1993; Akamine and Goo, 1973). In the ripening of 'Kensington Pride' mangoes, the climacteric rate gained a peak at day 4 (Lalel et al., 2003a). Fruit maturity affected the respiration rate in which only hard mature green stage showed the climacteric pattern (Lalel et al., 2003c). Mitcham and McDonald (1993) reported that heat treatment at 46 °C and 48 °C enhanced the respiration of mature green fruit. Moreover, the respiration of 'Kensington Pride' fruit was inhibited by aminoethoxyvinylglycine (AVG) and 1-methylcyclopropane (1-MCP), while exogenous ethephon enhanced it (Lalel et al., 2003d).

2.3.2 Ethylene production and the ripening process

Ethylene is important in triggering the ripening process of mango (Medlicott et al., 1987). Mangoes are climacteric fruit in which ethylene production facilitates the ripening process (Brecht and Yahia, 2009). According to Burdon et al. (1996), the mango ripening process was associated with increased ethylene production. Lalel et al. (2003c) showed ethylene production of 'Kensington Pride' mango fruit harvested at the hard mature green stage followed a typical climacteric rise during ripening. However, there was no increase at the half ripe or ripe stage as they were post-climacteric at the time of harvest. Lalel et al. (2004) reported that an increase in ripening temperature from 15 to 30 °C enhanced ethylene production in green mature mango fruit. In contrast, ripening at 35 °C lowered ethylene production slightly due evidently to temperature sensitivity of aminocyclopropane-1-carboxylic acid synthase (ACS) and aminocyclopropane-1-carboxylic acid oxidase (ACO).

It is important to modulate ethylene production because the increase in ethylene production during mango fruit ripening coordinates the ripening process (Burdon et al., 1996). Monitoring ethylene production has been recorded in many studies. Application of ethephon to green mature 'Kensington Pride' mangoes stimulated ethylene production during ripening. Conversely, aminoethoxyvinylglycine (AVG) at 500 and 1000 mg/l or 1-methylcyclopropane (1-MCP) at 1, 10 and 25 µl/l significantly inhibited ethylene biosynthesis (Lalel et al., 2003d). Additionally, Burdon et al. (1996) suggested increased ethylene production may be suppressed by acetaldehyde through inhibition of ACO.

2.4 Metabolic and compositional changes during ripening

2.4.1 Cell wall and structural polysaccharides and texture

Mango fruit softening is an important indicator of ripe and ready to eat fruit. Mango texture is mainly related to changes in cell wall composition, including degradation of pectin (Muda et al., 1995; Roe and Bruemmer, 1981). During ripening of 'Ngowe' mangoes, Brinson et al. (1988) showed that degradation of pectin involved marked declines in arabinose, galactose and galacturonic acid contents of mesocarp cell walls. Muda et al. (1995) examined changes in cell wall polymers during ripening of 'Tommy Atkins' mangoes. An overall reduction of galactosyl and deoxyhexosyl residues during ripening process was associated with degradation of pectin components.

The pectolytic enzymes polygalacturonase, pectin methyl esterase, galactanase, arabinanase and β -galactosidase are involved in 'Alphonso' mango ripening (Prasanna et al., 2003). The increase in polygalacturonase and cellulase activities was associated with softening and ripening of 'Keitt' mangoes (Roe and Bruemmer, 1981). Also, cell wall degrading glycanases and glycosidases in combination with decreased mannose content may contribute to textural softening during the ripening process (Yashoda et al., 2007).

2.4.2 Organic acids

During mango ripening, there is a substantial decrease in organic acids mainly due to the loss of citric acid (Medlicott and Thompson, 1985) that has important impact on palatability. Lalel et al. (2003c) reported that the acid content in 'Kensington Pride' ripe fruit, harvested at hard green mature stage was significantly higher than those in fruit collected at later stages of maturity.

2.4.3 Soluble sugars

Yashoda et al. (2006) reported the total soluble solids content of 'Alphonso' mango fruit increased from 7% to 20% over the unripe to ripe stage. Medlicott and Thompson (1985) reported that there was an increase in the contents of glucose, fructose and sucrose during ripening of 'Keitt'. Sucrose is predominant throughout ripening, with fructose being the major reducing sugar. Castrillo et al. (1992) found that sucrose phosphate synthase (SPS) may be involved in the regulation of sucrose metabolism during ripening. Similarly, Wongmetha et al. (2012) found that sucrose accumulation was associated with a decrease in SPS.

Soluble sugars that contribute mango sweetness are accumulated through carbon supplied by both photosynthesis and starch degradation during ripening (Peroni et al., 2008; Silva et al., 2008). Peroni et al. (2008) showed that starch was converted into sucrose, the most abundant sugar of the 'Keitt' ripe fruits, by α - and β -amylases. According to Lalel et al. (2003c), maturity stage at harvest has no considerable effect on the contents of non-reducing sugars when fruit ripen. However, markedly lower concentrations of reducing and total sugar were found in ripe fruit harvested at the hard mature green as compared to other later stages of maturity.

2.4.4 Colour and pigments

2.4.4.1 Skin colour changes

Mango skin colour change, typically from green to yellow, is an important indicator to consumers of fruit ripeness level. The change from green to yellow or orange is often augmented by red blush, depending on the variety (Medlicott et al., 1986). Peel colour in 'Tommy Atkins' and 'Keitt' mango cultivars has a stronger red blush than in green or yellow-skinned mangoes. 'Tommy Atkins' mangoes develop more red and yellow pigmentation in peel than 'Keitt' mangoes (Mitcham and McDonald, 1992). Ornelas-Paz et al. (2008) characterized gradual changes in epidermis pigmentation from green to yellow in 'Manila' and 'Ataulfo' mango cultivars during ripening.

Mango peel ground colour changes during ripening are due to the degradation of chlorophyll and the accumulation of carotenoid (Medlicott et al., 1986). Ketsa et al. (1999) observed that the breakdown of chlorophyll was catalysed by chlorophyllase and peroxidase. Lalel et al. (2003c) established that 'Kensington Pride' ripe fruit harvested at the hard mature green stage had lower a^* value than those harvested at other later maturity stages.

2.4.4.2 Flesh colour changes

The development of yellow-orange colouration in the mesocarp during ripening of most mango cultivars is due to carotenoid accumulation (Vásquez-Caicedo et al., 2005). A range of carotenoids have been detected in fruit of different mango cultivars, but only a few constitute large portions (Chen et al., 2004b; Mercadante et al., 1997; Ornelas-Paz et al., 2008). Mercadante et al. (1997) found that *all-trans*-violaxanthin, *all-trans*- β -carotene and *9-cis*violaxanthin are major carotenoids in 'Keitt' mango pulp. In 'Manila' and 'Ataulfo' fruit mesocarp, *all-trans*- β -carotene and the dibutyrate of *all-trans*-violaxanthin and *9-cis*-violaxanthin were the predominant carotenoids (Ornelas-Paz et al., 2008). There was an increase of total carotenoid contents from the mature-green to the ripe stage of 'Keitt' and 'Tommy Atkins' mango fruit (Mercadante and Rodriguez-Amaya,

1998). In ‘Kensington Pride’, there was no significant effect of fruit maturity stage at harvest in carotenoid concentration (Lalel et al., 2003c).

2.4.5 Flavour (taste, aroma)

2.4.5.1 Flavour components

Flavour is a complex attribute of quality because it encapsulates taste, mouthfeel, and aroma properties. Taste includes ‘sweetness, sourness, bitterness, saltiness, and aroma’ (Baldwin, 2002). Mouthfeel is all ‘tactile (feel) properties’ perceived from the food’s physical and chemical interaction in the mouth (Guinard and Mazzucchelli, 1996). Fruit aroma is defined as ‘a complex mixture of a large number of volatile compounds including alcohols, aldehydes, and esters’ (Defilippi et al., 2009).

Differences in the composition of aroma volatile compounds among different mango cultivars have been found (Lalel et al., 2003a). ‘Kensington Pride’ mangoes produced 61 volatile components, of which 35 compounds had not been shown in previous studies (Lalel et al., 2003a). Three hundred and seventy-two volatile compounds have been identified that contributed to the volatile profiles of 20 Cuban mango cultivars (Pino et al., 2005). Although ripe mangoes contain more than 300 volatile compounds, only some are odour-active as key odourants which contribute significantly to aroma (Brecht and Yahia, 2009).

Esters together with some lactones contribute to peach flavour in ‘Kensington Pride’ (MacLeod et al., 1988). In sensory evaluation, ‘Kensington Pride’ was scored highly for terpene, fermented, buttery, tropical, and floral scent (Table 2.1). ‘R2E2’ had some fermented and buttery aroma notes, and was also high in melon scent (Smyth et al., 2008). MacLeod and Snyder (1985) reported that while α -terpinolene was described as contributing floral, scented and sweet (fragrant) scents, car-3-ene contributed pungent and mango leaf-like aromas in ‘Willard’ and ‘Parrot’ mangoes.

Terpene hydrocarbons represent the most abundant class of volatiles for most mango cultivars studied (Defilippi et al., 2009; Lalel and Singh, 2006; Lalel et al., 2003a; MacLeod and de Troconis, 1982; MacLeod et al., 1988; Pino et al., 2005). Monoterpenes was the major group among terpene hydrocarbons, contributing *ca* 49%, 50%, and 54% of total volatiles, respectively, in ‘Kensington Pride’ (MacLeod et al., 1988), ‘R2E2’ (Lalel and Singh, 2006) and Venezuelan mangoes (MacLeod and de Troconis, 1982). In comparison, sesquiterpene hydrocarbons were represented in lower concentration of 14% of total volatiles in Venezuelan mangoes (MacLeod and de Troconis, 1982).

Table 2.1 Odour threshold and odour quality description of major aroma compounds in ‘B74’ and ‘Kensington Pride’ mangoes. Odour threshold is defined as the minimum concentration of odour compound perceived by human sense of smell (Van Gemert, 2003).

Varieties	Aroma compounds	Odour quality description	Odour threshold ($\mu\text{g}/\text{kg}$)
Calypso	Hexanal ⁸	Green, fatty ⁶	4.5 ⁶
	Ethyl methacrylate ⁸	Fruity and ester aroma ⁹	n.a
	p-Cymene-8-ol ⁸	Herbal, slightly minty ³	11.4 ⁷
	1,3,8-p-Menthatriene ⁸	Turpentine odour ¹	n.a
	2,6-Nonadienal ⁸	Cucumber-like ⁶	0.01 ⁶
	γ -Octalactone ⁸	Coconut ⁶	7 ⁶
	Dimethyl trisulfide ⁸	Fishy, sulfur smell ⁹	7.3 ⁹
Kensington Pride	α -Terpinolene ^{2,5,8}	Floral, fragrant, piney ⁶	200 ⁶
	Hexanal ⁸	Green, fatty ⁶	4.5 ⁶
	p-Cymene-8-ol ⁸	Herbal, slightly minty ³	11.4 ⁷
	β -Myrcene ⁸	Fress, green grass ⁴ , resinous, balsamic ⁶	36 ⁶
	1,3,8-p-Menthatriene ⁸	Turpentine odour ¹	n.a
	Limonene ⁵	Citrus-like ⁶	210 ⁶
	α -Terpinene ⁵	Sweet ⁹	n.a
	3-Carene ⁵	Resinous, sweet ⁶	5 ⁶
	γ -Octalactone ⁵	Coconut ⁶	7 ⁶

Sources: ¹Laohakunjit et al. (2006), ²Lalel et al. (2003a); ³MacLeod and de Troconis (1982); ⁴MacLeod and Snyder (1985), ⁵MacLeod et al. (1988); ⁶Pino (2012); ⁷Pino et al. (2005); ⁸Sunarharum et al. (2007); ⁹Shivashankara et al. (2006). n.a, not available.

Among monoterpenes, α -terpinolene was the most abundant aroma compound in ‘Kensington Pride’ (Lalel et al., 2003a; MacLeod et al., 1988). α -Terpinolene has also been reported as the main aroma constituent in ‘B74’ (Singh et al., 2004), ‘R2E2’ (Lalel and Singh, 2006), ‘Bowen’ (Malundo et al., 2001), ‘Parrot’ and Willard’ (MacLeod and Snyder, 1985), Cuban cultivars including ‘Obispo’, ‘Corazón’ and ‘Huevo de toro’ (Pino et al., 2005), and Brazilian cultivars consisting of ‘Cheiro’, ‘Chana’, ‘Bacuri’, ‘Cameta’, ‘Gojoba’, ‘Carlota’, ‘Coquinho’, and ‘Comum’ (Andrade et al., 2000). 3-Carene was the second major monoterpene in ‘Kensington Pride’ (Lalel et al., 2003a)

and 'R2E2' (Lalel and Singh, 2006). δ -3-Carene was reported as the most abundant volatile in 'Haden', 'Manga Amarilla', 'Macho', 'Manga Blanca', 'San Diego', 'Manzano', 'Smith', 'Florida', 'Keitt', and 'Kent' cultivars (Pino and Mesa, 2006; Pino et al., 2005). Besides terpenes, esters represented an important class of volatiles in 'Kensington Pride' (Lalel et al., 2003a).

The variability in mango volatile compounds depends on many other factors including cultivar (i.e., genetics), maturity stage at harvest (Lalel et al., 2003c), part of the fruit (Lalel et al., 2003b), and ripening temperature (Lalel et al., 2004). Lalel et al. (2003c) suggested that harvesting at sprung green stage can result in greater production of aroma volatiles in ripe 'Kensington Pride' fruit. Lalel et al. (2003b) showed aroma volatiles presented more abundantly in the top mesocarp and outer parts of ripe 'Kensington Pride' mango fruit consisting of skin and outer mesocarp. Lalel et al. (2004) suggested that ripening temperature is a key factor directly affecting the biosynthesis of aroma volatile compounds in green mature 'Kensington Pride' mangoes. Twenty °C was found to be optimum for fruit ripening and aroma volatile biosynthesis in 'Kensington Pride'.

2.4.5.2 Volatiles metabolism

Aroma compounds are derived from many different pathways in fatty acid, amino acid, phenolic, and terpenoid metabolism (Baldwin, 2002) (Figure 2.1). Through β -oxidation and the lipoxygenase (LOX) pathway (Sanz et al., 1997), fatty acids have been considered precursors of many aroma volatile compounds of fruit, including terpenes, aldehydes, acids, alcohols, and esters (Bartley et al., 1985; Lalel et al., 2004; Tressl and Drawert, 1973). During ripening, there was an increase in fatty acid production in 'Kensington Pride' mango fruit (Lalel et al., 2003a). The biosynthesis of esters may be related with the biosynthesis of fatty acids in 'Kensington Pride' mangoes (Lalel et al., 2003a). Lalel et al. (2003d) reported that the biosynthesis of fatty acids seemed to be catalyzed by ethylene in 'Kensington Pride'. Higher ripening temperature up to 30 °C increased the level of all fatty acids, except palmitic, palmitoleic, and linolenic acids (Lalel et al., 2004).

Sanz et al. (1997) reported that only a few classes of aroma components were created directly from carbohydrate metabolism. The biosynthetic synthesis of monoterpenes and sesquiterpenes diterpenes is reported to be catalyzed by terpene synthases (Chen et al., 2004a; Nieuwenhuizen et al., 2009). Nortriterpenoids or norisoprenoids are aroma compounds which originated from carotenoids (Defilippi et al., 2009; Winterhalter and Rouseff, 2002). Cleavage of the carotenoid chain was catalyzed by carotenoid cleavage dioxygenases (CCD) (Booker et al., 2004; Kato et al., 2006). Pott et al. (2003) suggested that the expression of *ccd4* played the major role in the accumulation of carotenoids and carotenoid-derived volatiles in peach fruit flesh.

Amino acids are involved in aroma biosynthesis in fruits and vegetables as direct and indirect precursors to generate alcohols, carbonyls, acids, and esters (Defilippi et al., 2009; Sanz et al., 1997; Tressl and Drawert, 1973). Amino acids, including leucine, valine and phenylalanine are predominant direct precursors to generate aroma compounds in banana (Tressl and Drawert, 1973).

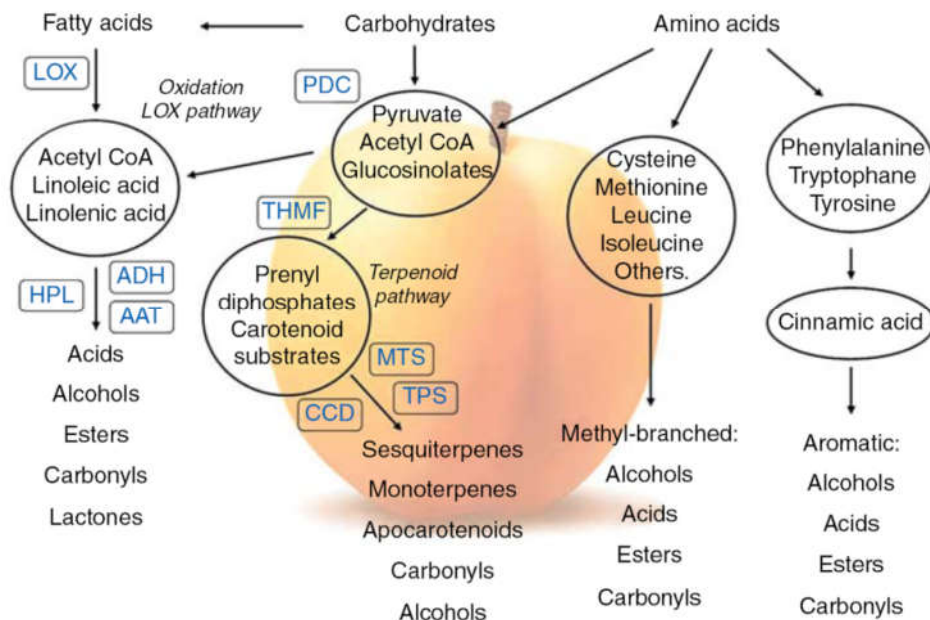


Figure 2.1 Biosynthesis pathways of major volatile compounds in fruits (from Defilippi et al., 2009). Abbreviations: LOX, lipoxygenase; HPL, fatty acid hydroperoxide lyase; ADH, alcohol dehydrogenase; AAT, alcohol acyl transferase; PDC, pyruvate decarboxylase; THMF, 3-ketoacyl-CoA thiolase; CCD, carotenoid cleavage dioxygenase; MTS, monoterpene synthase; TPS, terpene synthase; and, Acetyl CoA, acetyl coenzyme A.

2.5 Mango skin disorders

2.5.1 Lenticel discolouration

2.5.1.1 Origin and structure of lenticels

Lenticels are macroscopic openings on the mango fruit skin that can contribute to gaseous exchange and transpiration. Lenticels in mangoes typically derive from stomata. They enlarge during fruit growth, largely due to stretching of the fruit surface (Bezuidenhout et al., 2005; Rymbai et al., 2012). Bally (1999) confirmed that lenticel formation in ‘Kensington Pride’ was due to the rupturing of stomata.

Tamjinda *et al.* (1992) found that cells surrounding the lenticel were smaller than surrounding cells and had many intercellular spaces. Bezuidenhout *et al.* (2005) reported that cells below the stomatal cavity had thinner cell walls and larger intercellular spaces. Lenticels of different mango cultivars vary in size. For example, mature lenticels of ‘Kent’ are smaller in size than those of ‘Tommy Atkins’ and ‘Keitt’ cultivars (Bezuidenhout and Robbertse, 2004). In ‘Tommy Atkins’ mangoes, lenticels were the most organised with the largest lenticel cavity but the smallest aperture as compared with ‘Kent’ and ‘Keitt’ (Du Plooy *et al.*, 2009b).

2.5.2.1 Mechanisms of lenticel discolouration

Several studies on the mechanisms of mango LD have been conducted. Bezuidenhout *et al.* (2005) observed that discolouration occurred in the form of light purple areas due to pigment accumulation in the vacuoles of sub-lenticellular cells (Figure 2.2). The vacuolar deposition of phenolic compounds resulted in increased reddening, and eventual darkening of the lenticels (Du Plooy *et al.*, 2009a). Tamjinda *et al.* (1992) found that polyphenols were associated with discoloured cells. Brown or black discoloured lenticels were associated with accumulation of quinine in the cell walls. Red LD was associated with the production of anthocyanins in sub-lenticellular cells (Self *et al.*, 2006) and phenylpropanoid derivatives (Du Plooy *et al.*, 2009a).

Lenticel structure likely influences susceptibility to lenticel discolouration. Tamjinda *et al.* (1992) found that ‘Falan’ mango did not develop lenticel discolouration. This variety had a cork cambium which was considered to prevent cell wall shearing and maintain cell integrity. Bezuidenhout *et al.* (2005) concluded that the lack of a cork cambium and cork cells in lenticels of susceptible mango cultivars contributed to less organised lenticels and increased LD potential.

Cultivar-dependant susceptibility to LD was related to the totals of wax, suberin as well as cutin in the lenticels (Du Plooy *et al.*, 2004). LD susceptibility appears to increase by the bad weather that involves rain in the latter stages of the fruit growth (see below). Fruit wetting presumably allows water and other contaminants to stimulate a stress response in cells surrounding the lenticel cavity. This phenomenon may explain why cultivars with less organised lenticels are more susceptible to discolouration. Mango sap on the skin during desapping the fruit is also a cause of lenticel damage (O'Hare and Prasad, 1992). Mango sap is comprised of an oil fraction (largely alkenyl resorcinol) and a protein-polysaccharide fraction (Hassan *et al.*, 2009). Studies suggest that the oil fraction is the major contributor to lenticel damage (O'Hare, 1994; O'Hare *et al.*, 1999). LD can be induced by terpenes from the resin of both ‘Tommy Atkins’ and Keitt’ fruit. These terpenes evidently destroy the integrity of cell membranes (e.g. tonoplast) of the sub-lenticellular cells, thereby allowing

polyphenol oxidase direct contact with phenolic substrates in cell walls (Bezuidenhout et al., 2005). Bezuidenhout et al. (2005) also suggested that discolouration may be caused by sap migrating from resin canals to lenticels in close proximity, particularly during periods of high rainfall and fruit turgor (Figure 2.3).

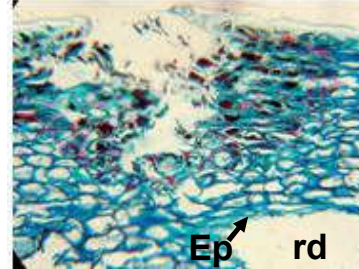
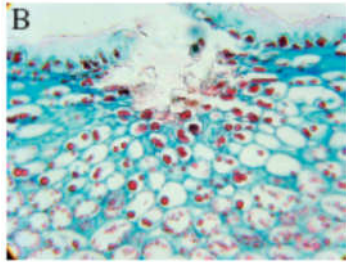


Figure 2.2 Vacuolar accumulation of pigmentation in sub-lenticular cells of mature 'Tommy Atkins' mango fruit stained with Safranin A (from Bezuidenhout et al., 2005). Figure 2.3 Damaged resin duct adjacent to discoloured lenticels of 'Keitt' mango exocarp (from Bezuidenhout et al., 2005). Ep, epithelial cells; rd, resin duct.

2.5.2 Under-skin browning

Under-skin browning (USB) is a visible injury under the epidermis of 'Honey Gold' mango fruit. It appears as diffuse areas on the fruit surface, but with no damage to the flesh (Hofman et al., 2010; Holmes et al., 2010). In some cases, the unaffected cuticle on the skin has an opaque appearance. The defect is usually not obvious at harvest, but develops as the fruit ripens. USB results in loss of value and reduced sales.

The mechanism regulating USB development is not clear. Hofman et al. (2010) found that delayed or slowed cooling before or after packing can reduce the incidence of USB on 'Honey Gold' mangoes. It is apparent that fruit from relatively hotter production areas are more susceptible, perhaps due to greater differences between field temperature and postharvest temperatures, including during cooling.

USB often occurs around areas of visible physical injury upon prolonged vibration during transport. Reducing fruit to fruit contact using the tray liners or tray with plastic reduced USB expression following road transport of 'Honey Gold' mango fruit from the Northern Territory to Brisbane (Marques et al., 2012). Light microscopy of affected areas on 'Honey Gold' mango skin revealed the retention or accumulation of starch around resin canals and discoloured cells in affected versus non-USB-affected epidermal tissue (Figure 2.4). The browning may be related to discolouration of latex vessels that spreads to surrounding cells (Hofman et al., 2010).

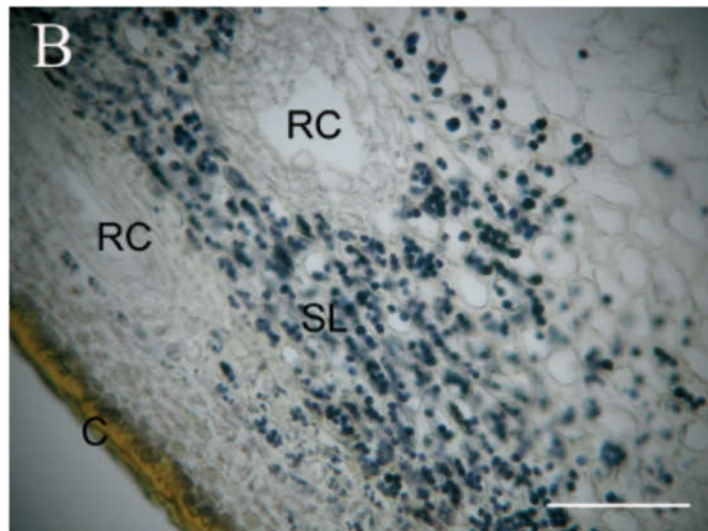


Figure 2.4 Retention or accumulation of starch granules revealed in starch-iodine stained USB-affected tissue (from Marques et al., 2012). C, cuticle; RC, resin canal; SL, starch layer. Scale bars = 0.1 mm.

2.5.3 Resin canal discolouration

Resin canal discolouration or disorder (RCD) has evidently become relatively more prominent on the domestic Australian market in recent seasons. This disorder presents as unsightly black streaks in the epidermal outer layers of the flesh. On the skin, the disorder progresses in severe cases as large blackened areas (Brann, 2013; Holmes et al., 2010). RCD is associated with the discolouration of the resin canals that contain the sap manifested in a finely branched pattern of sub-cuticular black streaks (Figure 2.5). The damage usually occurs on the distal ‘nose’ or the mid-region of the mango fruit.



Figure 2.5 Resin canal disorder symptom in ‘Kensington Pride’ mango fruit.

The causes are unknown, but are likely a combination of production factors increasing fruit susceptibility and postharvest factors causing fruit stress that contribute to disorder expression in these more susceptible fruit.

2.6 Pre-harvest management

Most postharvest skin defects/disorders are not obvious at harvest, but become so from several days after harvest to near ripe. Pre-harvest factors that can potentially influence skin disorders include cultivar, climate, soil, nutrition, and water stress. Relatively little has been published on these interactions, except for lenticel discolouration.

2.6.1 Cultivar

Cultivar plays an important role in susceptibility to disorders. According to Hofman et al. (2010), USB mainly occurs on 'Honey Gold' while RCD is often more obvious on 'Kensington Pride'. The susceptibility of mango to LD is also cultivar-dependant (Du Plooy et al., 2009b; Oosthuyse, 1999). For example, 'Tommy Atkins' and 'Keitt' are generally more susceptible to LD as compared with 'Kent' (Bezuidenhout and Robbertse, 2004; Oosthuyse, 1998). Also, LD is generally least noticeable on 'Honey Gold' as compared with 'Kensington Pride', 'R2E2', and 'B74' (authors observations). Relative cultivar susceptibility appears to be influenced by the degree of disorganisation of the cells around the lenticel cavity and also the presence and extent of suberisation and cutinisation on the cells lining the cavity (Du Plooy et al., 2004).

2.6.2 Moisture

Moisture can have a significant influence on fruit appearance, especially skin and flesh disorders. Duvenhage (1993) showed that LD in avocado was more severe when fruit were picked wet as compared to dry-harvested fruit. Also, wet-harvested fruit had higher incidence of black cold damage which was related to the response to low temperature. Hofman et al. (1997) reported that mango fruit from cooler growing locations and harvested in the rain were more susceptible to chilling injury.

The influence of both excess and deficit water stress on skin disorders depends on the timing and extent of water stress. Excess water in soil during fruit growth is one of main causes of translucent flesh disorder and gamboge disorder in mangosteen fruit (Sayan and Rawee, 2005). Incidence of both disorders can be reduced by controlling soil water potential at about -70 kPa. Simmons et al. (1998) reported that withholding irrigation for 12 weeks after flowering and from 7 weeks before

harvest significantly reduced lenticel spotting in ‘Kensington Pride’ mango, possibly by minimising stretching of the skin during fruit growth. In ‘Keitt’ and ‘Tommy Atkins’, LD decreased significantly as soil water potential decreased to -50 to -70 kPa (Cronje, 2009). Thus, reducing soil water potential in orchards with a history of LD can help reduce LD.

2.6.3 Nutrition

Nutrient balance influences many physiological disorders. Calcium (Ca) is an important mineral in the physiological and biochemical processes in plants and is particularly important in fruit disorders (Hewett, 2006), including skin disorders. For example, Dong et al. (2014) reported that Ca^{2+} deficiency can lead to the increased incidence of skin browning spot in ‘Huangguan’ pear fruit.

There is limited information on nutrient effects on mango skin disorders, but nutrient effects on flesh disorders such as soft nose and jelly seed are well documented (Cracknell Torres et al., 2002; Johnson and Hofman, 2009). In general, higher calcium and lower N treatments are associated with lessening of disorder severity. Disorders are also lessened by other factors that can influence the water, carbohydrate and mineral balance in the fruit, such as excess vegetative growth and water supply (Beverly et al., 1993). It is possible these factors also influence fruit susceptibility to USB.

2.6.4 Fruit bagging

Pre-harvest bagging can reduce disease and blemish. Chonhenchob et al. (2011) observed that bagging significantly reduced the severity of mango physical damage, skin browning, insect damage, and disease defects. Bagging ‘Sensation’ mango reduced fruit blemish (Shorter et al., 1997). Bagging with paper bags 70 days after flowering reduced the incidence of lenticel and browning blemish of ‘Apple’ mango (Mathooko et al., 2009). It is not known if bagging would affect USB development. It suggests that bagging treatment should be tested on ‘Honey Gold’ mango fruit to evaluate its potential effects on USB prevention.

2.7 Irradiation of mango fruit

Irradiation is recommended as an effective quarantine or phytosanitary treatment. The purpose of irradiation is to prevent the spread of regulated pests or diseases as it can kill or sterilize microorganisms or insects by damaging their DNA (FAO, 2008). In 1986, the U.S. Food and Drug Administration was amended to permit irradiation at specific doses to delay maturation, inhibit growth, and control disinfect foods, including vegetables and spices (FDA, 1986).

2.7.1 Sources

Sources of ionizing radiation include radioactive isotopes viz., ‘cobalt-60 or caesium-137’, ‘high-energy electrons from machine’, and ‘X-rays’ (FAO, 2008; Kilcast, 1995). Gray (Gy), which is the amount of ionizing radiation energy absorbed by the irradiated material in joules per kilogram of material, is the measurement unit (Huda, 1997). Hallman (2000) reported that X-ray radiation and gamma (γ) ray from isotopes can be used for pallet-loads. Additionally, electron beam radiation is limited to small products as it penetrates only a thin surface to just a few centimetres.

2.7.2 γ -Irradiation and mango fruit

The effectiveness of γ -irradiation in eliminating insect pests and controlling postharvest spoilage organisms on mangoes is reported in Table 2.2. For instance, Bustos et al. (2004) found that a dose of 150 Gy was sufficient to assure quarantine security against the infestation of fruit fly in ‘Ataulfo’, ‘Kent’, and ‘Keitt cultivars. Heather et al. (1991) determined that irradiation treatment with 74 - 101 Gy of ‘Kensington Pride’ mangoes was effective to inactivate eggs or larvae of *Bactrocera tryoni* (Froggatt) and *B. jarvisi* (Tryon). A dose of 100 Gy was sufficient to disinfest mango seed weevil in ‘Haden’ mangoes (Follett, 2001). Santos et al. (2010) showed that γ -radiation at 0.45 kGy inhibited the development of 'Tommy Atkins' mango rot caused by *Fusicoccum* incidence. A combination of dipping ‘Zebda’ mangoes in hot water (55 °C/5 min) with irradiation up to 1 kGy was an appropriate treatment for shelf life extension (El-Samahy et al., 2000).

For mangoes, irradiation has variable effects on quality characteristics. Moreno et al. (2006) found that irradiation at up to 1.0 kGy retained overall fruit quality attributes and extended the shelf life of ‘Tommy Atkins’ mangoes. Irradiations at medium (1.5 kGy) and high (3.1 kGy) dose levels were compromised by potential change to fruit texture; viz., softening. Sensory panelists indicated unacceptable overall quality, colour, texture, and aroma for fruit treated with 3.1 kGy. According to Cruz et al. (2012), starch degradation in Tommy Atkins mangoes was affected by irradiation. For example, fruit exposed to 0.4 and 1.0 kGy showed higher rates of starch hydrolysis than for non-irradiated fruit. However, there were no significant differences in total sugar content between treated and control fruit. Mitchell et al. (1990) found no significant effects on the carotene content in ‘Kensington Pride’ mango fruit irradiated at 0.75, 0.3 and 0.6 kGy.

Table 2.2 Effects of irradiation on insect pests and spoilage organisms.

Dose (Gy)	Variety	Effectiveness	Ref.
150	Ataulfo, Kent and Keitt	prevent adult emergence of Mexican fruit fly (<i>Anastrepha ludens</i>), West Indies fruit fly (<i>A. obliqua</i>), sapote fruit fly (<i>A. serpentina</i>), and Mediterranean fruit fly (<i>Ceratitidis capitata</i>)	Bustos et al. (2004)
74 - 101	Kensington	inactivate eggs or larvae of <i>Bactrocera tryoni</i> and <i>B. jarvisi</i>	Heather et al. (1991)
100	Haden	disinfest of mango seed weevil	Follett (2001)
450	Tommy Atkins	inhibit mango rot by <i>Fusicoccum</i>	Santos et al. (2010)
1000 + hot water treatment	Zebda	shelf life extension from 1 week to 2 weeks	El-Samahy et al. (2000)

2.8 Conclusions and perspectives

Although Australian mango production is not significant as compared to the world scale (AAG, 2006), mango is one of the major horticultural crops in Australia (AMIA, 2016). γ -Irradiation is an effective and approved post-harvest quarantine treatment in mango (Ducamp Collin et al., 2007; Follett, 2004). LD is the real issue facing by many cultivars in the world including Australian mango genotypes. Irradiation induced the development of LD in mango (Marques et al., 2016). However, limited study has been conducted on the effects of irradiation in term of appearance and internal quality attributes on importantly commercial Australian mango cultivars. Based on the literature review, the cultivar-dependant susceptibility of important Australian mangoes to LD should be evaluated in order to illustrate a better understanding the cultivar response to irradiation in terms of LD. In addition, the impact of irradiation on other ripening parameters, including external appearance attributes and flavour is an additional interest. Better visualization of the discolouration of entire lenticels provides a detailed understanding of morphology differences in LD.

The mechanism of the development of USB and RCD are still unclear. It is likely USB and RCD development may be related to different cell biology processes. USB is thought to be associated with the release of sap from resin canals (laticifers) within the exocarp. USB symptoms can develop

in response to physical stress (e.g. abrasion) and low temperature (e.g. < 12 - 14 °C) exposure during postharvest handling. Pre-harvest variables may also influence the occurrence of USB. In the context of this thesis, a postulated effect of harvest time over the diurnal cycle should be evaluated in regard to the propensity of fruit to develop USB. More importantly, the comparative anatomy of these browning disorders should be examined to receive a fuller understanding of the comparative biochemistry of these symptom expressions.

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CHAPTER 3. STABLE ISOTOPE DILUTION ASSAY (SIDA) AND HS-SPME-GCMS QUANTIFICATION OF KEY AROMA VOLATILES FOR FRUIT AND SAP OF AUSTRALIAN MANGO CULTIVARS

Abstract

Reported herein is a high throughput method to quantify in a single analysis the key volatiles that contribute to the aroma of commercially significant mango cultivars grown in Australia. The method constitutes stable isotope dilution analysis (SIDA) in conjunction with headspace (HS) solid-phase microextraction (SPME) coupled with gas-chromatography mass spectrometry (GCMS). Deuterium labelled analogues of the target analytes were either purchased commercially or synthesised for use as internal standards. Eight volatiles, hexanal, 2-carene, 3-carene, α -terpinene, p-cymene, limonene, α -terpinolene and ethyl octanoate, were targeted. The resulting calibration functions had determination coefficients (R^2) ranging from 0.93775 to 0.99741. High recovery efficiencies for spiked mango samples were also achieved. The method was applied to identify the key aroma volatile compounds produced by 'Kensington Pride and 'B74' mango fruit and by 'Honey Gold' mango sap. This method represents a marked improvement over current methods for detecting and measuring concentrations of mango fruit and sap volatiles.

3.1 Introduction

Mango fruits are highly desired by consumers because of their distinctive and evocative flavour as a function of their aroma and taste. Australia produces *ca.* 50,000 tonnes of mango fruit for domestic and export markets annually (AMIA, 2016). 'Kensington Pride', 'B74', 'Honey Gold' and 'R2E2' are the main cultivars grown commercially (Dillon et al., 2013). These Australian varieties are not grown widely overseas. Nonetheless, they are in high demand both domestically and as premium products in export markets due to their distinctive fruit odour profiles. The intense flavour of the fruit of Australian mango cultivars has been reported to be a crucial attribute that drives repeat purchases by consumers (Florkowski et al., 2014a). The mango industry has responded to this driver by focusing horticultural research on optimising agronomic and postharvest handling practices to maximise flavour production by fruit; for example, a 'guaranteed sweet' program (Florkowski et al., 2014a).

Several hundred volatile compounds have been identified in mango fruits (Andrade et al., 2000; Lalel and Singh, 2006; Lalel et al., 2003; MacLeod and de Troconis, 1982; MacLeod et al., 1988; MacLeod and Snyder, 1985; Pino et al., 2005; Shivashankara et al., 2006). The volatile profile

varies considerably among different mango cultivars. The selection of the eight targeted compounds was based on their presence in the headspace analysis and their contribution to the flavour of mango fruit. For example, α -terpinolene is considered a key aroma volatile responsible for the characteristic flavour of 'Kensington Pride', the most commonly grown cultivar in Australia (Lalel et al., 2003). It was also shown to be present in relatively high abundance in 'Cheiro', 'Chana', 'Bacuri', 'Cametá', 'Gojoba', 'Carlota', 'Coquinho' and 'Comum' mango fruits (Andrade et al., 2000). Other volatile compounds, including hexanal, 3-carene and limonene, are also considered important as they have been found in mango fruits at concentrations above their sensory thresholds (Pino, 2012). 3-Carene has a sweet and pungent odour and quantitatively represented the second main volatile compound produced by 'Kensington Pride' mango fruit (2003). In 'Kensington Pride' mango, α -terpinene was present as the major volatile component at the climacteric stage, while the highest concentrations of limonene and *p*-cymene were found during the pre-climacteric stage. 2-Carene was also highest among other monoterpenes at the over-ripe stage. Ethyl octanoate replaces α -terpinolene in dominance after fruit reach the fully ripe stage. Ethyl octanoate has a low odour activity value. Nonetheless, it is important in the aromatic equilibrium phase as the most abundant constituent, and is a potential indicator of 'Kensington Pride' mango fruit senescence.

Mango sap has two phases including an oily upper non-aqueous phase and a milky lower aqueous phase. It is interesting to note that the non-aqueous phase, which is rich in terpenoids, plays an important role in the plant's defence against bacteria and fungus (Negi et al., 2002). In this study, limonene was reported as the dominant terpenoid compound. Moreover, a 'sapburn' browning injury was largely due to sap exudation on the mango fruit surface (Loveys et al., 1992). This blemish reduces the visual quality of fruit and leads to the loss of market confidence. Terpinolene was abundant in the non-aqueous phase of 'Kensington Pride' mango sap that is evidently related with this browning (Loveys et al., 1992).

The purpose of the present study was to develop a rapid, accurate and precise analytical method utilising a combination of Stable Isotope Dilution Analysis (SIDA) and Head-space Solid phase microextraction (HS-SPME) in combination with gas chromatography mass spectrometry (GC-MS) to quantify characteristic aroma volatiles produced by fruits and sap of Australian mango cultivars to assist flavour optimisation research. This analytical work was conducted towards better understanding the role of sap components in antimicrobial activities and sapburn injury. Eight key aroma volatile compounds, hexanal, 2-carene, 3-carene, α -terpinene, *p*-cymene, limonene, α -terpinolene, ethyl octanoate, were targeted for ripe 'Kensington Pride' and 'B74' mango fruit and for 'Honey Gold' mango sap.

HS-SPME coupled with GC-MS is a relatively simple, rapid, inexpensive, and solvent-free technique for quantifying volatiles (Butkhup et al., 2011; Sánchez-Palomo et al., 2005). However, matrix effects and other potential sources of variation (e.g. MS detector, ionic strength and other matrices) can considerably impact the quantification of results, particularly their accuracy and precision (Yuan et al., 2013). SIDA provides an alternative approach to eliminate variability related to sample preparation and matrix effects and to improve the recovery of the analytes (Maraval et al., 2010). The combined analytical method of SIDA and HS-SPME-GC-MS is considered more accurate, precise, rapid and sensitive than other quantitative techniques, such as liquid–liquid extraction, especially for low volatile and polar odorants (Siebert et al., 2005).

Quantification methods have been developed previously for the analysis of volatiles in mango. The common examples are where analytical methods have been applied either to compare the most abundant aroma compounds between mango cultivars (Munafo et al., 2014; Nicola and Fontana, 2014; Pandit et al., 2009a; Pino et al., 2005), to measure differences in volatile aroma components between ripened and mature green mango fruits (Lalel et al., 2003; Pandit et al., 2009b; Shivashankara et al., 2006) or to quantify the major aroma-active compounds in tree-ripened mango fruit (Munafo et al., 2016). These methods only provide definite and accurate quantification results if recovery rates and response factors are measured for each compound against a suitable internal standard. Some studies (Loveys et al., 1992; John et al., 1999) demonstrate only the percentage composition of sap and not the quantitative concentrations of aroma volatiles in sap. Musharraf et al. (2016) quantified the volatile concentration of different Pakistani mango sap using gas chromatography triple quadrupole mass spectrometry. However, limited information is given regarding the effect of the sap matrix on the quantification. In most cases, such rigor in analytical method development has not been employed for the analysis of mango volatiles.

Ideally, in analytical methods development, the response of analytes should be calibrated to an internal standard that behaves very similarly both chemically and physically to the analyte in a given matrix. This is particularly important where SPME is employed due to variation in the adsorption properties of different analytes to the fibre. Techniques that overcome these challenges use either deuterium or carbon-13 labelled analogues of target analytes as internal standards. Deuterium labelled standards are commonly applied as they are readily accessible (Florkowski et al., 2014b) and relatively inexpensive as compared to ¹³C-labeled standards. This technique known as SIDA has been applied in combination with SPME for the analysis of volatiles in rice (Maraval et al., 2010), freeze-dried Cheonggukjang (Park et al., 2007), olive oil (Dierkes et al., 2012), juice and wine (Kotseridis et al., 2008), for which target analytes are successfully measured in concentrations ranging from µg/l down to ng/l. To date, however, the combination of SIDA and

HS-SPME-GC-MS has not been proven for quantitative analysis of key aroma volatiles in mango fruit and sap, which inspired our method development in this space.

3.2 Materials and methods

3.2.1 Materials

All solvents were HPLC grade and all reagents were purchased from Sigma-Aldrich, Australia. The standards used included hexanal (Sigma-Aldrich, Australia), 2-carene (Sigma-Aldrich, Australia), 3-carene (Sigma-Aldrich, Australia), α -terpinene (Sigma-Aldrich, Australia), *p*-cymene (Sigma-Aldrich, Australia), limonene (Sigma-Aldrich, Australia), α -terpinolene (Fluka, Australia) and ethyl octanoate (Sigma-Aldrich, Australia). d_{15} -Ethyl octanoate and d_{12} -hexanal were purchased from CDN Isotopes (CDN, Canada). Deuterium labelled internal standards d_6 - α -terpinolene and d_5 -limonene were synthesised in-house, as they were not commercially available.

3.2.2 Synthesis of isotopes

The commercially available methyl 4-methyl-cyclohex-3-ene-1-carboxylate was reacted with an excess of d_3 -methyl magnesium iodide to provide d_6 -terpineol (Ketter and Herrmann, 1990). Phosphoryl chloride mediated elimination of water (procedure for unlabelled terpineol given below) produced a mixture of d_6 -terpinolene and d_5 -limonene (Korstanje et al., 2012; Rudloff, 1961). Both compounds were partially separated by column chromatography on silver impregnated silica gel (Mander and Williams, 2016; Williams and Mander, 2001) to give 9:1 and 1:1 fractions of d_6 -terpinolene and d_5 -limonene. The fractions were characterised by high field ^1H and ^{13}C NMR as well as by Bruker AV400MHz mass spectrometry. For ^1H NMR, residual CHCl_3 in CDCl_3 was referenced at $\delta 7.26$ ppm. For ^{13}C NMR, the central peak of the CDCl_3 triplet ($\delta 77.0$ ppm) was used to reference chemical shifts. GC/MS was performed using a Shimadzu-17A GC equipped with J&W Scientific DB5 column (internal diameter 0.2mm; 30m) coupled to a Shimadzu QP5000 Mass Spectrometer (70eV).

d_6 - α -Terpinolene (Zou et al., 2008):

^1H NMR (CDCl_3 , 400 MHz): δ 1.65 (s, 3H, H₃₋₇), 1.99 (m, 2H), 2.30 (t, $J = 6.4$ Hz, 2H), 2.71 (m, 2H), 5.35 (m, 1H, H-2).

^{13}C NMR (CDCl_3 , 125 MHz): δ 23.4, 26.6, 29.5, 31.5, 120.8, 121.3, 127.6, 134.2.

d_5 -Limonene (Guo et al., 2016):

¹H NMR (CDCl₃, 400 MHz): δ 1.49 (m, 1H), 1.64 (br s, 3H, H₃₋₇), 1.73 – 2.12 (m, 4H), 2.30 (m, 1H), 2.71 (sextet, *J* = 2 Hz, 1H, H-4), 5.35 (m, 1H, H-2).

¹³C NMR (CDCl₃, 125 MHz): δ 23.4, 27.9, 30.6, 30.8, 41.0, 120.7, 133.8, 150.0.

Elimination of water from terpineol: A solution of α-terpineol (1.0 g, 6.5 mmol) in anhydrous pyridine (10 ml) was added in phosphoryl chloride (1.2 ml, 13.0 mmol) under a N₂ atmosphere at 0 °C. The reaction mixture was gradually warmed up to room temperature and stirred for 20 h. The reaction mixture was then added to cold water and extracted with petroleum spirit (2 × 10 ml). The organic phases were washed with a saturated solution of copper sulfate, dried over anhydrous magnesium sulfate and concentrated to give a yellow oil (0.75 g, 85%) consisting of terpinolene and limonene (1:1 by GC-MS) along with a small amount of chlorinated side product (8% by GC-MS).

3.2.3 Mango fruit

'B74' mango fruit were collected at commercial maturity (dry matter contents of 13.9, and 13.6%) from orchards near Dimbulah, Queensland, Australia (-17.149 °S, 145.111 °E) and Goodwood, Queensland, Australia (-25.10 °S, 152.37 °E), respectively. 'Kensington Pride' mango fruit were harvested at commercial maturity (dry matter contents of 13.1 and 13.6%) from orchards near Mareeba, Queensland, Australia (-16.992 °S, 145.422 °E) and Childers, Queensland, Australia (-25.17 °S, 152.17 °E), respectively. The dry matter of mango fruit was analysed destructively as described in Appendix 3. Thirty biological fruit replicates with three technical replicates per biological replicate were used for each 'B74' mangoes and 'Kensington Pride' mangoes. All fruit were de-stemmed in Mango Wash[®] (Septone, ITW AAMTech, Australia) for 2 min and graded for uniform quality as per commercial procedures. 'Nam Dok Mai' mango fruit for method validation were collected from the major commercial market at Rocklea near Brisbane, Queensland, Australia (-27.28 °S, 153.03 °E). The fruit were packed as a single layer into cardboard trays with plastic liners and transported within 24 h by airplane and / or car to the Maroochy Research Facility postharvest laboratory near Nambour, Queensland, Australia. They were then treated with 10 µl/l ethylene for 2 d at 20 °C to trigger ripening as per commercial practice. Thereafter, the fruit were maintained at 20 °C until ripe. The flesh of one cheek of each fruit was then removed using a sharp knife. A vertical section of flesh from the proximal stem end to the distal fruit tip was diced into small pieces and stored in glass bottles at -20 °C for 2 - 3 months pending analysis. Prior to instrumental analysis, samples were thawed and blended with an Ultra-Turrax[®] (IKA, Germany) stick blender ready for sampling directly into headspace vials.

3.2.4 Mango sap

'Honey Gold' mango fruit were harvested at commercial maturity from an orchard near Katherine, Northern Territory, Australia (14.28 °S, 132.16 °E). Fruit with a 2-5 cm-long pedicel attached were carefully transported to a nearby packing shed. The pedicel was broken at the abscission zone and the fruit inverted to allow the sap to drain over 4 - 5 min. The sap was collected into glass vials. The sap from 20 fruit was collected for each replication. The vial opening was covered with a piece of aluminium foil and closed with an aluminium screw-on cap. The collected sap was stored at -20 °C pending analysis. The aqueous phase of sap (lower phase) was separated using a 5810 R centrifuge (Eppendorf, Germany) at 3000 rpm for 10 min and used in the validation.

Aliquots of 0.1 g of sap, either whole sap or aqueous sap lower phase, were dissolved in 10 ml of distilled water. The diluted samples were mixed well using a ball mill MM400 (Retsch GmbH, Germany) for 30s and then diluted a further 50 times with distilled water. A volume of 2.5 ml diluted sap was then added to a 20 ml HS-SPME vial (Merck, Australia) containing 2.5 ml of saturated sodium chloride (NaCl) solution and a magnetic stir flea (5 x 2 mm) for aroma analysis. Five biological replicates with two technical replicates per biological replicate were prepared for analysis.

3.2.5 Calibration and validation of method

The calibration and validation of method were determined using the method of Siebert et al. (2005) with some modifications. Standard solutions were prepared using analytical reagent grade chemicals. A combined stock solution which contained each of the target analytes (viz., hexanal, 2-carene, 3-carene, α -terpinene, *p*-cymene, limonene, α -terpinolene and ethyl octanoate) in ethanol (Merck, Australia) was also prepared. Solutions were always made in duplicate from separately weighed reagents to ensure precision in their preparation. The stock concentration of target analytes were approximately 0.5 g/l (hexanal and *p*-cymene), 0.5 g/l (2-carene), 2 g/l (3-carene and α -terpinene), 10 g/l (limonene) and 20 g/l (α -terpinolene).

In duplicate, water, mango flesh and aqueous sap lower phase were spiked with increasing levels of the combined stock solution of analytes so that the resulting concentrations in water were approximately 0, 1.5, 3, 6, 15, 30, 60, 150, 300 and 600 μ g/l for hexanal and *p*-cymene, 0, 0.5, 1, 2, 5, 20, 50, 100, 200 and 400 for 2-carene, 0, 5, 10, 20, 50, 100, 200, 500, 1000 and 2000 μ g/l for 3-carene, α -terpinene and ethyl octanoate, 0, 25, 50, 100, 250, 500, 1000, 1500, 5 000 and 10 000 μ g/l for limonene, and 0, 50, 100, 200, 500, 1000, 2000, 5000, 10 000 and 20 000 μ g/l for α -terpinolene. A dilution of mango flesh of 1:400 was spiked with different amounts of α -terpinolene (0, 50, 100,

200, 500, 1000, 2000, 5000, 10 000 and 20 000 $\mu\text{g/l}$) in order to have a low α -terpinolene concentration for optimising the MS signal of α -terpinolene. The solutions were prepared in SPME vials for analysis ($n = 10 \times 2$). The concentrations of analytes in the samples were calculated from the peak area ratios for the unlabelled and labelled compounds versus the concentration ratio. Validation parameters including the calibration relative standard deviation (RSD) and recovery were calculated in spiked concentration levels of each standard compound. The limit of detection (LOD) and limit of quantification (LOQ) were calculated on the basis of $3.3\alpha/S$ and $10\alpha/S$, respectively (Musharraf et al., 2016). α is the standard deviation of the y-intercepts and S is the slope of the calibration curve.

3.2.6 Headspace sampling of volatiles for analysis

A sample of either 2.5 g homogenized mango flesh or 2.5 ml aqueous solution of standards was added to a 20 ml SPME vial (Merck, Australia) containing 2.5 ml of saturated sodium chloride (NaCl) solution and a magnetic stirrer flea (5 x 2 mm). Vials were immediately sealed with a screw cap fitted with a silicone/PTFE septum. Subsequently, 100 μl of combined internal standard solution was injected through the septum and the vial was shaken well. The deuterated internal standards were added at concentrations of 30 $\mu\text{g/l}$ for d_{12} -hexanal, 50 $\mu\text{g/l}$ for d_{15} -ethyl octanoate and 106 $\mu\text{g/l}$ for both d_6 - α -terpinolene and d_5 -limonene as equivalent to the amount of mango flesh in the vial. The vial and its contents were heated to 40 $^{\circ}\text{C}$ with stirring at 250 rpm for 2 min followed by extraction with a divinylbenzene/carboxen/polymethylsiloxane (DVB/CAR/PDMS, 'grey', 1 cm) 50/30 μm fibre (Supelco, USA) which was exposed to the headspace for 30 min. The SPME fibre was injected into the Programmable Temperature Vaporizing (PTV) inlet (Gerstel, Germany) set at 200 $^{\circ}\text{C}$ in splitless mode and the fibre was desorbed for 8 min.

3.2.7 Instrumental analysis of volatiles

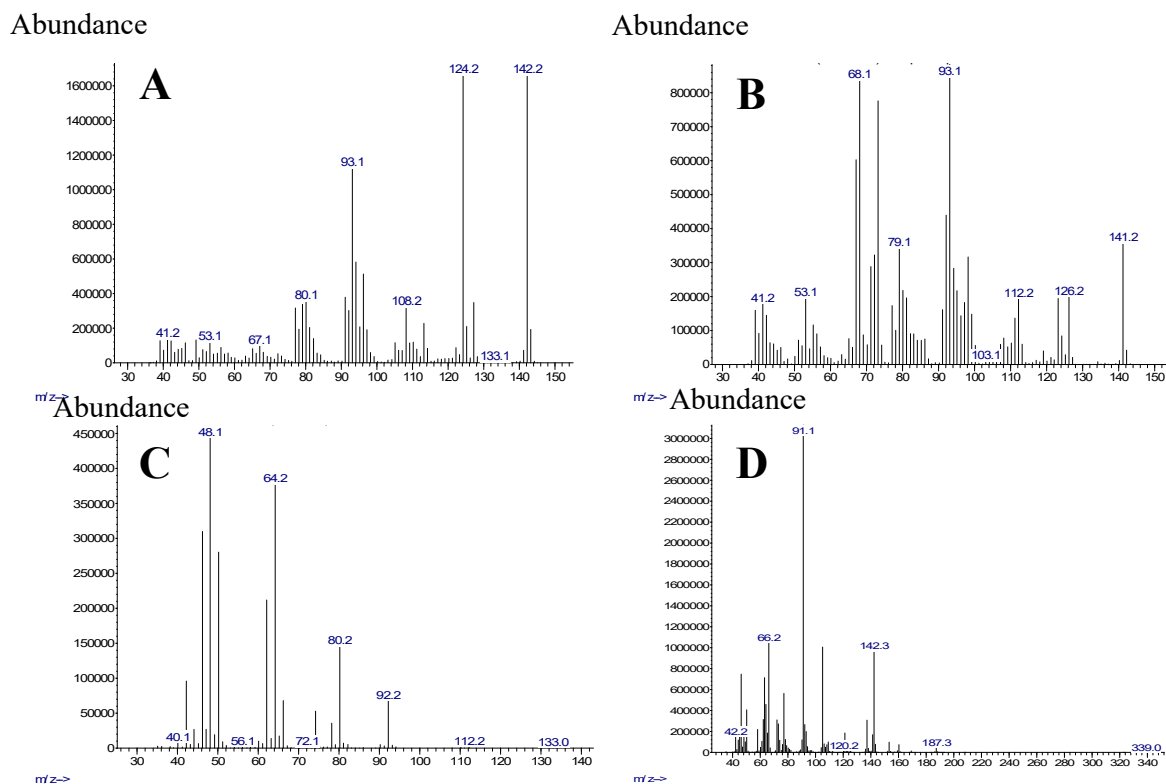
An Agilent 6890 gas chromatograph (Agilent Technologies, USA) equipped with a Gerstel MPS2XL multi-purpose sampler and coupled to a 5975N mass selective detector was used for the analysis of samples. The instrument was controlled by and the data analysed with MSD Chemstation E. 02. 02. 1431software (Agilent). The gas chromatograph was fitted with a ~ 30 m x 0.25 mm fused silica capillary ZB-5ms column (Phenomenex, Australia) with 0.25 μm film thickness. Helium carrier gas (BOC Gas) using a linear velocity of 44 cm/s with a constant flow rate of 1.5 ml/min was used. The pressure was 75.7 kPa and total flow was 70.5 ml/min. The oven had an initial temperature of 40 $^{\circ}\text{C}$ that was held for 2 min, increased to 80 $^{\circ}\text{C}$ at 2 $^{\circ}\text{C}/\text{min}$ and then to 220 $^{\circ}\text{C}$ at 40 $^{\circ}\text{C}/\text{min}$ and held for 5 min. The PTV inlet was fitted with a 0.75 mm borosilicate glass

SPME inlet liner (Agilent). The mass spectrometer quadrupole temperature was set at 230 °C, the source was set at 250 °C, and the transfer line at 280 °C. Ion electron impact spectra at 70 eV were recorded in selective ion monitoring (SIM) or scan (35-350 m/z) mode.

3.3 Results and Discussion

3.3.1 Isotope synthesis

The mass spectra of the internal standard compounds are characterized (Figure 3.1).



*d*₆- α -Terpinolene: *m/e* 142 (M^+), 127 ($M-CH_3$), 124 ($M-CD_3$), 93 ($M-C_3D_6H$).

*d*₅-Limonene: *m/e* 141 (M^+), 126 ($M-CH_3$), 123 ($M-CD_3$), 93 ($M-C_3D_5H$).

*d*₁₂-Hexanal: *m/e* 112 (M^+), 64 ($M-C_2D_4O$), 48 ($C_2D_4O^+$).

*d*₁₅-Ethyl octanoate: *m/e* 187 (M^+), 142 ($M-C_2H_5O$), 91 ($C_4D_3H_5O_2^+$).

Figure 3.1 Mass spectra and possible fragmentations of the internal standard compounds *d*₆- α -terpinolene (A) and *d*₅-limonene (B) as synthesised in-house and commercial *d*₁₂-hexanal (C) and *d*₁₅-ethyl octanoate (D).

The NMR data for d_6 -terpinolene matched those previously reported (Zou et al., 2008), except for the following observations: The signals for CH₃-9 and CH₃-10 were absent in the ¹H spectrum of d_6 -terpinolene and C-9 and C-10 appeared as complex multiplets slightly upfield of the reported shifts (Zou et al., 2008) in undeuterated terpinolene, as would be expected for C²H₃ moieties. The NMR spectral data obtained for d_5 -limonene was also in agreement with the literature values (Bollen and Emond, 2014), except that the CH₂-9 methylene and CH₃-10 methyl were largely absent from the ¹H NMR and the signals for C-9 and C-10 were vastly diminished in the ¹³C NMR spectrum. Analysis of the molecular ion region in the mass spectrum of d_6 -terpinolene indicated that it was 96% d_6 and 4% d_5 , and lower isotopomers were not observed. Similar analysis of d_5 -limonene indicated that it was 95% d_5 and 5% d_4 , and lower isotopomers were not observed.

3.3.2 Mango volatile analysis method and validation

Table 3.1 Analysis parameters for determination using SIM of eight important aroma volatiles.

Internal standard	t_R^a (min)	Target ion m/z	Qualifier ions m/z (%) ^b	Target compounds	RT (min)	Target ion m/z	Qualifier ions m/z (%) ^b
d_{12} -Hexanal	5.51	64	62 (46) 80 (46) 92 (6)	Hexanal	5.71	56	57 (70) 82 (22) 67 (11)
d_6 - α -Terpinolene	21.12	142	124 (98) 93 (71) 108 (18)	2-Carene	15.41	93	121 (93) 91 (84) 136 (52)
			124 (98) 93 (71) 108 (18)	3-Carene	16.01	93	91 (41) 77 (30) 79 (27)
			124 (98) 93 (71) 108 (18)	α -Terpinene	16.64	93	121 (103) 136 (89) 91 (55)
			124 (98) 93 (71) 108 (18)	p -Cymene	17.22	119	134 (27) 103 (5) 117 (16)
d_5 -Limonene	17.29	141	126 (56) 112 (54) 100 (14)	Limonene	17.48	136	121 (121) 107 (109) 93 (345)
d_6 - α -Terpinolene	21.12	142	124 (98) 93 (71) 108 (18)	α -Terpinolene	21.37	136	93 (160) 121 (198) 91 (87)
d_{15} -Ethyl octanoate	24.20	91	105 (32) 142 (29) 121 (6)	Ethyl octanoate	24.32	88	101 (38) 127 (29) 115 (9)

^a Retention time.

^b This % represents the associated ions relative % to the target ion shown in the previous column, not necessarily the base peak.

An accurate and precise analytical method involving a combination of HS-SPME-GCMS and SIDA was developed using selected ion monitoring (SIM) to quantify important aroma volatiles present in Australian mangoes. The concentration ranges targeted for each analyte were selected by considering both the sensorially active range of each compound by referring to the odour sensory threshold concentrations and the indicative concentration range as previously reported for that compound in mango (Table 3.5). The labelled internal standard used to quantify each target analyte, respective retention time and the target and qualifier ions used for SIM and relative percentages are given in Table 3.1. Schmarr et al. (2012) reported an inverse isotope effect whereby the heavier deuterated compounds elute prior to their non-labelled counterparts as was also observed in our study.

The precision of the method was thoroughly validated at various levels in mango fruit and sap matrices. Duplicate standard addition calibration equations were developed in water, mango flesh and sap matrices (Table 3.2) for each of the eight target volatile compounds. Separate standard addition calibration curves were created for mango fruit using 'Nam Dok Mai' fruit and mango sap using the non-aqueous sap phase of 'Honey Gold' mango. These were selected because of their naturally lower profile of these aroma volatiles.

The calibration equations developed for 'B74' and 'Kensington Pride' mango flesh, including their range of linearity and the coefficients of determination (R^2) achieved, are presented in Table 3.2. In general, the LOD and LOQ were relatively lower than its odour threshold. Therefore, the developed method showed very good sensitivity of detection. The calibration curves showed good linearity over the reported concentration range for all compounds with good correlation (R^2) from 0.93775 to 0.99741. The largest calibration range developed was for α -terpinolene at 50-20,200 $\mu\text{g/l}$ due to its relatively higher odour sensory threshold at 200 $\mu\text{g/l}$ and the higher concentration as is expected in mango (Lalel et al., 2004). The lowest calibration range developed was for *p*-cymene at 1.3-1,060 $\mu\text{g/l}$ since it has a much lower odour threshold and was expected to be found in mango at much lower concentration ranges. At higher concentrations in the spiked standards, *p*-cymene co-eluted with *d*₅-limonene which limited the range of calibration to \sim 550 $\mu\text{g/l}$. Nevertheless, this was still higher than the reported sensory threshold at 11.4 $\mu\text{g/l}$. The minimum LOD and LOQ were obtained for *p*-cymene at 0.824 and 2.498 $\mu\text{g/l}$, respectively. The validation was performed to confirm the calibration developed in the water matrix was suitable for application (Table 3.2). Generally, the method was accurate and precise for all eight compounds within the mango matrix. After this

verification, the analytes in mango flesh and sap samples were quantified using calibration curves obtained from a water matrix.

Table 3.2 Summary of calibration and main validation parameters for eight mango volatiles in water, mango flesh and mango sap matrices.

Analyte	Calibration		R^2	LOD ($\mu\text{g/l}$)	LOQ ($\mu\text{g/l}$)
	range ($\mu\text{g/l}$)	Calibration equation			
In a water matrix					
Hexanal	1.4-578	$y = 1.809 x$	0.99741	1.058	3.205
3-Carene	5.2-1,077	$y = 2.260 \times 10^{-1}x - 4.347 \times 10^{-2}$	0.95909	2.284	6.922
α -Terpinene	5.1-1,211	$y = 8.441 \times 10^{-1}x - 3.403 \times 10^{-2}$	0.93775	3.038	9.205
<i>p</i> -Cymene	1.3-548	$y = 3.158 x - 1.124 \times 10^{-1}$	0.97666	0.824	2.498
Limonene	25-5,041	$y = 1.919 \times 10^{-1}x - 1.315 \times 10^{-1}$	0.94393	12.870	38.999
α -Terpinolene	50-20,214	$y = 3.432 \times 10^{-2}x - 6.503 \times 10^{-2}$	0.97716	32.842	99.520
Ethyl octanoate	5.4-2,172	$y = 2.389 \times 10^{-1} x + 3.592 \times 10^{-1}$	0.98584	5.572	16.884
In a mango flesh matrix					
Hexanal	1.4-724	$y = 7.744 \times 10^{-1}x$	0.99249	1.939	5.875
3-Carene	5.2-2,300	$y = 3.695 \times 10^{-1}x + 1.347$	0.94224	0.379	1.149
α -Terpinene	5.1-2,220	$y = 1.601x + 9.669 \times 10^{-1}$	0.97366	5.721	17.336
<i>p</i> -Cymene	1.3-1,060	$y = 4.526x + 1.650$	0.90798	3.641	11.032
Limonene	25-10,082	$y = 5.226 \times 10^{-1}x + 5.637 \times 10^{-1}$	0.93014	3.391	10.275
α -Terpinolene	50-20,214	$y = 4.001 \times 10^{-2}x - 9.522 \times 10^{-2}$	0.98950	28.373	85.979
Ethyl octanoate	5.4-2,178	$y = 8.366 \times 10^{-1}x + 1.166 \times 10^{-2}$	0.98859	21.906	66.383
In a mango sap matrix					
Hexanal	1.4-724	$y = 3.769 \times 10^{-1}x$	0.99142	2.362	7.157
3-Carene	5.2-1,150	$y = 6.605 \times 10^{-2}x + 1.819 \times 10^{-2}$	0.89069	1.110	3.363
α -Terpinene	5.1-2,220	$y = 6.198 \times 10^{-1}x + 2.010 \times 10^{-1}$	0.90845	4.246	12.866
<i>p</i> -Cymene	1.3-530	$y = 2.346x - 2.098 \times 10^{-2}$	0.90143	7.025	21.288
Limonene	25-10,082	$y = 1.920 \times 10^{-1}x - 5.211 \times 10^{-2}$	0.90406	1.828	5.540
α -Terpinolene	50-20,214	$y = 6.979 \times 10^{-3}x + 4.775 \times 10^{-2}$	0.99336	17.505	53.045
Ethyl octanoate	5.4-1,122	$y = 5.139 \times 10^{-1}x + 2.972 \times 10^{-2}$	0.99632	16.656	50.474

Similarly, the validation curves constructed using the aqueous sap phase were linear throughout the calibration range, with good correlation (R^2) which varied from 0.89069 to 0.99142 (Table 3.2). As mango sap is a complex and variable matrix to quantify accurately all these compounds versus their labelled internal standards, the correlation efficiency (R^2) was lower for 3-carene (0.89069).

The recoveries were also demonstrated by spiking a known amount of analyte into the ‘Nam Dok Mai’ mango matrix at five replicate additions for each analyte. Recoveries of the spiked concentrations are reported in Table 3.3. All the relative standard deviations (RSD) were < 11.3% for all eight spiked analyte compounds.

Table 3.3 Recoveries and relative standard deviation (RSD) of compounds spiked into a mango fruit.

Samples with spiked compounds	Matrix spike concentration ($\mu\text{g/l}$)	Recovery (%)	RSD (%)
Hexanal	290	107-121	8.8
2-Carene	90	98-131	11.3
3-Carene	909	95-104	3.9
α -Terpinene	854	97-119	7.9
<i>p</i> -Cymene	419	72-88	8.2
Limonene	4,102	80-97	7.7
α -Terpinolene	4,028	99-114	5.9
Ethyl octanoate	898	86-106	8.0

$n = 5$ (replication)

3.3.3 Analysis of key aroma volatiles in mango fruits

A range of mango fruits within the two varieties were collected, ripened and prepared for analysis so that the analytical method could be validated for its applied usefulness. A summary of the concentration data for the eight key aroma volatile compounds produced by ripe ‘B74’ and ‘Kensington Pride’ mango fruit is reported in Table 3.4. Overall, the variety ‘Kensington Pride’ produced the highest average concentration of α -terpinolene at 19,719 $\mu\text{g/l}$, which is in agreement with published data (Lalel et al., 2004). ‘B74’ had an average terpinolene content (11,272 $\mu\text{g/l}$) lower than ‘Kensington Pride’ mango fruit. 3-Carene and limonene were the second and third, respectively, most abundant volatiles produced by both ‘B74’ and ‘Kensington Pride’ fruits. 3-Carene and limonene have been reported to be responsible for a ‘resinous, sweet, leafy, green,

pungent' (MacLeod and Snyder, 1985; Pino, 2012; Shivashankara et al., 2006) and 'citrus-like' aroma (Pino, 2012). *p*-Cymene, with a characteristic 'herbal or minty' odour (MacLeod and Snyder, 1985), was present in 'B74' and 'Kensington Pride' fruits at average concentrations of 12 and 14 µg/l, respectively, and above its odour perception threshold. The lowest average concentration of ethyl octanoate was measured in 'Kensington Pride' at 0.5 µg/l, while the lowest average contents of hexanal and ethyl octanoate were found in 'B74' mango fruit at 6.0 and 0.6 µg/l, respectively. There was a variation of the aroma volatile composition between the parts of fruit of 'Kensington Pride' mangoes (Lalel et al., 2003). Therefore, the application of a glass chamber integrated into a GC would be useful to measure the volatiles of whole fruit.

Table 3.4. Aroma compounds concentrations in ripe 'B74' and 'Kensington Pride' mango fruits.

Compounds	Concentration (µg/l)				
	Mean	Min	Max	S.D. ^a	CV ^b
In 'B74' fruit					
Hexanal	6	3	13	2	41
2-Carene	29	0.0	168	37	130
3-Carene	929	34	4,122	986	106
α-Terpinene	23	1	72	19	84
<i>p</i> -Cymene	12	2	25	6	55
Limonene	216	89	769	155	72
α-Terpinolene	11,272	329	49,122	11,966	106
Ethyl octanoate	0.6	0.2	1.4	0.3	51
In 'Kensington Pride' fruit					
Hexanal	54	14	188	47	87
2-Carene	31	0.0	173	41	132
3-Carene	383	33	1,777	418	109
α-Terpinene	43	2	202	47	109
<i>p</i> -Cymene	14	5	40	9	66
Limonene	264	93	881	198	75
α-Terpinolene	19,719	672	87,900	21,624	110
Ethyl octanoate	0.5	0.0	2.5	0.6	108

^a Standard deviation. ^b Coefficient of variation.

n.d., not detected; *n* = 30 (replication).

In order to compare the odour contribution and relative importance of each compound to the aroma of 'B74' and 'Kensington Pride', the odour activity value (OAV) was calculated for each compound (Table 3.5). The OAV is the ratio between the concentration of an aroma compound and its odour threshold (Pino and Febles, 2013). Reported odour sensory thresholds and the nature of the odour of each target analyte are also shown in Table 3.5. The compounds α -terpinolene, 3-carene, *p*-cymene and limonene were found to be odour active in all mango fruits analysed (i.e., OAV>1).

Table 3.5 Odour activity values in ripe 'B74' and 'Kensington Pride' mango fruits ($n = 30$).

Compounds	Odour description	Odour threshold ^a ($\mu\text{g/l}$)	OAV ^b				
			Mean	Min	Max	S.D.	CV
In 'B74' fruit							
Hexanal	Green, fatty ¹	4.5 ¹	1.31	1	3	1	41
2-Carene	Orange like ⁶	4000	0.01	0	0.04	0.01	130
3-Carene	Leafy ²	5 ¹	186	7	824	197	106
α -Terpinene	Citrus, lemon-like ³	85 ^{4,5}	0	0	1	0	84
<i>p</i> -Cymene	Herbal, minty ⁶	11.4 ⁷	1	0	2	1	55
Limonene	Citrus-like ¹	210 ¹	1	0	4	1	72
α -Terpinolene	Piney ¹	200 ¹	56	2	246	60	106
Ethyl octanoate	Slightly nutty coconut ⁶	194 ⁷	0	0	0	0	51
In 'Kensington Pride' fruit							
Hexanal	Green, fatty ¹	4.5 ¹	12	3	42	10	87
2-Carene	Orange like ⁶	4000	0.01	0	0.04	0.01	132
3-Carene	Leafy ²	5 ¹	77	7	355	84	109
α -Terpinene	Citrus, lemon-like ³	85 ^{4,5}	1	0.02	2	1	109
<i>p</i> -Cymene	Herbal, minty ⁶	11.4 ⁷	1	0	4	1	66
Limonene	Citrus-like ¹	210 ¹	1	0	4	1	75
α -Terpinolene	Piney ¹	200 ¹	99	3	439	108	110
Ethyl octanoate	Slightly nutty coconut ⁶	194 ⁷	0.003	0	0.013	0.003	108

^a Each sensory (odour) threshold reported was determined in a water matrix.

^b Odour activity values were calculated by dividing the concentrations by the respective odour threshold.

¹ Pino (2012); ² Shivashankara et al. (2006); ³ Costa et al. (2008); ⁴ Du et al. (2010); ⁵ Vincente et al. (2014); ⁶ MacLeod and Snyder (1985); ⁷ Pino and Mesa (2006).

The application of OAV's to compare and contrast odour volatile contribution has been reported previously (Du et al., 2011). However, the method utilised in that study could not approach the OAV of some compounds due to the lack of a suitable internal standard to detect aroma volatiles present at low levels. The accuracy and precision realised in the present work allow for meaningful comparisons to be made using OAV's, because the concentration data collected is sensitive and reliable.

3.3.4 Analysis of key aroma volatiles in mango sap

The SIDA HS-SPME-GCMS method was applied to analyse 'Honey Gold' mango fruit sap with some modification in the preparation steps, namely a dilution of a small amount of sap to reduce the initial concentration of all volatiles. Following that modification, the ensuing steps for sap analysis were the same as were utilised for mango flesh. Thereby, this modified SIDA HS-SPME-GCMS method is easily employed to quantify these analytes in different matrices, such as 'Honey Gold' mango sap (Table 3.6).

Table 3.6. Aroma compounds concentrations in 'Honey Gold' mango sap.

Compounds	Concentration (mg/g) of 'Honey' mango sap				
	Mean	Min	Max	S.D. ^a	CV ^b
Hexanal	n.d.				
2-Carene	0.6	0.4	0.9	0.2	33.1
3-Carene	4.2	3.0	5.7	1.2	28.8
α -Terpinene	1.5	1.0	2.1	0.5	30.7
<i>p</i> -Cymene	0.1	0.1	0.2	0.0	30.7
Limonene	1.1	0.5	1.6	0.5	45.9
α -Terpinolene	59.0	36.9	73.9	18.0	30.5
Ethyl octanoate	n.d.				

^a Standard deviation. ^b Coefficient of variation.

n.d., not detected. *n* = 5 (replication)

Quantitative data of the predominant volatile compounds in 'Honey Gold' mango sap are shown in Table 3.6. α -Terpinolene was the most abundant component in 'Honey Gold' mango sap. It is most likely responsible for mango sapburn, being present at levels notably higher than its perception threshold (Loveys et al., 1992). A relatively higher concentration of terpinolene (70 mg/g) was found in 'Kensington' mango sap (Loveys et al., 1992), while an average amount of 59 mg/g was found in 'Honey Gold' mango sap. Terpinolene was significantly more abundant in 'Honey Gold' mango sap than in 'B74' and 'Kensington Pride' mango fruit (Table 3.6). The average concentrations of 3-carene and of limonene in 'Honey Gold' sap varied from 3.0 to 5.7 mg/g and from 0.5 to 1.6 mg/g, respectively. α -Terpinene was found at average concentration of 1.5 mg/g. The mean content of β -cymene was 0.1 mg/g. It is notable that hexanal and ethyl octanoate were not detected in the sap samples. Considerable differences in major volatile constituents in sap exist between mango cultivars. For example, there were significant variations in the volatile sap components of Australian and Indian mango cultivars. Terpinolene and 3-carene were found to be the major components in Australian 'Kensington' and 'Irwin' (Loveys et al., 1992) fruit sap, whereas neither of these compounds was identified in any of the Indian cultivars (John et al., 1999). For this study, SIDA HS-SPME-GCMS served as a rapid, accurate and precise method for determination of key aroma compounds in sap.

3.4 Conclusion

An accurate and precise method for the analysis of key volatiles was developed in a model system and subsequently validated with both Australian mango fruit and sap. The method of combining SIDA with HS-SPME-GC-MS was found to be highly sensitive and allowed high throughput of samples. It produced calibrations with high coefficients of determination and excellent linearity across a range of concentrations relevant to mango flavour. This method represents a pronounced improvement over current methods for detecting and measuring the concentration of mango fruit and sap volatiles. It could be of wider benefit in future studies aimed at exploring and optimising the flavour of commercial mango varieties, their parent lines and also wild types.

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CHAPTER 4. γ -IRRADIATION EFFECTS ON APPEARANCE, PHYSICO-CHEMICAL PROPERTIES AND AROMA OF FOUR AUSTRALIAN MANGO FRUIT CULTIVARS

Abstract

γ -Irradiation is recommended as an effective quarantine or phytosanitary treatment. This study determined the response of four Australian cultivars ('B74', 'Honey Gold', 'Kensington Pride' and 'R2E2') to γ -irradiation. Fruit were harvested from north and south east Queensland during the 2012/2013 and 2013/2014 seasons. In both seasons, γ -irradiation of green mature fruit at 0.5 (commercial dose) and 1.0 kGy (twice the commercial dose) generally retarded softening in the early stages of ripening, but had little effect after 7 - 9 d of shelf life. γ -Irradiation also retarded the loss of green colour that is typically associated with ripening, resulting in both seasons in ripe fruit with less yellow skin colour. 'Honey Gold' was the least affected by irradiation, with 'Kensington Pride' generally having the least yellow colour at ripe. Irradiation at 0.5 kGy significantly ($P \leq 0.05$) increased lenticel discolouration (LD), with generally little increase between 0.5 and 1.0 kGy. 'Honey Gold' had the least LD in both seasons. Irradiation did not affect total soluble solids (TSS) at ripe, but reduced the titratable acidity (TA) decrease during ripening. There were significant reductions ($P \leq 0.05$) of the concentrations of all of the measured volatiles in 'Kensington Pride' and 'R2E2' exposed 0.5 kGy and 1.0 kGy as compared to no irradiation, while there were no considerable effects ($P > 0.05$) of irradiation on the volatile contents of 'B74' and 'Honey Gold'. It is possible that irradiation may also diminish the flavour of 'Kensington Pride' and 'R2E2' by increasing titratable acidity and reducing volatiles concentrations at ripe.

4.1 Introduction

Mango (*Mangifera indica* L.) is one of the most popular tropical fruits in the world, second only to banana (AAG, 2006). In Australia, mango is an economically significant domestic horticultural crop. The average production between 2011 and 2016 was 53,500 tonnes (AMIA, 2016). 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' are the major commercial cultivars in Australia (Dillon et al., 2013). Other cultivars cultivated in Australia include 'Brook', 'Haden', 'Irwin', 'Kent', 'Keitt' and 'Palmer' (AMIA, 2014; Bally et al., 2000).

Many countries impose strict regulatory and physical barriers to the importation of fruit that are susceptible to infestation by economically important quarantined insect pests (Follett, 2009). Exposure to relatively low doses of gamma (γ) irradiation can be an effective postharvest treatment against pest presence (Ducamp Collin et al., 2007; Follett, 2004). γ -Irradiation of ‘Ataulfo’, ‘Kent’ and ‘Keitt mango fruits at up to 0.15 kGy can provide quarantine security against fruit flies such as *Anastrepha obliqua*, *Anastrepha serpentina*, *Anastrepha ludens* and *Ceratitis capitata* (Bustos et al., 2004). Similarly, a minimum dose of 228 Gy can disinfest ‘Red Fuji’ apple fruit of peach fruit moth (*Carposina sasakii*) (Zhan et al., 2014). The mango seed weevil (*Cryptorhynchus mangiferae* F.) can be eliminated upon application of γ -irradiation at 0.1 kGy (Follett, 2001). Also, Santos et al. (2015) reported that low dose irradiation (0.45 kGy) of ‘Tommy Atkins’ mango reduced the incidence of rot caused by *Lasiodiplodia theobromae*.

While γ -irradiation can be an effective quarantine treatment, there is concern by mango packers and shippers about potential negative effects on fruit appearance and eating quality. ‘Tommy Atkins’ mangoes treated with the relatively high dose of 3.1 kGy were rated by a sensory panel to exhibit unacceptable general visual quality, colour, texture and aroma as compared to non-treated control fruit (Moreno et al., 2006). Reyes and Cisneros-Zevallos (2007) reported that ‘Tommy Atkins’ mango fruit irradiated at high doses (≥ 1.5 kGy) expressed higher levels of peel scalding and void formations as compared to non-irradiated control fruit. γ -Irradiation at 1.0 kGy initiated pulp browning and softer texture of ‘Tommy Atkins’ mango fruit (Sabato et al., 2009). Exposure to γ -irradiation from 0.3 up to 10kGy reduced the loss of green peel colour during ripening and gave a relatively lighter pulp colour to Indian mango fruit cvs. ‘Dushehri’ and ‘Fazli’ after storage (Mahto and Das, 2013).

Although the effects of irradiation on fruit physicochemical properties and quality have been extensively studied (Lacroix et al., 1992; Lacroix et al., 1993; McLauchlan et al., 1989; Thomas and Janave, 1975), there is limited published information on the response of Australian mango cultivars to γ -irradiation on appearance in terms of LD and skin degreening. For example, ‘B74’ mango fruit showed a significant increase ($P \leq 0.05$) in LD incidence after exposure to 0.543 kGy (Hofman et al., 2010c). According to McLauchlan et al. (1989), irradiation at 0.3 and 0.6 kGy increased LD and decreased the rate of ripening-related green skin colour loss in ‘Kensington Pride’ mango fruit as compared to the control.

Aroma is an important fruit quality attribute. Nonetheless, the effects of irradiation on aroma volatiles have been studied on only a few mango cultivars (Blakesley et al., 1979; Gholap et al., 1990; Laohakunjit et al., 2006). Irradiation of ‘Alphonso’ mangoes at 0.25 kGy and ‘Kent’ mango fruit at 0.75 kGy did not affect the aroma volatile profile (Blakesley et al., 1979; Gholap et al., 1990). In contrast, irradiation of applied chitosan or non-chitosan ‘Chok Anan’ mango fruit at 0.3 kGy decreased terpene production after 30 d of storage at 13 °C as compared to the non-irradiated control (Laohakunjit et al., 2006). There has been no published work on the effects of irradiation on aroma production by Australian mango cultivars. Among Australian cultivars, there are relatively different aroma descriptions (Smyth et al., 2008; Sunarharum et al., 2007).

The aim of the present study was to investigate the effects of γ -irradiation on the appearance, physicochemical properties and aroma volatile production of four (‘B74’, ‘Kensington Pride’, ‘Honey Gold’, ‘R2E2’) commercially important mango cultivars in Australia. We hypothesised that irradiation would differentially induce LD, delay the loss of green skin colour, and diminish aroma volatile production among these four cultivars (Dillon et al., 2013). The effects of γ -irradiation at 0.5 (commercial dose) and 1.0 kGy (twice the commercial dose) were evaluated using fruit harvested from north Queensland and south east Queensland over two seasons.

4.2 Materials and methods

4.2.1 Plant material

The fruit of four mango (*Mangifera indica* L.) cultivars (‘B74’, ‘Honey Gold’, ‘Kensington Pride’, ‘R2E2’) were harvested on day 0 (designated) at commercial maturity (dry matter content: 13 - 19%) from orchards in north Queensland (NQ) and south east Queensland (SEQ), Australia over two seasons 2012/13 and 2013/2014 (Table 4.1 and 4.2). Fifty-five fruit of each cultivar were harvested to provide 15 replicate fruit per irradiation treatment and ten fruit for dry matter measurement.

Immediately after harvest, all fruit were de-stemmed in 2.5 g/l Mango Wash[®] (Septone, ITW AAMTech, Australia) solution for 2 min. They were taken to a nearby commercial packing house and passed over a packing line under standard commercial conditions that included fungicide treatment (0.55 ml/l Sportak[®], a.i. prochloraz, Bayer Crop Science, Australia), brushing, drying, and sorting (Hofman et al., 2010b). The fruit were then graded for uniform quality and size as per commercial procedures. They were packed into single layer cardboard trays with polyethylene liners. NQ fruit were air-freighted to Brisbane from NQ in 2 h and then transported from Brisbane airport

by car (ca. 22 °C) to the Ecosciences Precinct laboratory in Brisbane, Queensland, Australia (27.49 °S, 153.03 °E) in 1 - 2 h. Fruit harvested in SEQ were transported in an air-conditioned vehicle (ca. 22 °C) to the lab in 3 - 4 h.

Table 4.1 Harvest location and dry matter content of green mature ‘B74’, ‘Honey Gold’, ‘Kensington Pride’ and ‘R2E2’ mango fruit. Fruit were harvested from north Queensland (NQ) and south east Queensland (SEQ) in the 2012/2013 season. $n = 10$ except ‘R2E2’ from SEQ ($n = 6$).

Cultivar	Location	Dry matter (%)
‘B74’	Orchard 1, Dimbulah, NQ (7.149 °S, 145.111 °E)	13.5 ± 1.2
	Orchard 2, Childers, SEQ (25.17 °S, 152.17 °E)	13.8 ± 0.7
‘Honey Gold’	Orchard 3, Mareeba, NQ (16.992 °S, 145.422 °E)	19.6 ± 1.6
	Orchard 4, Childers, SEQ (25.17 °S, 152.17 °E)	19.0 ± 1.4
‘Kensington Pride’	Orchard 5, Mareeba, NQ (16.992 °S, 145.422 °E)	16.1 ± 1.6
	Orchard 6, Childers, SEQ (25.17 °S, 152.17 °E)	13.5 ± 1.2
‘R2E2’	Orchard 7, Dimbulah, NQ (17.149 °S, 145.111 °E)	17.4 ± 1.6
	Orchard 6, Childers, SEQ (25.17 °S, 152.17 °E)	15.3 ± 1.2

Table 4.2 Harvest location and dry matter content of green mature ‘B74’, ‘Honey Gold’, ‘Kensington Pride’ and ‘R2E2’ mango fruit ($n = 7$). The fruit were harvested from north Queensland (NQ) and south east Queensland (SEQ) in the 2013/2014 season.

Cultivar	Location	Dry matter (%)
‘B74’	Orchard 1, Dimbulah, NQ (17.149 °S, 145.111 °E)	13.9 ± 1.7
	Goodwood, SEQ (25.10 °S, 152.37 °E)	13.6 ± 1.2
‘Honey Gold’	Orchard 2, Dimbulah, NQ (17.149 °S, 145.111 °E)	18.2 ± 0.4
	Electra, SEQ (24.98 °S, 152.11 °E)	20.8 ± 1.2
‘Kensington Pride’	Mareeba, NQ (16.992 °S, 145.422 °E)	13.1 ± 0.4
	Childers, SEQ (25.17 °S, 152.17 °E)	14.2 ± 1.1
‘R2E2’	Orchard 2, Dimbulah, NQ (17.149 °S, 145.111 °E)	16.6 ± 0.8
	Childers, SEQ (25.17 °S, 152.17 °E)	15.3 ± 0.8

4.2.2 Treatments

At the laboratory, fruit were randomly assigned to treatment lots and labelled with a treatment number. Fruit of the different cultivars and for the various treatments were randomly assigned to

single layer cardboard mango trays. Dosimeters (Opti-chromic detectors FWT-200SN2162, Far West Technology Inc., USA) were placed in two diagonally opposite corners of each tray. The open trays were each covered with 4 mm-thick plywood lids lined with 15 mm-thick low density foam to prevent fruit and dosimeter movement. The lids were secured to trays with sticky tape.

For exposure to γ -irradiation, the fruit were transported within 1 h on the same day of arrival in the lab (day 1) by an air-conditioned (ca. 22 °C) car to Steritech in Narangba, Queensland. To ensure more consistent doses between trays, they were stood on their sides and positioned in a rack designed to fit on 1 m high bins. The fruit in their trays were exposed to 0 kGy (control), the commercial dose of about 0.5 kGy, or twice the commercial dose of about 1.0 kGy from a Cobalt 60 source within 1 - 2 h of arrival. The actual minimum, maximum and average doses recorded for each experiment are shown in Table 4.3 and 4.4.

Immediately after irradiation, the fruit were transported back to the laboratory. All fruit were then treated with 10 μ l/l ethylene for 2 d at 20 °C inside a closed ripening room. Thereafter, they were allowed to ripen at 20 °C and 90 - 100% RH in the absence of exogenous ethylene. Fifteen replicate fruit per irradiation treatment were used in this experiment. Individual fruit were the replicates.

Table 4.3 The average and minimum (min) and maximum (max) doses recorded for green mature mango fruit ($n = 15$) harvested from north Queensland (NQ) and south east Queensland (SEQ) in the 2012/2013 season and exposed to γ -irradiation at 0.5 (commercial dose) and 1.0 kGy (twice the commercial dose).

Farm location	Cultivar	Dose (kGy)			
		Target 0.5 kGy		Target 1.0 kGy	
		Average	Min-max	Average	Min-max
NQ	‘B74’ and ‘Kensington Pride’	0.548	0.525 - 0.568	1.040	1.001 - 1.073
	‘Honey Gold’	0.581	0.502 - 0.656	1.047	1.033 - 1.061
	‘R2E2’	0.548	0.525 - 0.568	1.040	1.001 - 1.073
	SEQ	‘B74’	0.541	0.506 - 0.653	1.037
	‘Honey Gold’	0.541	0.568 - 0.591	1.037	1.204 - 1.266
	‘Kensington Pride’	0.580	0.568 - 0.591	1.235	1.204 - 1.266
	‘R2E2’	0.580	0.506 - 0.653	1.235	1.025 - 1.049

Table 4.4 The average and minimum (min) and maximum (max) doses recorded for green mature mango fruit ($n = 15$) harvested from north Queensland (NQ) and south east Queensland (SEQ) in the 2013/2014 season and exposed to γ -irradiation at 0.5 (commercial dose) and 1.0 kGy (twice the commercial dose).

Farm location	Cultivar	Dose (kGy)			
		Target 0.5 kGy		Target 1.0 kGy	
		Average	Min-max	Average	Min-max
NQ	‘B74’, ‘Honey Gold’, ‘Kensington Pride’ and ‘R2E2’	0.342	0.320 - 0.381	0.833	0.824 - 0.846
SEQ	‘B74’, ‘Honey Gold’, ‘Kensington Pride’ and ‘R2E2’	0.520	0.493 - 0.577	1.043	1.003 - 1.079

4.2.3 Quality assessments

In the 2012/2013 mango season, fruit were assessed for firmness (subjective only), skin colour (subjective and objective) and LD (subjective only) on day 0 and then every 2 d after irradiation. Once ripe (based on firmness; see below), the fruit were then assessed for total soluble solids (TSS), titrable acidity (TA), total chlorophyll (Chls), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids.

In the 2013/2014 mango season, fruit were assessed for firmness (subjective and objective), skin colour (subjective and objective) and LD (subjective) on day 0 and then every 2 d after irradiation. The fruit at ripe were then assessed for TSS, TA, total skin and flesh Chls, Chl *a*, Chl *b* and carotenoids as well as aroma volatiles.

Fruit firmness

Fruit firmness was assessed using the following subjective hand firmness rating scale: 0 = hard, 1 = rubbery, 2 = sprung, 3 = firm soft and 4 = soft (Hofman et al., 2010a). Fruit firmness was also determined objectively with a Sinclair firmness tester (Sinclair International Ltd., UK) for non-destructive displacement (Shmulevich et al., 2003).

Skin colour development

Skin colour was objectively measured using a CR 400 Chroma Meter (Konica Minolta, Japan). Colour was determined with two measurements per fruit (stem end and central region) and repeated every second day at the same position. Values were obtained for lightness (L) and chroma (C) and hue angle (h°) as calculated using 'a' and 'b' values by LabLchConversions (Semba et al., 2002). L value represents the lightness of the colour with 0 for black and 100 for white. C data represents colour saturation for h° representing colour space chroma position where red-purple is an angle of 0° , yellow is 90° , bluish-green is 180° , and blue is 270° (McGuire, 1992; Semba et al., 2002).

Skin colour was also subjectively rated using the following scale: 1 = 0 - 10% of the non-blushed skin with yellow colour, 2 = 11 - 30% yellow, 3 = 31 - 50% yellow, 4 = 51 - 70% yellow, 5 = 71 - 90% yellow and 6 = 91 - 100% yellow (Hofman et al., 2010a).

Lenticel discolouration (LD)

LD on each fruit was determined using the following rating scale (Hofman et al., 2010a): 0 = no lenticels discoloured, 1 = light spots on not more than 25% of the surface or dense pronounced spots on not more than 5% of the surface, 2 = light spots on not more than 50% of the surface or dense pronounced spots on not more than 10% of the surface, 3 = scattered pronounced spots on not more than 50% of the surface or dense pronounced spots on not more than 25% of the surface, 4 = dense pronounced spots on not more than 50% of the surface, and, 5 = dense pronounced spots on more than 50% of the surface.

TSS

When the fruit were ripe, the two 'cheeks' from each were removed using a knife. A vertical section of the flesh from the proximal stem end to the distal base was chopped into small pieces and frozen at -20°C . Prior to instrumental analysis, the samples were thawed and squeezed through two layers of cheesecloth to extract the juice. The TSS ($^\circ\text{Brix}$) of juice samples was determined at room temperature using a PAL-1 digital hand-held "Pocket" refractometer (Atago, Japan).

TA

Flesh samples were collected and stored as above. Prior to instrumental analysis, they were thawed and blended with a stick blender. TA of 10 g samples of the homogenised pulp in 50 ml distilled

water and then determined with an automatic titrator (Metrohm, Switzerland) using 0.1 M NaOH (Melrose Laboratories Pty Ltd, Australia) as the titrant. TA was expressed as the citric acid equivalent (%) in each sample.

Chls and carotenoids in mango skin

The Chls and carotenoid concentration were determined using the method of Ketsa et al. (1999) with minor modification. Peel samples on the non-blushed area of each fruit were sampled with a vegetable peeler when fruit reached ripe. They were frozen in liquid nitrogen and stored at -20 °C for 2 - 4 weeks and then at -80 °C for 3 months.

About 3 g of the frozen mango peel was ground to a powder in liquid nitrogen. A 0.2 - 0.3 g sub-sample of the powdered peel was extracted and diluted 100-fold with 80% cold (4 °C) acetone for 2012/2013 fruit samples and 100% cold acetone for 2013/2014 fruit samples. A solvent of 100% acetone was used in the second season in order to inhibit the conversion of chlorophyll into chlorophyllide (Hu et al., 2013). The extracts were transferred to 50 ml falcon tubes and kept in the dark overnight in a cool room at 4 °C. The sample extracts were clarified by centrifugation at 4000 rpm in a refrigerated centrifuge (Eppendorf, Germany) for 15 min at -9 °C. The absorbance of supernatant samples was measured at 662, 645 and 710 nm using a DU 530 UV Vis spectrophotometer (Beckman Coulter, USA). Chl *a*, Chl *b*, total Chls and carotenoids were expressed as µg/g fresh weight (Hartmut, 1983). Five fruit from 15 individual mango fruit were analysed per treatment for each cultivar in both locations.

Chls and carotenoids in mango flesh

To examine the effects of irradiation on Chls and carotenoids in mango flesh, the Chls and carotenoid concentration were determined using the method of (Ketsa et al., 1999) with modification. Flesh samples of each fruit were excised when fruit reached ripe as per the TSS section above and frozen in liquid nitrogen. They were stored at -20 °C for 2 - 4 weeks, then at -80 °C for 3 months.

Approximately 4 g of mango flesh (2013/2014) was ground to in 100% 4 °C cold acetone (Hu et al., 2013). The extract was diluted 100-times with 100% 4 °C cold acetone and transferred to a 50 ml falcon tube. Then the samples were kept in the dark in the cool room at 4 °C and processed as described in the mango skin section above.

Aroma volatiles production

The flesh of one cheek of each fruit was removed using a sharp knife. A vertical section of flesh from the proximal stem end to the distal fruit tip was diced into small pieces and stored in glass bottles at -20 °C for 2 - 3 months pending analysis. The samples were then analysed as described in section 3.2.5, 3.2.6 and 3.2.7 (Chapter 3). The concentrations of eight volatiles in the mango samples were referenced to standard curves and calculated from the peak area ratios for the unlabelled and labelled compounds versus the concentration ratio. The concentrations of volatiles including hexanal, 2-carene, 3-carene, α -terpinene, p-cymene, limonene, α -terpinolene and ethyl octanoate were expressed as $\mu\text{g}/\text{kg}$.

4.2.4 Statistical analysis

Daily observation data were subjected to two-way analysis of variance (ANOVA) with repeated measures between doses and time using Genstat 16th edition software (VSN international Ltd, U.K). One way ANOVA was used for the three irradiation treatments comparisons of ripe fruit. Differences between means were tested using least significant differences (Fisher's protected LSD) at $P < 0.05$.

4.3 Results

4.3.1 External appearance and physical properties during ripening

Fruit firmness

As determined by hand pressure and relative to control (i.e. 0 kGy) fruit in both the 2012/2013 and 2013/2014 seasons, exposure of 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' fruit to 0.5 and 1.0 kGy irradiation generally reduced initial rates of softening (Figures 4.1 and 4.2). In most cases and in both seasons, irradiated fruit were more firm in the early stages of ripening compared with no irradiation. However, there were no differences in firmness at ripe. In general, there was no significant difference between the two doses of 0.5 and 1.0 kGy (Figures 4.1 and 4.2). Exceptions were that non-irradiated 'Honey Gold' mango from NQ and SEQ in the 2012/2013 season (Figure 4.1) and also 'Honey Gold' mango fruit from NQ were softer in the early stages of ripening than irradiated fruit in the 2013/2014 season (Figure 4.2).

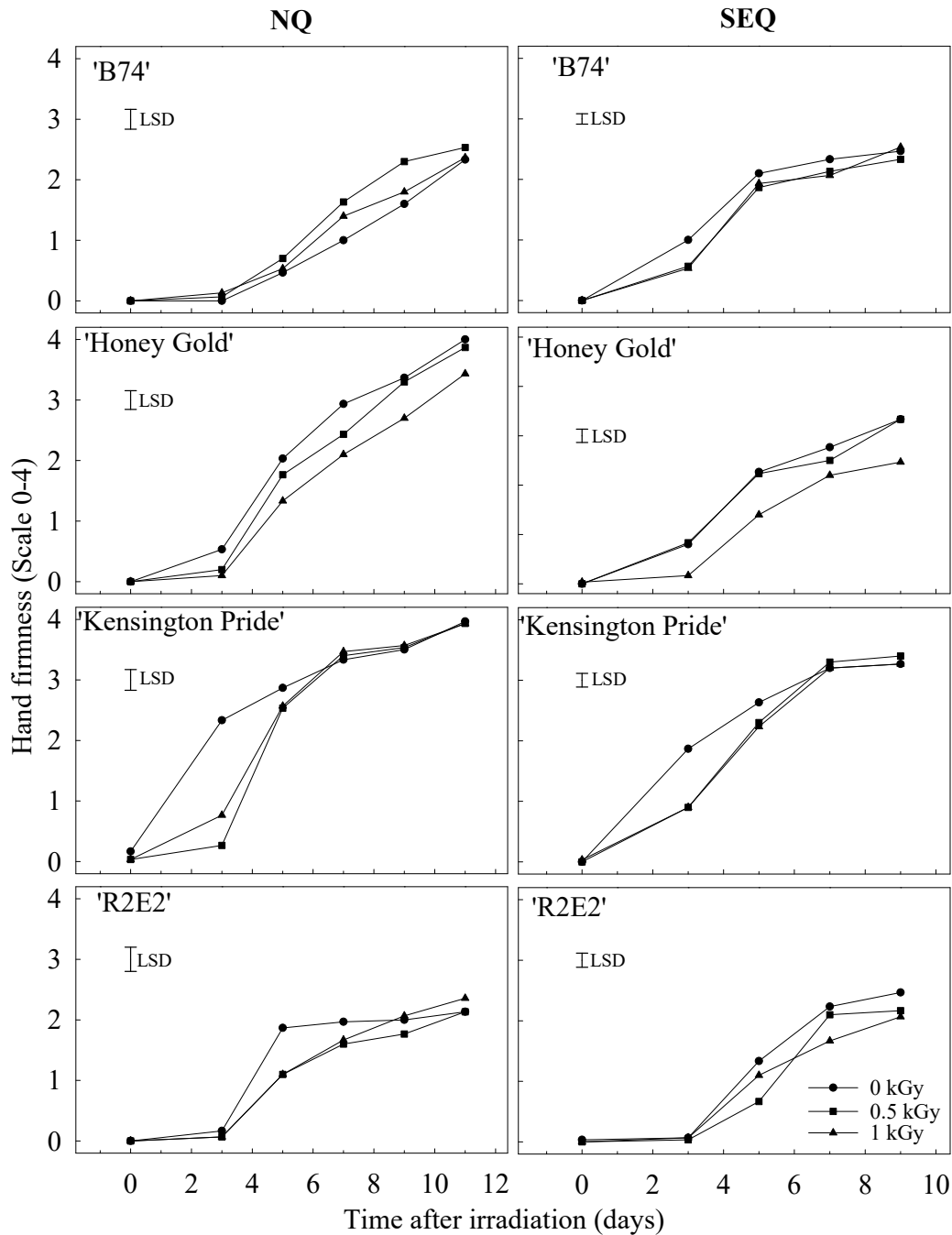


Figure 4.1 Effect of irradiation (0, 0.5, 1.0 kGy) on hand firmness of 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' mango fruit ($n = 15$) during shelf life at 20 °C. Green mature fruit were harvested from north Queensland (NQ) and south east Queensland (SEQ) in the 2012/2013 season and exposed to γ -irradiation. Fruit were treated with 10 $\mu\text{l/l}$ ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe. The LSD ($P = 0.05$) bars are for factorial effect of dose and time.

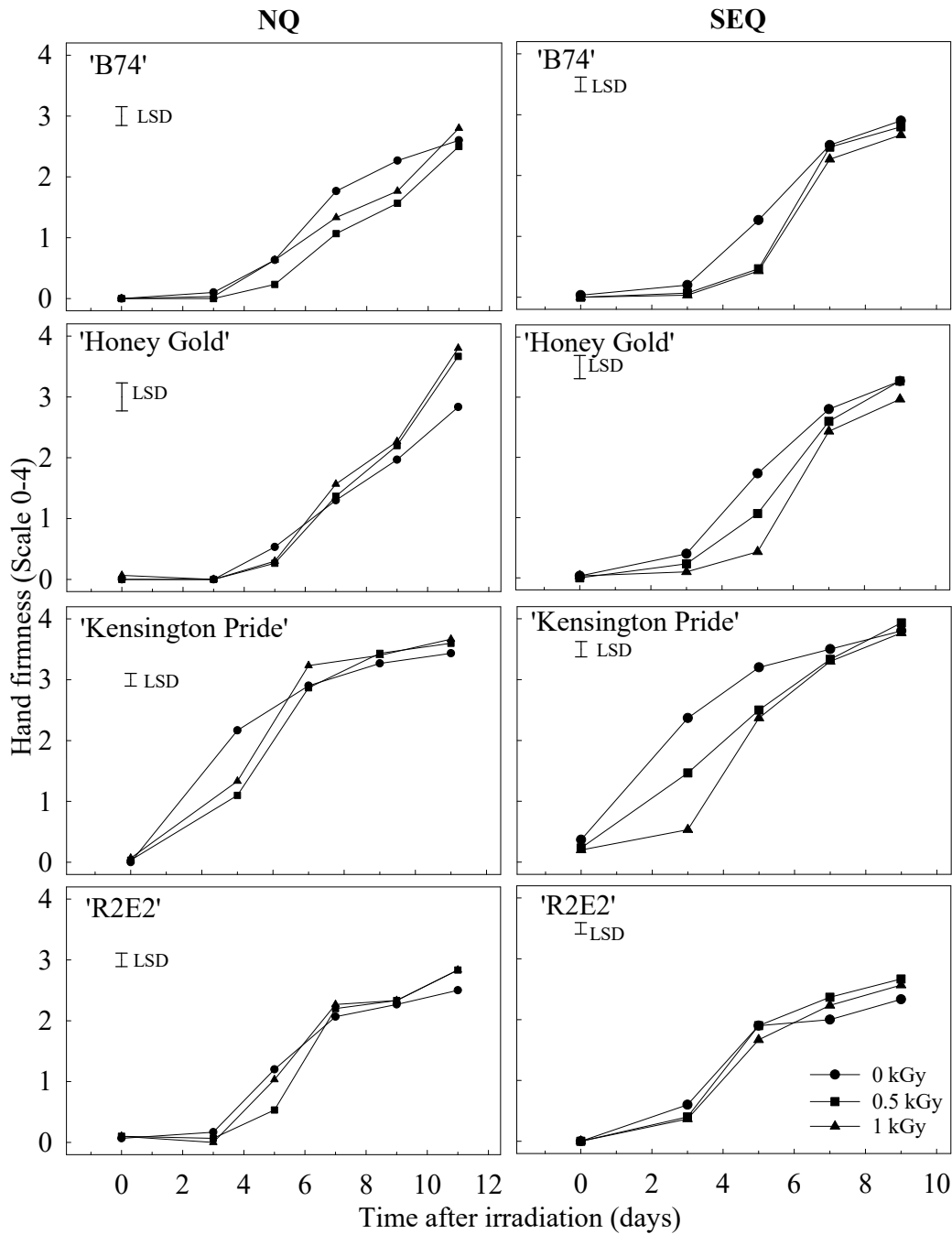


Figure 4.2 Effect of irradiation (0, 0.5, 1.0 kGy) on hand firmness of 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' mango fruit ($n = 15$) during shelf life at 20 °C. Green mature fruit were harvested from north Queensland (NQ) and south east Queensland (SEQ) in the 2013/2014 season and exposed to γ -irradiation. Fruit were treated with 10 $\mu\text{l/l}$ ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe. The LSD ($P = 0.05$) bars are for factorial effect of dose and time.

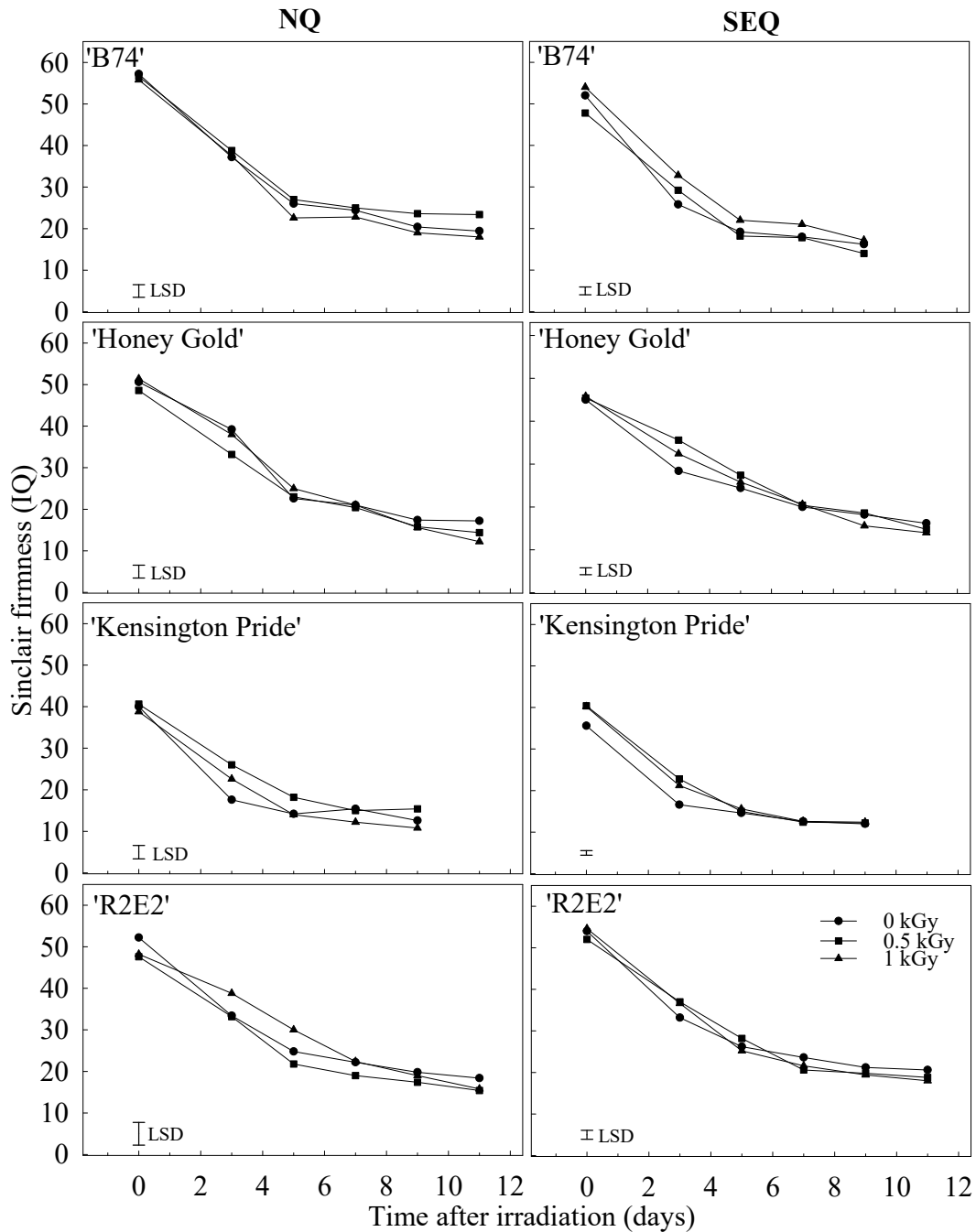


Figure 4.3 Effect of irradiation (0, 0.5, 1.0 kGy) on Sinclair IQ firmness tester assessments of ‘B74’, ‘Honey Gold’, ‘Kensington Pride’ and ‘R2E2’ mango fruit ($n = 5$) during shelf life at 20°C. Green mature fruit were harvested from north Queensland (NQ) and south east Queensland (SEQ) in the 2013/2014 season and exposed to γ -irradiation. Fruit were treated with 10 μ l/l ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe. The LSD ($P = 0.05$) bars are for factorial effect of dose and time.

In terms of method of assessment, similar patterns in firmness changes over time were detected with the Sinclair firmness tester (Figure 4.3). There was no significant difference in firmness between irradiated and non-irradiated fruit by 9 and 11 d of shelf life. The irradiation treatment effect diminished as fruit progressively ripened.

Mango skin colour development

As judged by subjective skin colour assessment, irradiation of 'B74', 'Kensington Pride' and 'R2E2' fruit at either 0.5 or 1.0 kGy significantly reduced loss of green skin colour during ripening as compared with non-irradiated controls (Figures 4.4 and 4.5). This effect was less pronounced in 'Honey Gold' fruit, particularly those harvested from SEQ in the 2012/2013 season and NQ in 2013/2014. There was generally no significant difference in skin colour response between 0.5 and 1.0 kGy.

Measured L , C , h° colour parameters during ripening corresponded with subjective colour rating results as reported above. L values increased as the fruit became lighter at the end of ripening. The L values were significantly higher for irradiated (i.e. 0.5 and 1 kGy) 'B74', 'Kensington Pride' and 'R2E2' fruit when fruit reached ripe in both seasons (Figures 4.6 and 4.9). In contrast, there was no significant difference in L between ripe control and irradiated 'Honey Gold' fruit. Decreases in L for irradiated 'Kensington Pride' in the 2012/2013 season and 'R2E2' fruit in the 2012/2013 (Figure 4.6) and 2013/2014 (Figure 4.9) at days 9 and 11 of shelf life was associated with skin browning.

C values were significantly lower for the skin of fruit exposed to 0.5 and 1.0 kGy than for control fruit (Figures 4.7 and 4.10). Relatively higher C values are associated with brighter colour and so these irradiated fruit had a duller colour.

Exposure to irradiation resulted in significantly higher h° values for the skin of 'B74', 'Kensington Pride' and 'R2E2' fruit as compared to the control (Figures 4.8 and 4.11). These irradiated fruit exhibited delayed colour development in higher h° as compared to the control, which was prominent from day 5 after irradiation. For 'Honey Gold', h° values were generally not significantly different between irradiated and non-irradiated fruit (Figure 4.8).

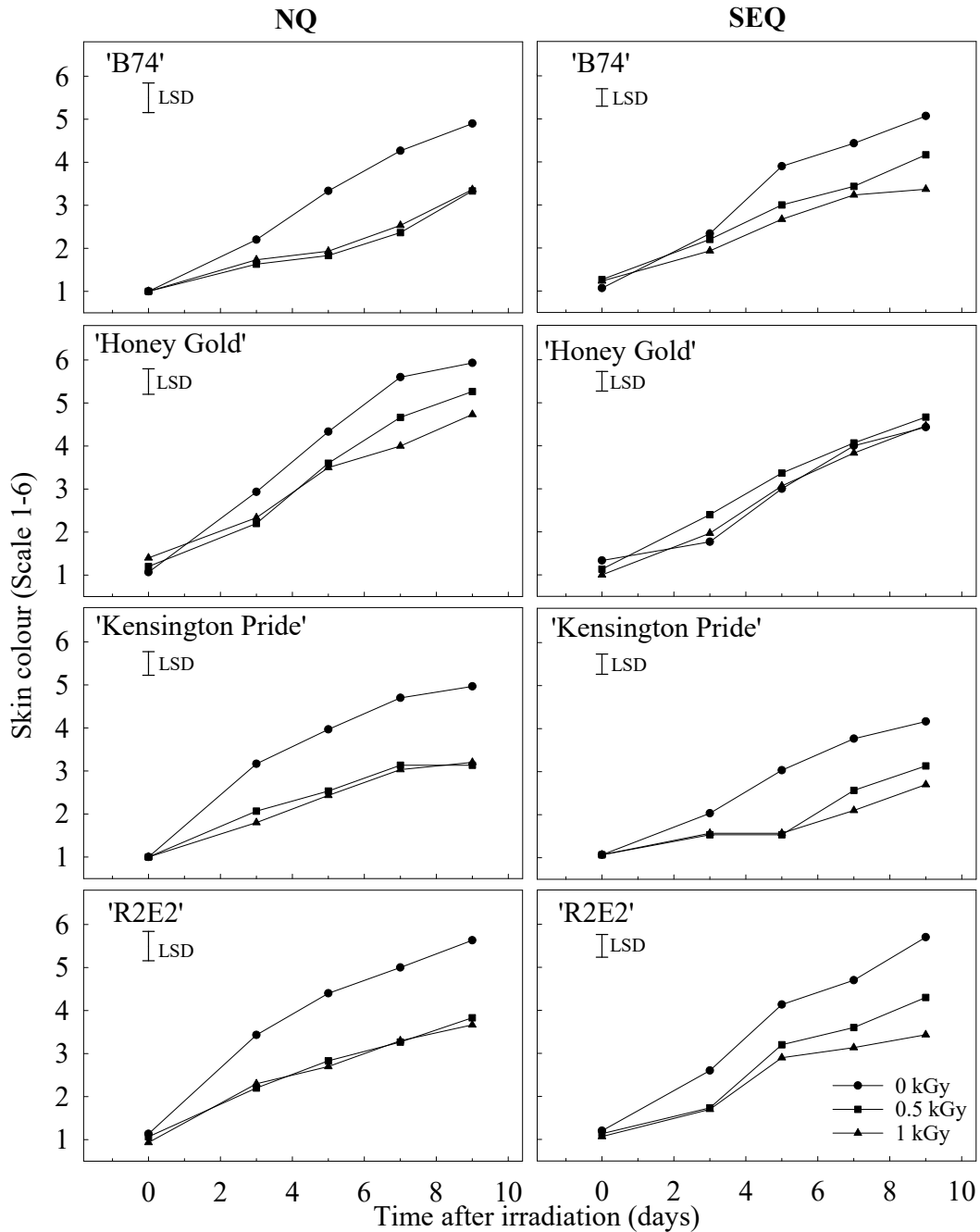


Figure 4.4 Effect of irradiation (0, 0.5, 1.0 kGy) on skin colour of ‘B74’, ‘Honey Gold’, ‘Kensington Pride’ and ‘R2E2’ mango fruit ($n = 15$) during shelf life at 20 °C. Green mature fruit were harvested from north Queensland (NQ) and south east Queensland (SEQ) in the 2012/2013 season and exposed to γ -irradiation. Fruit were treated with 10 μ l/l ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe. The LSD ($P = 0.05$) bars are for factorial effect of dose and time.

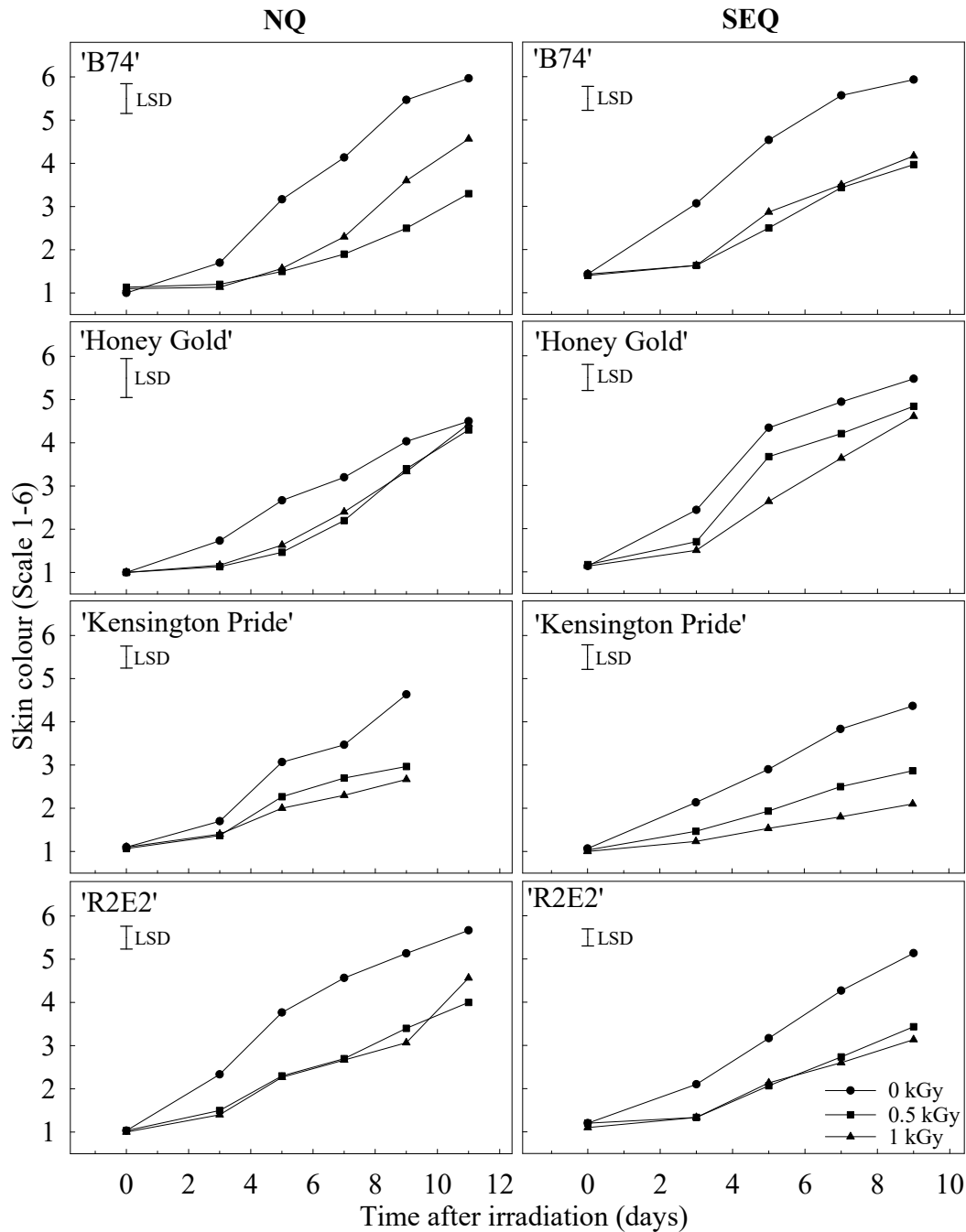


Figure 4.5 Effect of irradiation (0, 0.5, 1.0 kGy) on skin colour of ‘B74’, ‘Honey Gold’, ‘Kensington Pride’ and ‘R2E2’ mango fruit ($n = 15$) during shelf life at 20 °C. Green mature fruit were harvested from north Queensland (NQ) and south east Queensland (SEQ) in the 2013/2014 season and exposed to γ -irradiation. Fruit were treated with 10 μ l/l ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe. The LSD ($P = 0.05$) bars are for factorial effect of dose and time.

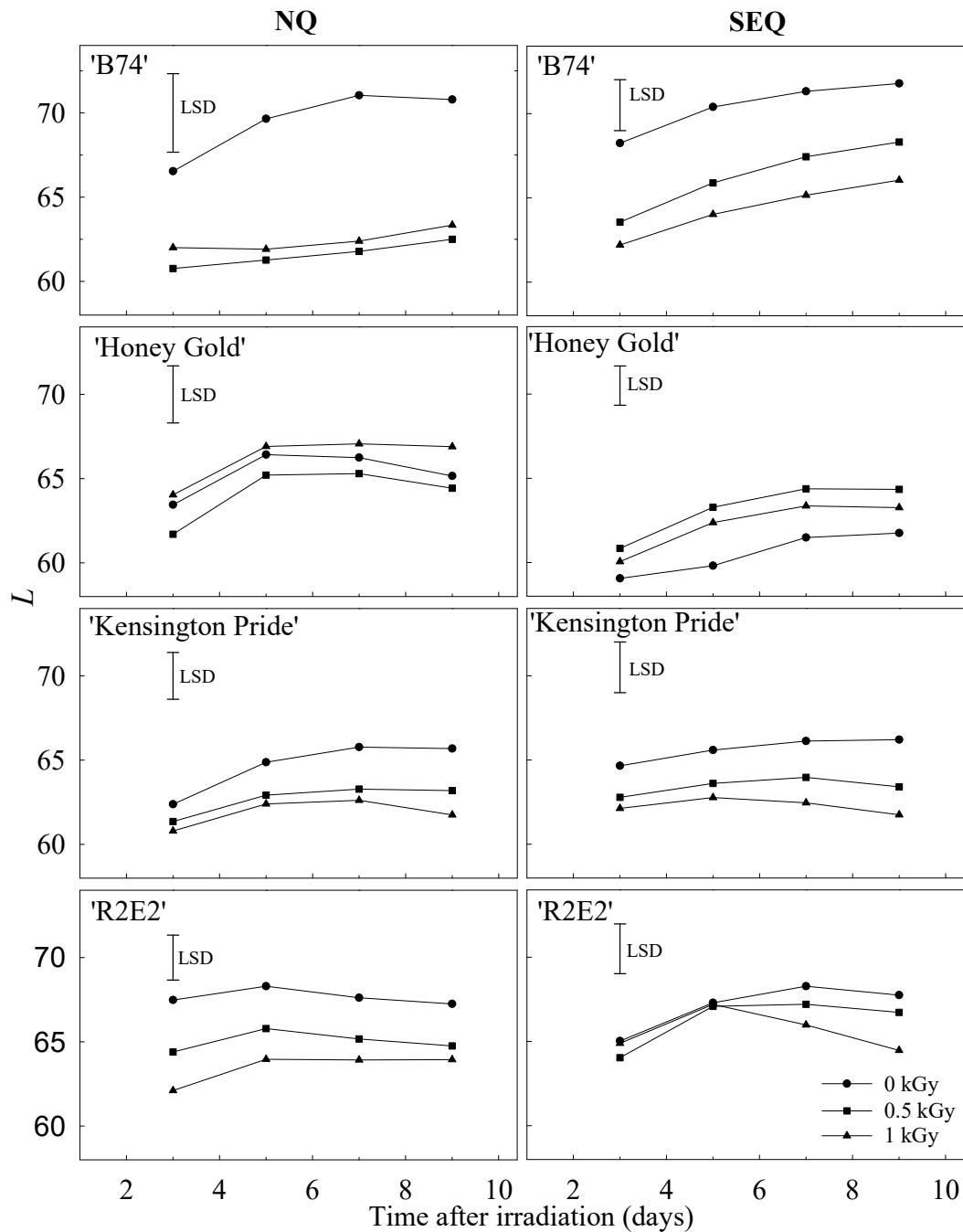


Figure 4.6 Effect of irradiation (0, 0.5, 1.0 kGy) on lightness (L) of 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' mango fruit during shelf life at 20 °C. Green mature fruit were harvested from north Queensland (NQ)¹ and south east Queensland (SEQ)² in the 2012/2013 season and exposed to γ -irradiation. Fruit were treated with 10 μ l/l ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe. The LSD ($P = 0.05$) bars are for factorial effect of dose and time. (¹ $n = 15$ and ² $n = 10$).

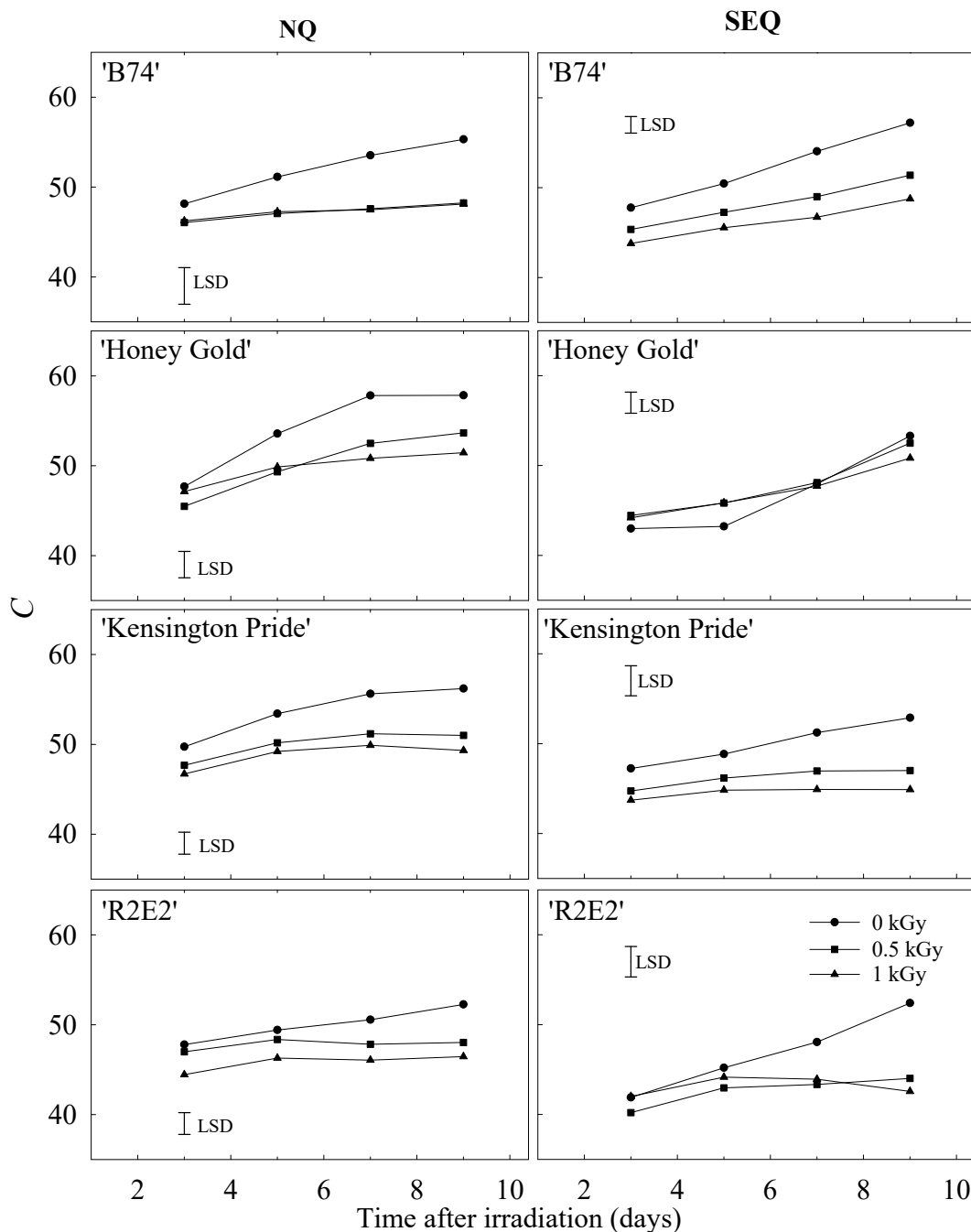


Figure 4.7 Effect of irradiation (0, 0.5, 1.0 kGy) on chroma (C) of 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' mango fruit during shelf life at 20 °C. Green mature fruit were harvested from north Queensland (NQ)¹ and south east Queensland (SEQ)² in the 2012/2013 season and exposed to γ -irradiation. Fruit were treated with 10 μ l/l ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe. The LSD ($P = 0.05$) bars are for factorial effect of dose and time. (¹ $n = 15$ and ² $n = 10$).

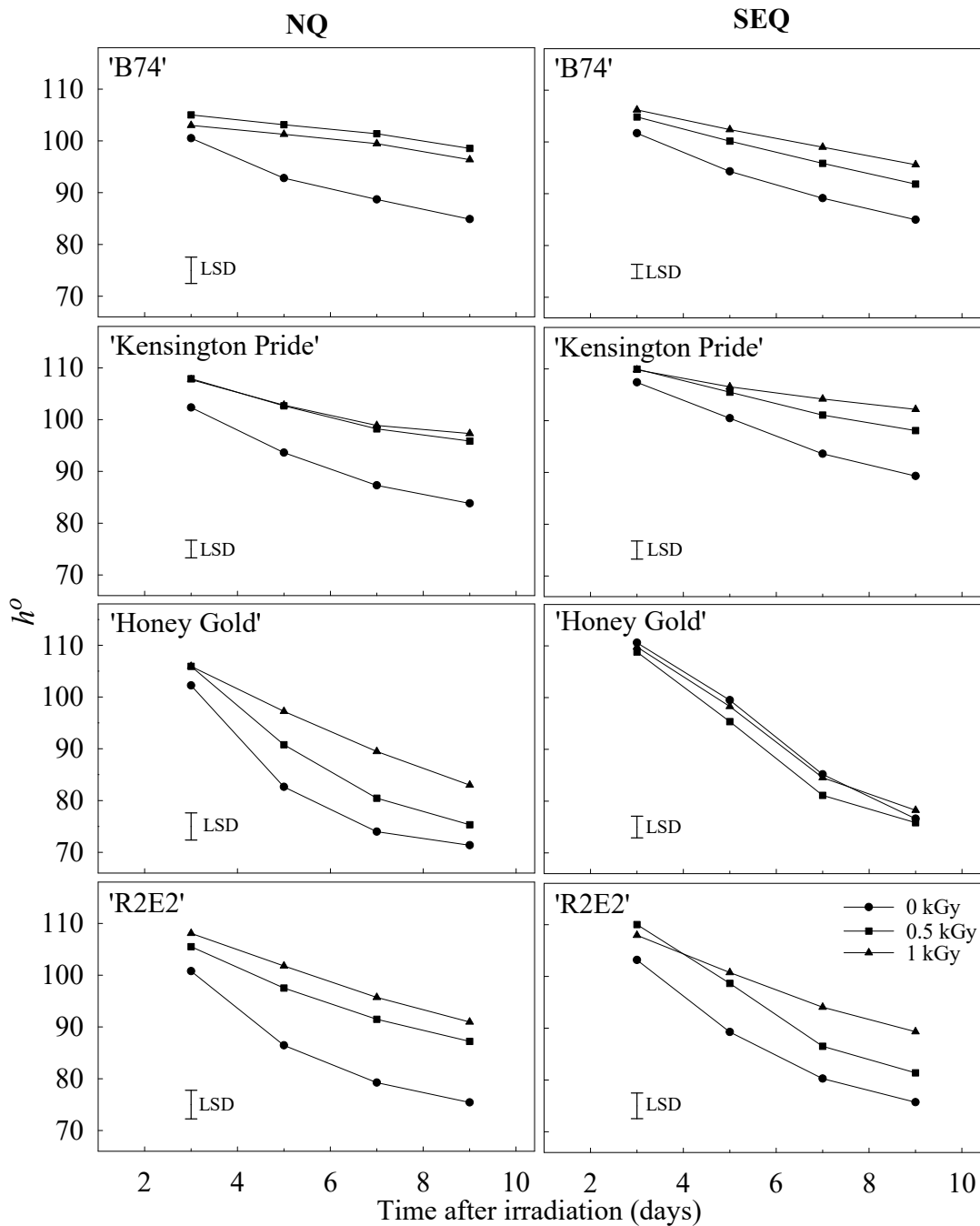


Figure 4.8 Effect of irradiation (0, 0.5, 1.0 kGy) on h° of 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' mango fruit during shelf life at 20 °C. Green mature fruit were harvested from north Queensland (NQ)¹ and south east Queensland (SEQ)² in the 2012/2013 season and exposed to γ -irradiation. Fruit were treated with 10 μ l/l ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe. The LSD ($P = 0.05$) bars are for factorial effect of dose and time. (¹ $n = 15$ and ² $n = 10$).

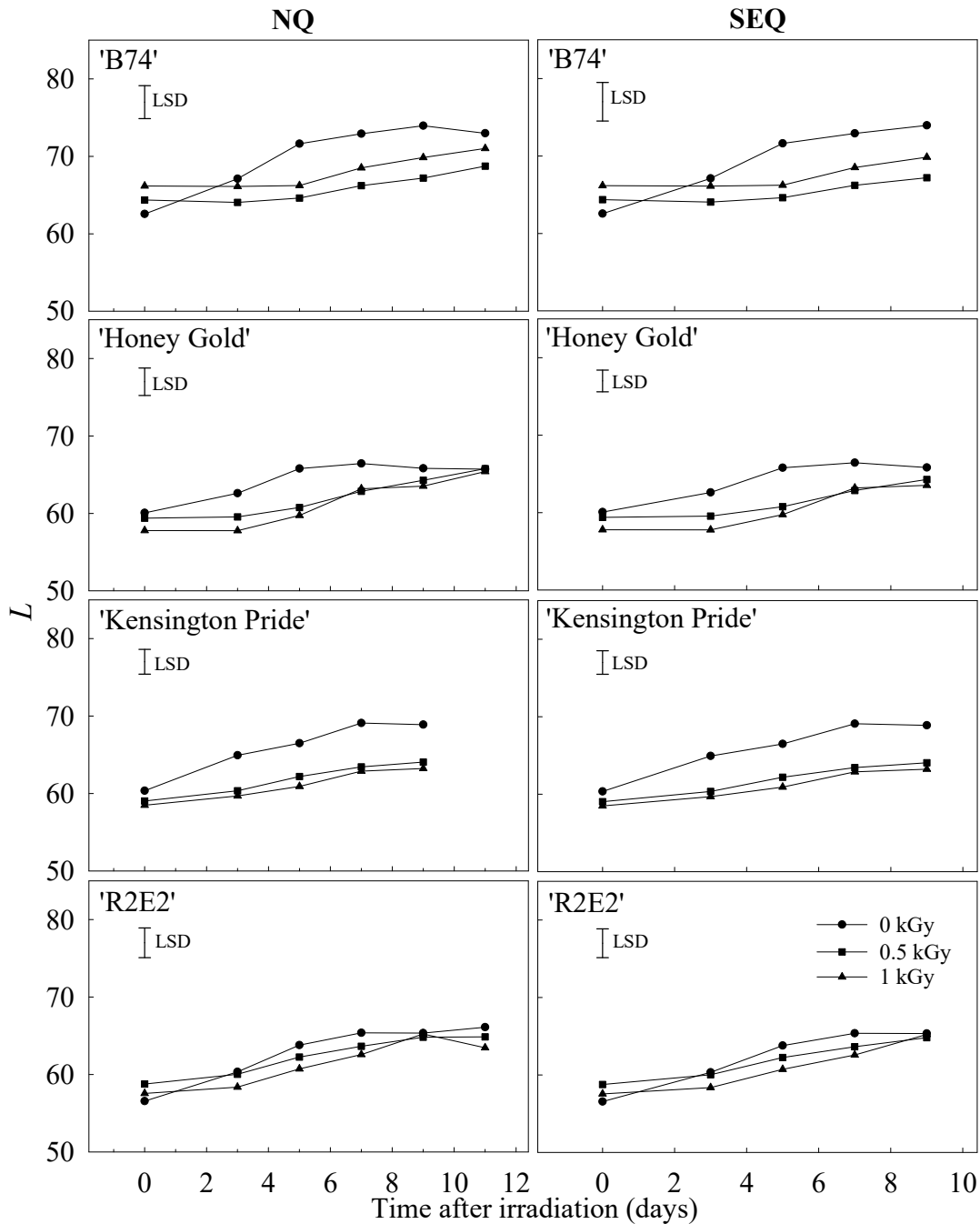


Figure 4.9 Effect of irradiation (0, 0.5, 1.0 kGy) on lightness (L) of 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' mango fruit ($n = 10$) during shelf life at 20 °C. Green mature fruit were harvested from north Queensland (NQ) and south east Queensland (SEQ) in the 2013/2014 season and exposed to γ -irradiation. Fruit were treated with 10 $\mu\text{l/l}$ ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe. The LSD ($P = 0.05$) bars are for factorial effect of dose and time.

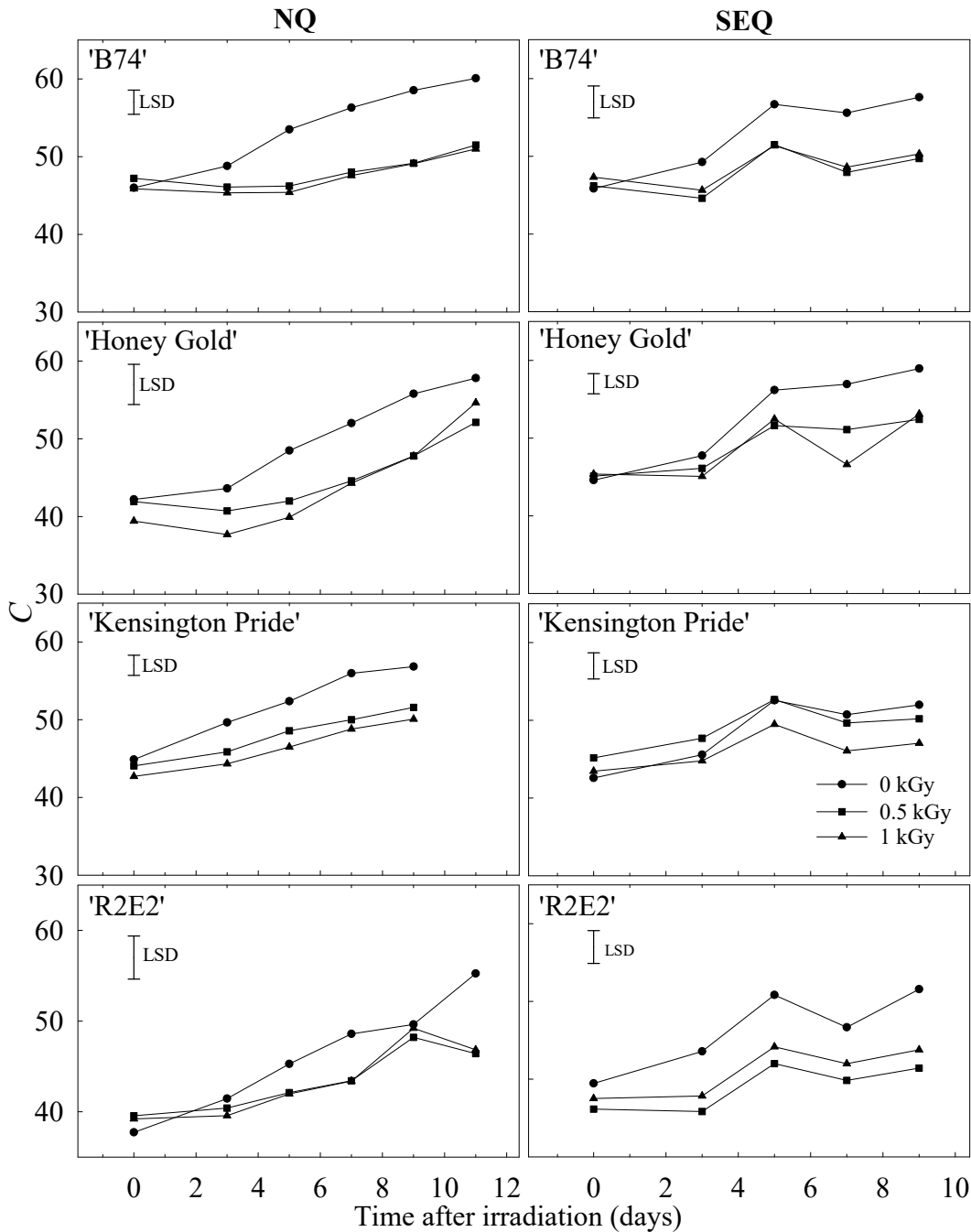


Figure 4.10 Effect of irradiation (0, 0.5, 1.0 kGy) on chroma (C) of 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' mango fruit ($n = 10$) during shelf life at 20 °C. Green mature fruit were harvested from north Queensland (NQ) and south east Queensland (SEQ) in the 2013/2014 season and exposed to γ -irradiation. Fruit were treated with 10 μ l/l ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe. The LSD ($P = 0.05$) bars are for factorial effect of dose and time.

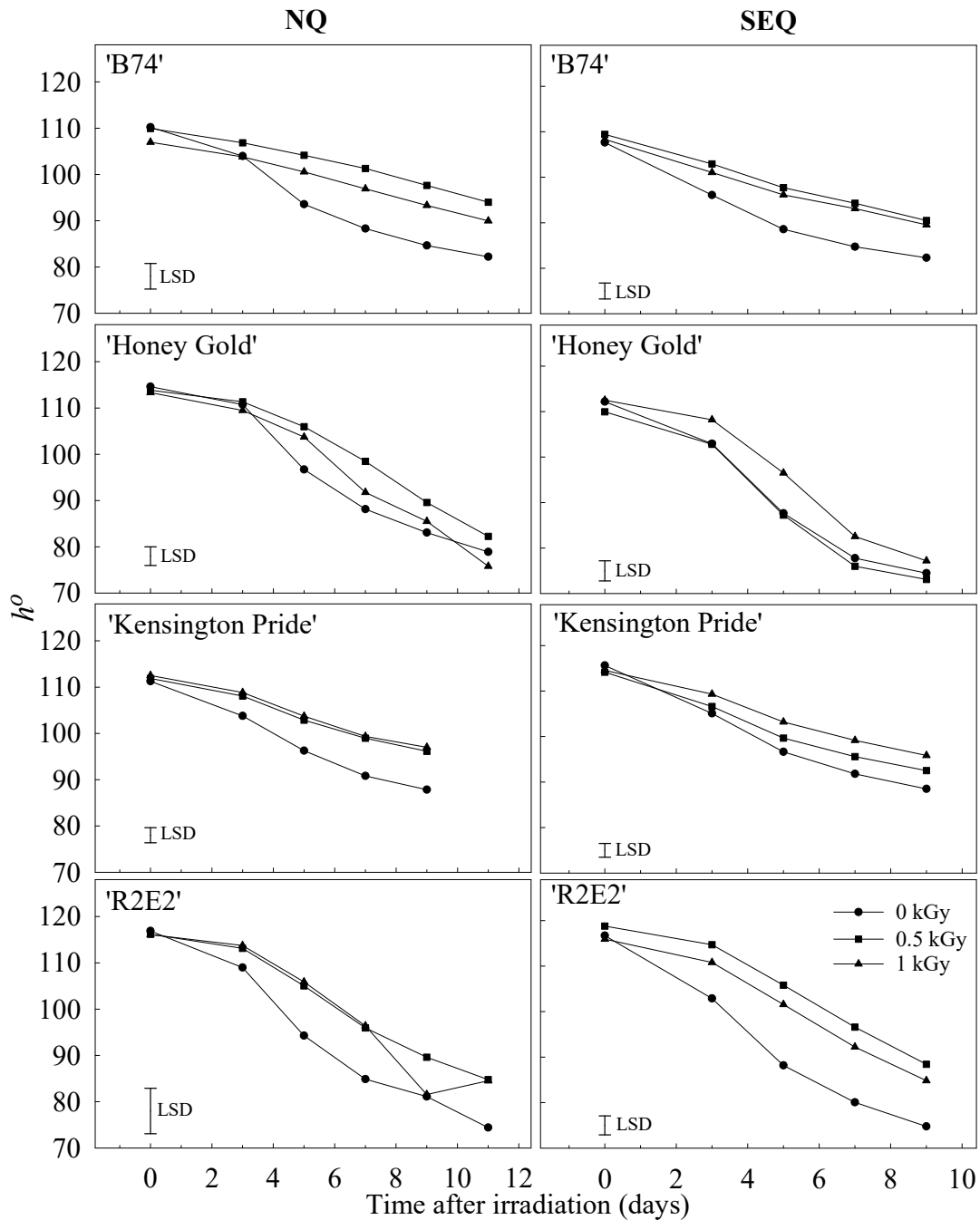


Figure 4.11 Effect of irradiation (0, 0.5, 1.0 kGy) on h° of 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' mango fruit ($n = 10$) during shelf life at 20 °C. Green mature fruit were harvested from north Queensland (NQ) and south east Queensland (SEQ) in the 2013/2014 season and exposed to γ -irradiation. Fruit were treated with 10 μ l/l ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe. The LSD ($P = 0.05$) bars are for factorial effect of dose and time.



Figure 4.12 Typical symptoms of skin browning on ‘Kensington Pride’ (B) and ‘R2E2’ (D) mango fruit exposed to 1.0 kGy as compared to non-irradiated ‘Kensington Pride’ (A) and ‘R2E2’ (C) mango fruit. Arrows point to affected regions.

LD

Exposure of ‘B74’, ‘Honey Gold’, ‘Kensington Pride’ and ‘R2E2’ mango fruit to 0.5 and 1.0 kGy stimulated development of more severe LD than on non-irradiated control fruit in both seasons (Figures 4.13 and 4.14). LD developed rapidly within 2 - 3 d of exposure to irradiation. Thereafter, LD development was either relatively slow in development towards the end of shelf-life or did not increase at all as fruit ripened.

The severity of LD on ‘B74’ mangoes from NQ and SEQ was markedly affected by the irradiation doses of 0.5 and 1.0 kGy (Figures 4.13 and 4.14). From day 3, ‘B74’ fruit irradiated at 0.5 and 1.0 kGy also had much higher LD scores than did control samples. Fruit dosed at 1.0 kGy had the highest LD rating score.

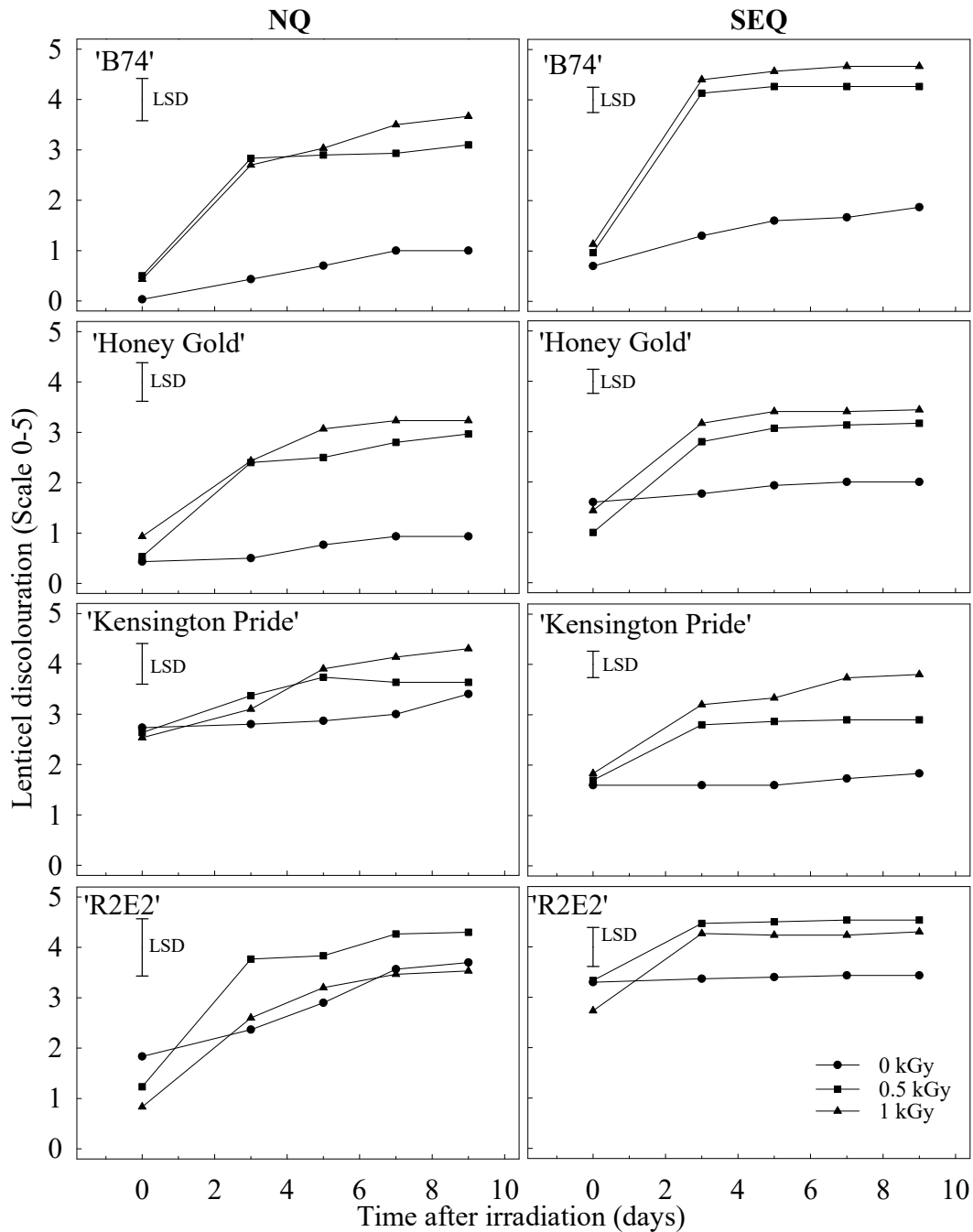


Figure 4.13 Effect of irradiation (0, 0.5, 1.0 kGy) on lenticel discoloration of 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' mango fruit ($n = 10$) during shelf life at 20 °C. Green mature fruit were harvested from north Queensland (NQ) and south east Queensland (SEQ) in the 2012/2013 season and exposed to γ -irradiation. Fruit were treated with 10 μ l/l ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe. The LSD ($P = 0.05$) bars are for factorial effect of dose and time.

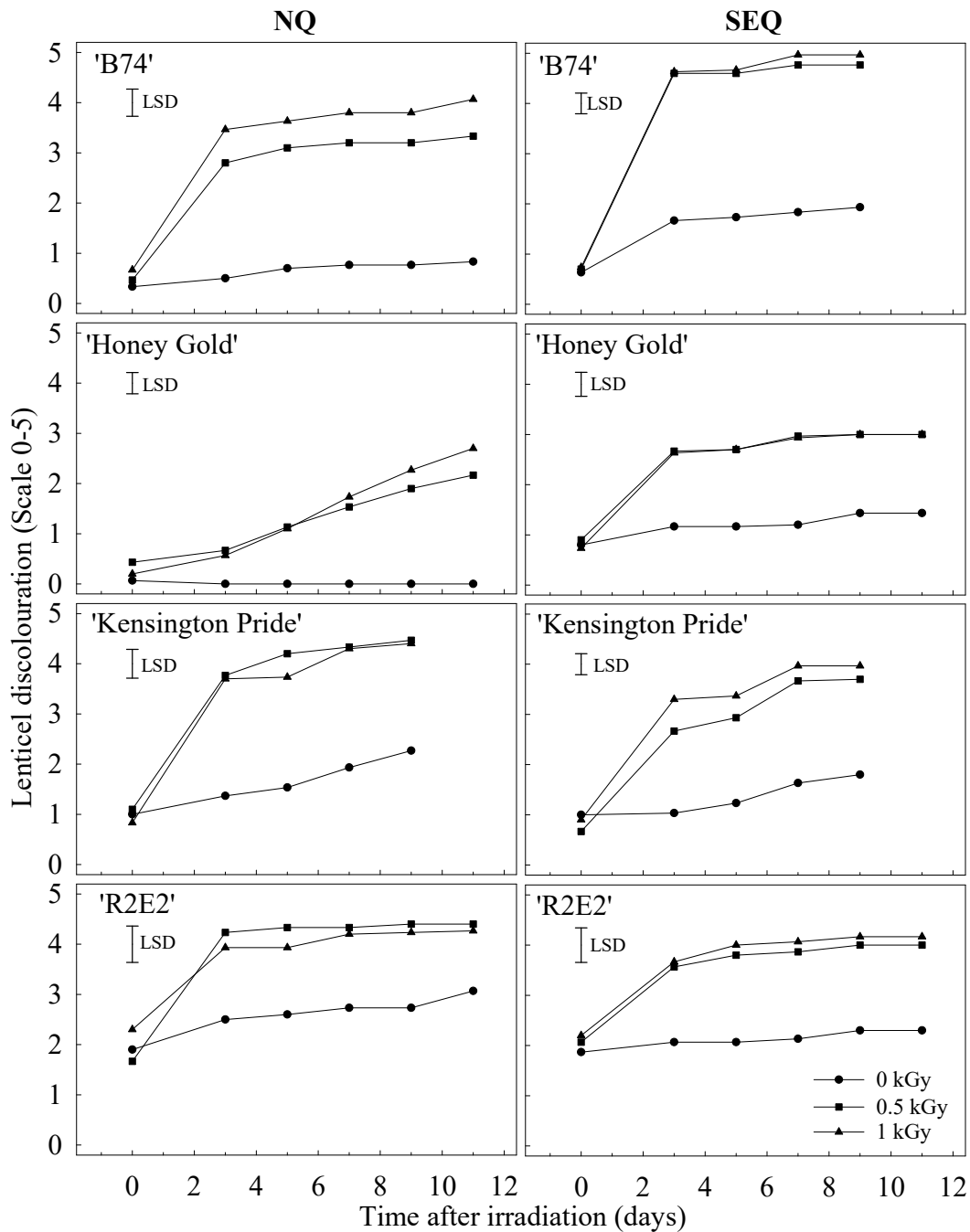


Figure 4.14 Effect of irradiation (0, 0.5, 1.0 kGy) on lenticel discoloration of 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' mango fruit ($n = 15$) during shelf life at 20 °C. Green mature fruit were harvested from north Queensland (NQ) and south east Queensland (SEQ) in the 2013/2014 season and exposed to γ -irradiation. Fruit were treated with 10 $\mu\text{l/l}$ ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe. The LSD ($P = 0.05$) bars are for factorial effect of dose and time.

4.3.2. Taste and chemical properties of ripe fruit

Flesh TSS

Across irradiation treatments and production locations, ‘B74’ and ‘Honey Gold’ mango fruit had the lowest and highest, respectively, TSS at ripe (Tables 4.5 and 4.6). Exposure of ‘B74’, ‘Kensington Pride’, ‘Honey Gold’ and ‘R2E2’ mango fruit to irradiation at 0.5 and 1.0 kGy generally did not affect TSS at ripe (Table 4.5). In the 2012/2013 season, ‘Honey Gold’ fruit from NQ treated with 1.0 kGy showed a significant ($P \leq 0.05$) decrease in TSS content as compared to samples exposed to 0 and 0.5 kGy.

Flesh TA and TSS/TA ratio

In the 2012/2013 season, the TA of ‘B74’ fruit from SEQ and ‘Honey Gold’ from NQ and SEQ was unaffected by irradiation (Table 4.5). In contrast, irradiation at 0.5 and 1.0 kGy reduced rates of ripening-related decreases in TA for ‘B74’ from NQ, ‘Kensington Pride’ and ‘R2E2’ as compared to their non-irradiated controls. With no irradiation, TA was highest in ‘Honey Gold’ (Table 4.5) in combination with a high TSS. Irradiation generally reduced the rate of acidity loss, except with ‘Honey Gold’ where there was no irradiation effect.

Table 4.5 Effect of γ -irradiation (0, 0.5, 1.0 kGy) on total soluble solids (TSS), titrable acidity (TA) and TSS/TA ratio of ripe ‘B74’, ‘Honey Gold’, ‘Kensington Pride’ and ‘R2E2’ mango fruit. Green mature fruit were harvested from north Queensland (NQ) and south east Queensland (SEQ) in the 2012/2013 season and exposed to γ -irradiation. Fruit were treated with 10 μ l/l ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe.

Dose (kGy)	NQ			SEQ		
	TSS (°Brix)	TA (%)	TSS/TA	TSS (°Brix)	TA (%)	TSS/TA
‘B74’						
0	12.1	0.15 a	86.1 b	11.3	0.12	105.0 b
0.5	11.9	0.21 ab	63.1 a	11.1	0.21	55.0 a
1.0	12.5	0.23 b	56.0 a	11.1	0.25	45.8 a
LSD ($P = 0.05$)	n.s.	0.03	12.1	n.s.	n.s.	26.1

Table 4.5 (Continued).

Dose (kGy)	NQ			SEQ		
	TSS (°Brix)	TA (%)	TSS/TA	TSS (°Brix)	TA (%)	TSS/TA
‘Honey Gold’						
0	17.0 b	0.49	35.8	16.2	0.49	36.2 b
0.5	17.4 b	0.45	37.8	15.2	0.61	25.7 a
1.0	15.7 a	0.46	36.6	16.2	0.56	30.0 ab
LSD ($P = 0.05$)	1.0	n.s.	n.s.	n.s.	n.s.	7.2
‘Kensington Pride’						
0	15.8	0.27 a	62.9 b	12.4	0.21 a	62.2 b
0.5	15.8	0.89 b	22.1 a	13.1	0.68 b	21.4 a
1.0	15.2	0.69 b	25.2 a	12.4	0.60 b	21.6 a
LSD ($P = 0.05$)	n.s.	0.26	13.5	n.s.	0.15	8.7
‘R2E2’						
0	14.7	0.16 a	98.7 b	14.0	0.16 a	98.2 b
0.5	14.6	0.27 b	57.5 a	13.5	0.35 b	40.3 a
1.0	15.2	0.31 b	53.1 a	13.1	0.25 c	62.3 a
LSD ($P = 0.05$)	n.s.	0.07	15.9	n.s.	0.07	27.2

Data represent means for a total of $n = 10$. Means within assessed parameter columns with different lower case letters differ significantly ($P \leq 0.05$) by Fisher's protected LSD. n.s. indicates no significant difference ($P > 0.05$).

In 2013/2014, similarly higher TA contents were found for all irradiation treatments for ‘B74’ from SEQ and ‘R2E2’ from SEQ as compared to the control (Table 4.6). However, neither a significant general decrease nor differences in TA between the treatments were seen for ‘B74’ and ‘R2E2’ from NQ or for ‘Honey Gold’ and ‘Kensington Pride’ from both locations. Irradiated fruit showed a decrease in TSS/TA ratios for ‘B74’, ‘Kensington Pride’ and ‘R2E2’, while there were no significant differences in TSS/TA ratio between control and treated ‘Honey Gold’.

Table 4.6 Effect of irradiation (0, 0.5, 1.0 kGy) on total soluble solids (TSS), titrable acidity (TA) and TSS/TA ratio of ripe ‘B74’, ‘Honey Gold’, ‘Kensington Pride’ and ‘R2E2’ mango fruit. Green mature fruit were harvested from north Queensland (NQ) and south east Queensland (SEQ) in the 2013/2014 season and exposed to γ -irradiation. Fruit were treated with 10 μ l/l ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe.

Dose (kGy)	NQ			SEQ		
	TSS (°Brix)	TA (%)	TSS/TA	TSS (°Brix)	TA (%)	TSS/TA
‘B74’						
0	12.9	0.2	65.5	12.9	0.2 a	73.8 b
0.5	12.5	0.2	54.4	12.9	0.3 b	49.8 a
1.0	12.9	0.2	55.8	12.7	0.3 b	49.9 a
LSD ($P = 0.05$)	n.s	n.s	n.s	n.s	0.04	8.5
‘Honey Gold’						
0	15.7	1.06	22.4	18.5	0.46	42
0.5	15.7	1.84	13.6	18.7	0.79	35
1.0	15.4	2.16	7.3	18.2	0.96	28
LSD ($P = 0.05$)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
‘Kensington Pride’						
0	12.6	0.2 a	69.4 b	13.1	0.3 a	40.2 b
0.5	13.2	1.7 b	11.5 a	13.9	1.1 b	14.4 a
1.0	12.2	1.8 b	13.4 a	13.4	1.1 b	13.7 a
LSD ($P = 0.05$)	n.s	1.3	15.9	n.s	0.5	7.1
‘R2E2’						
0	14.4	0.38	59.6	14.4	0.15 a	104.7 b
0.5	15.4	0.29	55.7	13.8	0.31 c	46.3 a
1.0	14.6	0.28	54.4	13.9	0.24 b	58.0 a
LSD ($P = 0.05$)	n.s.	n.s.	n.s.	n.s.	n.s.	37.1

Data represent means for a total of $n = 5$. Means within assessed parameter columns with different lower case letters differ significantly ($P \leq 0.05$) by Fisher's protected LSD. n.s. indicates no significant difference ($P > 0.05$).

Skin Chl and carotenoid concentrations

Irradiation of fruit at 0.5 and 1.0 kGy inhibited the ripening-related degradation of Chl *a*, Chl *b* and total Chls in the skins of ‘B74’, ‘Kensington Pride’ and ‘R2E2’ mangoes in both 2012/2013 (Table 4.7) and 2013/2014 (Tables 4.8 and 4.9) seasons relative to non-irradiated fruit. In contrast, there was no significant effect of irradiation on Chl *a*, Chl *b* and total Chls concentrations in the skin of ‘Honey Gold’. Irradiation at 0.5 to 1.0 kGy generally had no considerable effect on carotenoid concentrations of mango skin (Tables 4.7, 4.8 and 4.9).

The Chls levels are also reflected in h^o (Figures 4.8 and 4.11). The h^o values were higher in mango fruits exposed to 0.5 and 1.0 kGy as compared to control fruit. For example, doses of 0.5 kGy and 1.0 kGy resulted in 1.5 to 2-fold lower total Chls than in ripe control ‘B74’ samples from SEQ in the 2013/2014 season (Table 4.9). For ‘Kensington Pride’, irradiation treatments resulted in 2-fold greater retention of total Chls as compared to the non-irradiated fruit. Irradiated ‘R2E2’ fruit at 0.5 kGy and 1.0 kGy also showed 2-fold higher content of total Chls as compared to the control fruit.

Flesh Chl and carotenoid concentrations

In general, there was no major effect of the irradiation treatments on the Chl *a*, Chl *b* and Chl concentrations ($P > 0.05$) of mango flesh as compared to control fruit (Tables 4.10 and 4.11). As shown in Table 4.10, non-irradiated ‘B74’, ‘Kensington Pride’ and ‘R2E2’ mangoes from NQ had a high carotenoid level in their flesh as compared to mangoes exposed to 0.5 and 1.0 kGy. Irradiation at 0.5 and 1.0 kGy did not affect the carotenoid concentration of the ‘Honey Gold’ flesh samples.

Carotenoid contents were unaffected by irradiation for ‘B74’, ‘Honey Gold’, ‘Kensington Pride’ and ‘R2E2’ fruit at ripe from SEQ (Table 4.11). A similar effect was observed by Reyes and Cisneros-Zevallos (2007), where irradiation at 1 - 3.1 kGy did not affect total carotenoid concentrations of ‘Tommy Atkins’ mango flesh.

Table 4.7 Effect of irradiation (0, 0.5, 1.0 kGy) on Chl *a*, Chl *b*, total Chls and carotenoid concentrations of ripened ‘B74’, ‘Honey Gold’, ‘Kensington Pride’ and ‘R2E2’ mango skin. Green mature fruit were harvested from south east Queensland (SEQ) in the 2012/2013 season and exposed to γ -irradiation. Fruit were treated with 10 μ l/l ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe. The skin was collected from non-blush area using a fruit peeler.

Dose (kGy)	Concentration (μ g/g)			
	Chl <i>a</i>	Chl <i>b</i>	Total Chls	Carotenoids
‘B74’				
0	5.9 a	6.7 a	12.6 a	67.0 c
0.5	21.7 b	6.0 a	27.8 a	41.8 b
1.0	37.5 c	20.7 b	58.2 b	31.0 a
LSD ($P = 0.05$)	8.9	12.0	19.9	10.2
‘Honey Gold’				
0	9.9	8.1	18.0	63.8
0.5	16.5	16.0	32.5	57.0
1.0	16.2	14.0	30.2	64.8
LSD ($P = 0.05$)	n.s.	n.s.	n.s.	n.s.
‘Kensington Pride’				
0	15.2 a	11.2 a	29.3 a	68.9
0.5	42.3 b	21.7 ab	63.9 b	60.6
1.0	55.1 b	27.1 b	82.2 b	56.1
LSD ($P = 0.05$)	18.0	12.5	28.0	n.s.
‘R2E2’				
0	6.8 a	7.5	14.2 a	63.5
0.5	25.5 b	11.3	36.8 b	59.3
1.0	29.9 b	9.5	39.4 b	54.8
LSD ($P = 0.05$)	15.3	n.s.	18.6	n.s.

Data represent means for a total of $n = 5$ plus two laboratory replicates per replication. Means within assessed parameter columns with different lower case letters differ significantly ($P \leq 0.05$) by Fisher's protected LSD. n.s. indicates no significant difference ($P > 0.05$).

Table 4.8 Effect of irradiation (0, 0.5, 1.0 kGy) on Chl *a*, Chl *b*, total Chls and carotenoid concentrations of ripened ‘B74’, ‘Honey Gold’, ‘Kensington Pride’ and ‘R2E2’ mango skin. Green mature fruit were harvested from north Queensland (NQ) in the 2013/2014 season and exposed to γ -irradiation. Fruit were treated with 10 μ l/l ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe. The skin was collected from non-blush area using a fruit peeler.

Dose (kGy)	Concentration (μ g/g)			
	Chl <i>a</i>	Chl <i>b</i>	Total Chls	Carotenoids
‘B74’				
0	7.4	10.6	18	76.3
0.5	31.4	20.2	51.6	42.4
1.0	28.2	19.5	47.7	42.9
LSD ($P = 0.05$)	n.s.	n.s.	n.s.	n.s.
‘Honey Gold’				
0	11.5 a	19.4	30.9	141.2
0.5	15.6 ab	14.8	30.4	111.4
1.0	21.8 b	17.3	39.1	127.6
LSD ($P = 0.05$)	7.3	n.s.	n.s.	n.s.
‘Kensington Pride’				
0	11.7 a	12.6 a	24.3 a	54.9 a
0.5	61.4 b	31.4 b	92.8 b	65.0 ab
1.0	62.1 b	32.3 b	94.4 b	69.8 b
LSD ($P = 0.05$)	19.6	7.7	26.7	11.7
‘R2E2’				
0	9.0 a	11.9 a	20.9 a	155.4
0.5	35.6 b	26.3 b	61.8 b	106.6
1.0	36.5 b	25.4 b	61.9 b	118.1
LSD ($P = 0.05$)	9.7	6.3	14.6	n.s.

Data represent means for a total of $n = 5$ plus two laboratory replicates per replication. Means within assessed parameter columns with different lower case letters differ significantly ($P \leq 0.05$) by Fisher's protected LSD. n.s. indicates no significant difference ($P > 0.05$).

Table 4.9 Effect of irradiation (0, 0.5, 1.0 kGy) on Chl *a*, Chl *b*, total Chls and carotenoid concentrations of ripened ‘B74’, ‘Honey Gold’, ‘Kensington Pride’ and ‘R2E2’ mango skin. Green mature fruit were harvested from south east Queensland (SEQ) in the 2013/2014 season and exposed to γ -irradiation. Fruit were treated with 10 μ l/l ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe. The skin was collected from non-blush area using a fruit peeler.

Dose (kGy)	Concentration (μ g/g)			
	Chl <i>a</i>	Chl <i>b</i>	Total Chls	Carotenoids
‘B74’				
0	23.3 a	19.3 a	42.5 a	67.6
0.5	33.9 ab	25.1 ab	59.0 ab	69.7
1.0	49.7 b	30.4 b	80.1 b	69.3
LSD ($P = 0.05$)	18.7	8.2	26.3	n.s.
‘Honey Gold’				
0	18.6	28.7	47.4	154.7
0.5	20.2	27.7	47.9	155.3
1.0	25.2	31.1	56.3	167.5
LSD ($P = 0.05$)	n.s.	n.s.	n.s.	n.s.
‘Kensington Pride’				
0	30.1 a	28.8 a	58.9 a	108.0
0.5	80.6 b	39.9 ab	120.5 b	79.8
1.0	90.6 b	42.1 b	132.7 b	83.3
LSD ($P = 0.05$)	21.5	11.4	31.1	n.s.
‘R2E2’				
0	19.7 a	29.0 a	48.7 a	155.2
0.5	52.4 b	38.6 b	90.9 b	179.2
1.0	45.8 b	34.8 ab	80.6 b	149.8
LSD ($P = 0.05$)	8.0	7.3	14.0	n.s.

Data represent means for a total of $n = 5$ plus two laboratory replicates per replication. Means within assessed parameter columns with different lower case letters differ significantly ($P \leq 0.05$) by Fisher's protected LSD. n.s. indicates no significant difference ($P > 0.05$).

Table 4.10 Effect of irradiation (0, 0.5, 1.0 kGy) on Chl *a*, Chl *b*, total Chls and carotenoid concentrations of ripened ‘B74’, ‘Honey Gold’, ‘Kensington Pride’ and ‘R2E2’ mango flesh. Green mature fruit were harvested from north Queensland (NQ) in the 2013/2014 season and exposed to γ -irradiation. Fruit were treated with 10 μ l/l ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe.

Dose (kGy)	Concentration (μ g/g FW)			
	Chl <i>a</i>	Chl <i>b</i>	Total Chls	Carotenoids
‘B74’				
0	8.1	14.0	22.1	14.7 b
0.5	8.1	13.7	21.7	12.6 b
1.0	8.2	14.1	22.2	9.6 a
LSD ($P = 0.05$)	n.s.	n.s.	n.s.	2.7
‘Honey Gold’				
0	3.3	5.4	8.7	22.7
0.5	3.5	5.7	9.2	17.7
1.0	3.8	6.9	10.7	18.8
LSD ($P = 0.05$)	n.s.	n.s.	n.s.	n.s.
‘Kensington Pride’				
0	5.6	9.7	15.3	22.3 b
0.5	2.5	4.1	6.6	15.8 a
1.0	4.8	8.3	13.1	15.4 a
LSD ($P = 0.05$)	n.s.	n.s.	n.s.	3.5
‘R2E2’				
0	6.4	10.9	17.4	18.2 b
0.5	4.3	7.8	12.0	8.4 a
1.0	6.2	10.7	16.8	13.7 ab
LSD ($P = 0.05$)	n.s.	n.s.	n.s.	6.2

Data represent means for a total of $n = 5$ plus two laboratory replicates per replication. Means within assessed parameter columns with different lower case letters differ significantly ($P \leq 0.05$) by Fisher's protected LSD. n.s. indicates no significant difference ($P > 0.05$).

Table 4.11 Effect of irradiation (0, 0.5, 1.0 kGy) on Chl *a*, Chl *b*, total Chls and carotenoid concentrations of ripened ‘B74’, ‘Honey Gold’, ‘Kensington Pride’ and ‘R2E2’ mango flesh. Green mature fruit were harvested from south east Queensland (SEQ) in the 2013/2014 season and exposed to γ -irradiation. Fruit were treated with 10 μ l/l ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe.

Dose (kGy)	Concentration (μ g/g FW)			
	Chl <i>a</i>	Chl <i>b</i>	Total Chls	Carotenoids
‘B74’				
0	7.3	12.1	19.4	8.9
0.5	10.1	16.9	26.9	9.0
1.0	13.1	21.8	35.0	10.0
LSD ($P = 0.05$)	n.s.	n.s.	n.s.	n.s.
‘Honey Gold’				
0	3.4	6.1	9.5	33.5
0.5	3.1	5.8	8.9	26.5
1.0	4.2	7.5	11.7	27.2
LSD ($P = 0.05$)	n.s.	n.s.	n.s.	n.s.
‘Kensington Pride’				
0	8.5	14.6	23.0	23.8
0.5	5.1	8.6	13.7	16.3
1.0	8.4	13.8	22.2	21.2
LSD ($P = 0.05$)	n.s.	n.s.	n.s.	n.s.
‘R2E2’				
0	10.5	18.5 ab	29.0 ab	29.7
0.5	14.6	25.5 b	40.1 b	28.6
1.0	8.1	13.7 a	21.7 a	24.4
LSD ($P = 0.05$)	n.s.	9.1	14.4	n.s.

Data represent means for a total of $n = 5$ plus two laboratory replicates per replication. Means within assessed parameter columns with different lower case letters differ significantly ($P \leq 0.05$) by Fisher's protected LSD. n.s. indicates no significant difference ($P > 0.05$).

Aroma volatiles

Exposure of green mature fruit to irradiation generally reduced biosynthesis rates of the important aroma volatile 2-carene, 3-carene, α -terpinene, *p*-cymene, limonene, α -terpinolene, and ethyl octanoate in the ripened pulp of 'Kensington Pride' and 'R2E2' mango fruit from NQ, except hexanal (Table 4.12). For example, irradiation of 'Kensington Pride' mango fruit at 0.5 and 1.0 kGy reduced the production of α -terpinolene, the most abundant compound in the mango pulp, by approximately 66% and 76%, respectively, as compared to the control (i.e. 0 kGy) fruit. Production of 3-carene, the second major volatile component in ripe 'Kensington Pride', was reduced by 53% for 0.5 kGy and about 77% for 1.0 kGy in irradiated fruit relative to controls. There was significant reduction in limonene production in 'Kensington Pride' mango fruit exposed to the commercial dose (0.5kGy) and high dose (1.0kGy). α -Terpinene concentration in the pulp of 'Kensington Pride' fruit with doses up to 1.0 kGy was lower than that in the control fruit. Similar decreases at the two irradiation doses were found, for example, in the aromatics 2-carene, β -cymene and ethyl octanoate (Table 4.12).

Similarly, the pulp of 'R2E2' mango fruit irradiated at 0.5 and 1.0 kGy exhibited significantly reduced production of aroma volatiles (i.e., 2-carene, 3-carene, α -terpinene, *p*-cymene, limonene, α -terpinolene) in the pulp of the ripe fruit as compared to the control, except for hexanal and ethyl octanoate. There was no significant effect of irradiation on ethyl octanoate production. Ethyl octanoate concentration was minor as compared to other compounds analysed in 'R2E2'. The concentration of α -terpinolene in irradiated (0.5 and 1.0 kGy) 'R2E2' mango fruit was 0.6–0.8-fold lower than in the control fruit. In contrast, irradiation with 0.5 and 1.0 kGy did not affect the concentration of the aroma volatiles hexanal, 2-carene, 3-carene, α -terpinene, β -cymene, limonene, α -terpinolene and ethyl octanoate as produced by ripe 'B74' and 'Honey Gold' cultivars from NQ.

In SEQ, similar findings were found in 'Kensington Pride' and 'R2E2' responses to irradiation treatment (Table 4.13). Irradiation had a negative impact on aroma production by the pulp with marked decreases of aroma concentrations while maintaining minor hexanal concentrations. The content of α -terpinolene was significantly lower by 58 and 80% less, respectively, in 'Kensington Pride' fruit subjected to 0.5 and 1.0 kGy as compared with the non-irradiated fruit. α -Terpinolene also decreased by 63% for 0.5 kGy and 75% for 1.0 kGy in 'R2E2' fruit relative to their controls.

Table 4.12 Effect of irradiation (0, 0.5, 1.0 kGy) on the aroma production of ripened ‘B74’, ‘Honey Gold’, ‘Kensington Pride’ and ‘R2E2’ mango flesh. Green mature fruit were harvested from north Queensland (NQ) in the 2013/2014 season and exposed to γ -irradiation. Fruit were treated with 10 μ l/l ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe.

Dose (kGy)	Compound (μ g/kg)							
	Hexanal	2-Carene	3-Carene	α -Terpinene	β -Cymene	Limonene	α -Terpinolene	Ethyl octanoate
‘Kensington Pride’								
0	27	96 a	990 a	111 a	28 a	556 a	51,293 a	1.0 a
0.5	37	9 b	337 b	38 b	13 b	246 b	17,617 b	0.1 b
1.0	64	7 b	272 b	26 b	12 b	188 b	12,213 b	n.d.
LSD ($P = 0.05$)	n.s.	54	606	67	11	270	30,308	0.7
‘B74’								
0	4	13	475	15	10	160	6,489	0.4
0.5	5	16	703	19	12	184	8,938	0.7
1.0	5	6	1263	34	17	263	15,934	0.3
LSD ($P = 0.05$)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Honey Gold’								
0	6	8	74	11	7	136	2,864	0.3
0.5	6	6	68	9	7	124	2,642	n.d.
1.0	7	4	62	7	7	137	2,121	0.1
LSD ($P = 0.05$)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 4.12 (Continued)

Dose (kGy)	Compound ($\mu\text{g}/\text{kg}$)							
	Hexanal	2-Carene	3-Carene	α -Terpinene	β -Cymene	Limonene	α -Terpinolene	Ethyl octanoate
R2E2'								
0	40	15 a	112 a	11 a	11 a	127 a	4,807 a	1.3
0.5	44	3 b	44 b	5 b	7 b	99 b	1,085 b	0.8
1.0	32	8 b	51 b	6 b	7 b	103 b	1,844 b	0.4
LSD ($P = 0.05$)	n.s.	5	44	4	3	22	2,434	n.s.

Data represent means for a total of $n = 5$ plus three laboratory replicates per replication. Means within assessed parameter columns with different lower case letters differ significantly ($P \leq 0.05$) by Fisher's protected LSD. n.s. indicates no significant difference ($P > 0.05$). n.d.: not detected (it belows limit of detection).

The pulp of 'Kensington Pride' non-irradiated fruit also produced 2 to 21-fold higher concentrations of 2-carene, 3-carene, α -terpinene, β -cymene and limonene than for those irradiated at 0.5 and 1.0 kGy. Similarly, 'R2E2' non-irradiated fruit showed 2 to 6-fold higher concentrations of 2-carene, 3-carene, α -terpinene, β -cymene and limonene as compared to those irradiated at 0.5 and 1.0 kGy.

Table 4.13 Effect of irradiation (0, 0.5, 1.0 kGy) on the aroma production of ripened ‘B74’, ‘Honey Gold’, ‘Kensington Pride’ and ‘R2E2’ mango flesh. Green mature fruit were harvested from south east Queensland (SEQ) in the 2013/2014 season and exposed to γ -irradiation. Fruit were treated with 10 μ l/l ethylene for 2 d at 20 °C inside a ripening room and maintained at 20 °C and 90 - 100% RH until ripe.

Dose (kGy)	Compound (μ g/kg)								
	Hexanal	2-Carene	3-Carene	α -Terpinene	β -Cymene	Limonene	α -Terpinolene	Ethyl octanoate	
‘Kensington Pride’									
0	21 b	42 a	411 a	48 a	15 a	297 a	22,993 a	1.0 a	
0.5	69 ab	2 b	195 b	20 b	7 b	171 b	9,607 b	0.6 b	
1.0	106 a	12 b	93 b	13 b	7 b	126 b	4,577 b	0.5 b	
LSD ($P = 0.05$)	51	18	190	20	5	108	10,933	0.5	
‘B74’									
0	8	50	1205	24	10	273	14,629	0.5	
0.5	6	64	893	25	11	205	10,752	0.7	
1.0	8	24	1035	21	10	211	10,887	0.7	
LSD ($P = 0.05$)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
Honey Gold’									
0	2	9	100	10	7	117	3,454	n.d.	
0.5	2	6	50	7	6	107	1,698	n.d.	
1.0	4	1	42	6	5	96	1,263	n.d.	
LSD ($P = 0.05$)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.d.	

Table 4.13 (Continued)

Dose (kGy)	Compound ($\mu\text{g}/\text{kg}$)							
	Hexanal	2-Carene	3-Carene	α -Terpinene	β -Cymene	Limonene	α -Terpinolene	Ethyl octanoate
R2E2'								
0	39	16 a	104 a	10 a	11 a	125 a	4,348 a	1.2
0.5	53	3 b	44 b	5 b	7 b	101 b	1,585 b	0.8
1.0	36	6 b	47 b	5 b	7 b	99 b	1,067 b	0.5
LSD ($P = 0.05$)	n.s.	5	38	4	3	19	2,150	n.s.

Data represent means for a total of $n = 5$ plus three laboratory replicates per replication. Means within assessed parameter columns with different lower case letters differ significantly ($P \leq 0.05$) by Fisher's protected LSD. n.s. indicates no significant difference ($P > 0.05$). n.s indicates no significant difference. n.d: not detected (it belows limit of detection).

4.4 Discussion

γ -Irradiation at 0.5 and 1.0 kGy generally reduced initial rates of softening for 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' mango fruit relative to their controls, but there were no differences in firmness at ripe (Figures 4.1 and 4.2). These data are in general agreement with the observations of Mahto and Das (2013) who reported that 'Dushehri' and 'Fazli' mangoes exposed to low doses (0.3 – 1 kGy) showed lower rates of initial softening as compared with non-irradiated fruit. Boag et al. (1990) found that irradiation at 0.75 kGy did not affect softening of 'Kensington pride' mangoes after storage at 20 °C. In contrast, 'Tommy Atkins' mangoes irradiated at 1.5 and 3.1 kGy showed higher rates of softening and stiffness as compared with control fruit during storage at 12 °C for 21 d. (Moreno et al., 2006). The firmness of 'Tommy Atkins' mangoes exposed to 1.0 kGy were lower throughout storage at 11 °C for 10 d and at 22 °C for 12 d as compared to control fruit (Sabato et al., 2009). This suggested that fruit irradiated at above 1.0 kGy softened more rapidly than their controls after long storage at low temperature. The observation that higher softening rates in irradiated fruit at above 1.5 kGy be explained by the proposition of Moreno et al. (2006) that fruit softening induced by irradiation at above 1.5 kGy was due to the changes in the cell structure, surface depressions and cell disruption.

Exposure of green mature 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' mango fruit to 0.5 and 1.0 kGy doses significantly reduced external appearance quality by retarding the loss of green skin colour and enhancing the development of LD (Figures 4.4 - 4.14). However, 'Honey Gold' showed the least pronounced effects in term of green skin colour retention. Similar findings were reported by Boag et al. (1990), where irradiation of 'Kensington Pride' fruit at 0.75 kGy retarded the loss of green skin colour relative to non-irradiated controls. Moreno et al. (2006) also observed that the green skin colour loss from 'Tommy Atkins' mango was retarded by exposure to an irradiation dose of 3.1 kGy after storage. Green skin colour in irradiated 'Tommy Atkins' fruit contributed to significantly higher *L* value as compared to non-irradiated fruit.

'B74' and 'Honey Gold' fruit usually showed the greatest and least LD, respectively, in response to irradiation as compared with the other cultivars, but this trend was not consistent across all seasons and locations (Figures 4.13 - 4.15). This response variation was likely due to the nature of genotypic differences (Bally et al., 1996; Dillon et al., 2013). This result accords with Hofman et al. (2010c), who found a significant increase of LD of 'B74' mangoes exposed to 0.543 kGy relative to non-irradiated fruit. Furthermore, severe skin browning was observed towards the end of shelf life in some irradiated 'Kensington Pride' and 'R2E2' fruit (Figure 4.12).

Irradiation at 0.5 and 1.0 kGy generally did not affect TSS at ripe (Tables 4.5 and 4.6). Likewise, Lacroix et al. (1990) and Sabato et al. (2009) found that irradiating 'Keitt' or 'Tommy Adkins' mango fruit at 1.0 kGy of γ -irradiation did not significantly affect TSS. Moreno et al. (2006) reported that exposure to 1.0 and 3.1 kGy irradiation delayed the ripening-related increase in TSS of 'Tommy Adkins' fruit.

γ -Irradiation doses of up 1.0 kGy showed different cultivar dependant responses on other physico-chemical properties of mango fruit (Tables 4.5 - 4.11). The lower skin colour rating of greener colour and significantly higher TA in irradiated 'Kensington Pride' and 'R2E2' fruit at ripe suggests that rates of these processes were slowed more by irradiation as compared with the other tested cultivars, 'B74' and 'Honey Gold'. The net result is greener more acidic fruit at ripe. Palafox-Carlos et al. (2012) showed that both flesh acidity and green skin colour decrease during mango fruit ripening. El-Samahy et al. (2000) found that irradiation resulted in 'Zebda' mango fruit with higher TA at ripe stage.

'B74', 'Kensington Pride' and 'R2E2' mango fruit exposed to 0.5 and 1.0 kGy showed higher level of Chl *a*, Chl *b* and Chl contents at ripe as compared to the fruit without irradiation in both harvest seasons (Tables 4.7 - 4.9). Although reductions in rates of de-greening presumably represent a decrease in chlorophyll degradation, this still needs to be confirmed in terms of the biochemistry. The losses of green chlorophyll pigment during mango ripening to reveal yellow carotenoid pigments is typically associated with increased chlorophyllase and peroxidase activities (Ketsa et al., 1999). Moreover, doses of 0.5 and 1.0 kGy did not affect the carotenoid concentration of 'Honey Gold' flesh samples from both locations (Tables 4.10 - 4.11). A similar effect was observed by Reyes and Cisneros-Zevallos (2007), where irradiation in the range of 1.0 - 3.1 kGy did not affect the total carotenoid concentration of 'Tommy Atkins' mango flesh.

Irradiation at 0.5 kGy and 1.0 kGy reduced aroma volatile production in 'Kensington Pride' and 'R2E2' mangoes (Tables 4.12 and 4.13). Retardation of aroma volatile production by irradiation at 0.5 and 1.0 kGy was associated with the inhibition of chlorophyll degradation and carotenoid synthesis (Figure 4.15). The reduced concentrations of aroma volatiles in irradiated fruit may in fact be due to the inhibition of normal ripening-related skin yellowing and the decrease in chlorophyll degradation. The inhibition of de-greening was presumably an irradiation-induced suppression of the synthesis or activity of enzyme systems, like chlorophyllase, as involved in the regulation of Chl breakdown (Kaewsuksaeng et al., 2011). The development of yellow-orange skin colouration during mango ripening is due to carotenoids accumulation in the mesocarp (Vásquez-Caicedo et al., 2005). The relationship between pigments and aroma volatiles is expected to be relatively strong

(Defilippi et al., 2009). Some aroma components such as norisoprenoids are derived from carotenoids (Defilippi et al., 2009; Winterhalter and Rouseff, 2002).

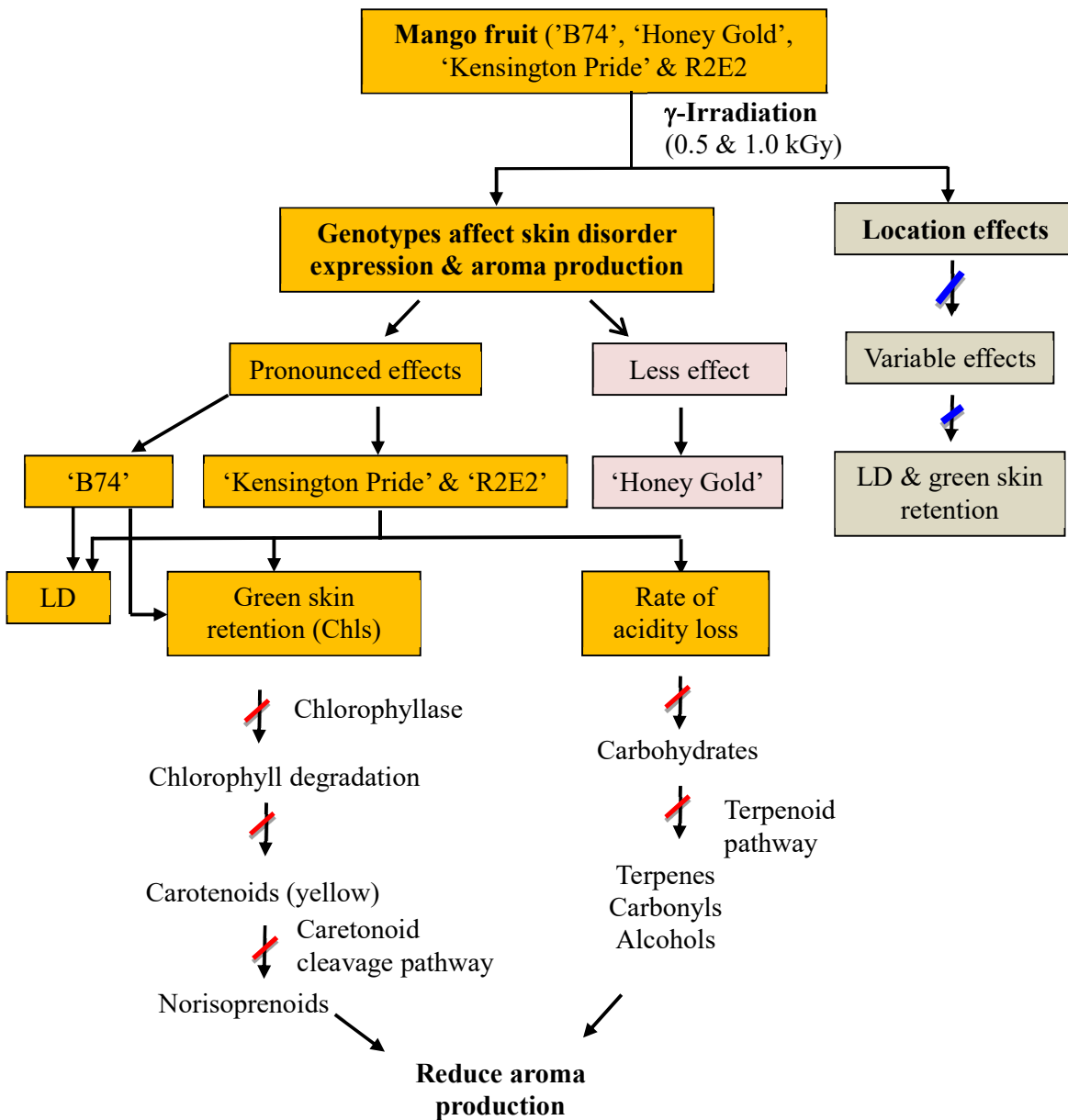


Figure 4.15 A diagrammatic model of γ -irradiation effects on appearance, physico-chemical properties and aroma of four Australian mango fruit cultivars based on currently findings (this chapter) and published work (Defilippi et al., 2009; Schwab et al., 2008; Winterhalter and Rouseff, 2002). Chls, chlorophylls; blue slashes, suppressed; red slashes, suppressed.

On the other hand, there was no significant irradiation effect on volatile biosynthesis by 'B74' and 'Honey Gold' from SEQ (Tables 4.12 and 4.13). Neither a significant general increase nor differences of Chl *a*, Chl *b* nor Chl contents between the treatments were discerned for 'Honey

Gold' mango skin (Tables 4.7 - 4.9). These findings suggest that 'B74' and 'Honey Gold' are relatively less sensitive to irradiation-induced reduction in rates of volatile production. It is possible that irradiation has a more dramatic impact on fruit that are typically higher in volatile components to begin with. Conversely, irradiation may have little or impact on fruits that typically have low volatiles composition. It suggests that the use of irradiation as a disinfestation treatment should be manipulated by further post-harvest practices (suitable fruit ripeness stage for irradiation or proper coatings) to optimise the irradiation procedure and deliver high-quality fruit to the market. In future refinement of this work, complimentary sensory evaluation is recommended towards fully understanding the impacts of irradiation-induced changes in, for example, TA and aroma on consumer satisfaction.

4.5 Conclusion

In order to sterilise insect pests and meet quarantine requirements, irradiation doses of 0.4+ kGy are applied in the horticultural industry. However, γ -irradiation at mature green for both the recommended commercial level of 0.5 and higher for research purposes (viz. positive control) at 1.0 kGy generally reduced the visual appearance quality of 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' mango fruit, particularly by inhibiting chlorophyll degradation and by increasing LD at ripe. γ -Irradiation at 0.5 and 1.0 kGy elicited different cultivar dependant responses in the physico-chemical properties of the fruit. Increased TA and retarded chlorophyll degradation of irradiated and ripened 'Kensington Pride' and 'R2E2' fruit at 0.5 kGy coupled with reduction in volatiles with a higher dose at 1.0 kGy may translate into diminished flavour. In contrast, there were no notable effects of irradiation treatment at 0.5 and 1.0 kGy on the 'Honey Gold' cultivar. Knowing the relative susceptibility to irradiation damage in terms of appearance and physical-chemical properties for various mango cultivars, in this case Australian, represents opportunity for the mango industry to assure the quality of irradiated fruit. Moreover, understanding irradiation effects on mango fruit appearance (e.g. LD, skin de-greening), taste (e.g. TSS, TA) and flavour (e.g. aroma synthesis) responses should inform postharvest practices to optimise the overall quality of irradiated fruit.

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CHAPTER 5. ANATOMICAL OBSERVATIONS OF LENTICEL DISCOLOURATION IN AUSTRALIAN MANGO FRUIT SKIN

Abstract

Lenticel discolouration (LD) is a disorder of concern to the mango industry. The disorder is typically characterised by brown to black lenticels on the surface of mango fruit visible as small round spots sometimes comprised of a bordered centre surrounded by a white corona. To describe anatomical features of non-coloured and discoloured lenticels towards better understanding the mechanisms of LD in the Australian mango fruit cultivars including 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2, binocular microscope observations, free-hand sectioning, light microscopy (LM) and serial block-face scanning electron microscopy (SBF-SEM) were employed. There was no pronounced difference in the morphology of lenticels between these four cultivars. Morphological examination with LM showed LD clearly evident as browning in the cell wall of sub-lenticellular cells around the cavity. No such cell browning was observed in non-discoloured lenticels. Based on SBF-SEM results, the browning was related to phenolic deposition in the cell walls and of cytoplasm of sub-lenticellular cells surrounding the lenticel cavity. Three different types of phenolic deposition were evident as polymerised electron-dense phenolic depositions in the cell walls and cytoplasm and a discrete deposit of phenolics in the cytoplasm of sub-lenticellular cells surrounding the cavity of discoloured lenticels. Only a discrete deposit of phenolics characterised by spherical dark granules was observed in the non-coloured lenticel. Greater knowledge of exterior, central and periphery regions of the lenticels and detailed ultrastructural changes in the partitioning of phenolics as associated with LD has contributed to an improved understanding of the mechanism of LD.

5.1 Introduction

Skin appearance is an important quality criterion for fresh fruit. Surface defects can reduce perceived quality with undesirable effects on customers' value decisions to purchase. Lenticel discolouration (LD) is an important mango fruit skin disorder in terms of reduction in fruit appearance and economic value (Bezuidenhout et al., 2005).

Lenticels in mango fruit are macroscopic pits on the skin (peel) that contribute or contributed to gaseous exchange and transpiration. Lenticels in mango can derive from stomata (Bally, 1999). They enlarge during fruit growth due largely to stretching of the fruit surface; i.e. epidermis (Bezuidenhout et al., 2005; Rymbai et al., 2012). LD on mango skin produces brown, black or red

discoloured spots in and around lenticels. The brown and black discolouration involves vacuolar deposition of phenolic compounds in cells surrounding the lenticel cavity (Du Plooy et al., 2009). Brown or black lenticels were also associated with accumulation of quinine in cell walls (Bezuidenhout and Robbertse, 2005). Red lenticel spotting was related to production of anthocyanins in sub-lenticel cells (Self et al., 2006).

Micro-structural properties of typical lenticels have been described for some mango cultivars. For instance, lenticel development and subsequent discolouration in ‘Tommy Atkins’ and ‘Keitt’ mango fruit were studied using LM by Bezuidenhout et al. (2005). The discoloured lenticels were associated with natural pigment deposition such as quinine in the cell walls (Bezuidenhout et al., 2005; Tamjinda et al., 1992). In terms of discolouration, differences of LD localisation and chemical changes in ‘Tommy Atkins’ mango fruit were characterised by Du Plooy et al. (2006) using transmission electron microscopy (TEM). In this study, LD was associated with the deposition of electron-dense materials from the cell wall to the inside of cells.

Physicochemical, including micro-structural properties of LD on Australian mango cultivars are largely undescribed. This is perhaps in part because key micro-morphological methodology (viz., LM, SEM, TEM, etc.) is an intricate and expensive approach to gaining structural functional information on biological tissues (Fowke et al., 1994). In this light, SBF-SEM offers a relatively new cost-effective method to align stacks of serial images, including gaining insight in three-dimensional (3D) structure (Feng et al., 2016). Accordingly, comparative visualisation of non-discoloured and coloured lenticels using SBF-SEM could contribute to better understanding the physiochemistry of LD.

To better understand the physicochemical mechanism of LD on fruit of the Australian mango cultivars ‘B74’, ‘Honey Gold’, ‘Kensington Pride’ and ‘R2E2’, this study sought to: (1) comparatively describe the general features of non-discoloured and coloured lenticels on a microscopic scale; and, (2) comparatively describe ultrastructural properties in the morphology of the non-coloured and discoloured lenticels of ‘B74’ mango fruit. In due course, it is anticipated that this basic information will help to inform commercial practices to mitigate post-harvest losses due to this disorder.

5.2 Materials and methods

5.2.1 Materials

'B74' (dry matter content of $13.9 \pm 1.7\%$), 'Honey Gold' (dry matter content of $18.2 \pm 0.4\%$) and 'R2E2' (dry matter content of $16.6 \pm 0.8\%$) mango (*Mangifera indica* L.) fruit were collected at commercial green maturity stages from orchards near Dimbulah (17.149°S , 145.111°E), North Queensland (NQ), Australia. 'Kensington Pride' mango fruit (dry matter content of $13.1 \pm 0.4\%$) was harvested at commercial maturity from near Mareeba (16.992°S , 145.422°E), NQ, Australia.

All fruit were de-sapped in 2.5 g/l Mango Wash[®] (Septone, ITW AAMTech, Australia) solution for 2 min. They were then taken to a nearby commercial packing house and passed through the pack line under standard commercial practices, including fungicide treatment (Sportak[®], a.i. prochloraz, Bayer Crop Science, Australia), brushing, drying and sorting (Hofman et al., 2010). The fruit were then graded for uniform quality and size as per commercial procedures (Hofman et al., 2010). They were packed into single-layer cardboard trays with polyethylene liners. The fruit were air-freighted to Brisbane over 2 h and then transported by car at ca. 22°C over about 1 - 2 h to the Maroochy Research Facility postharvest laboratory in Nambour, Queensland, Australia (26.62°S , 152.95°E). There, they were treated with 10 $\mu\text{l/l}$ (ppm) ethylene as Ripegas (BOC, Kunda Park, Australia) for 2 d inside a closed ripening room at 20°C and 90 - 100% RH.

5.2.2 Binocular microscope observations

Rectangle skin tissues of B74' mango fruit ~ 2 cm width, $\sim 3 - 4$ mm length bearing non-coloured and discoloured lenticels were excised with a razor blade. The symptoms were photographed using a digital camera (Canon DOS40D, Canon Inc., Japan) fitted with fitted with a Canon macro-zoom len EF-S 60 mm (Canon Inc., Tokyo, Japan). The lenticels were then examined using an Olympus SZH10 research stereo microscope (Olympus, Japan) equipped with Micro Publisher 3.3 RTV digital camera (Q-Imaging, Germany).

5.2.3 Free-hand sectioning

Non-coloured and brown lenticels on 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' fruit ($n = 3$) were compared at the cellular level. Free-hand sections (approximate 0.038 mm) were cut through the excised tissues after Ruzin (1999). They were transferred onto a drop of distilled water on a glass microscope slide and covered with glass cover slip. The free-hand sections were viewed and photographed with an Olympus BX61 light microscope equipped with a DP 70 camera (Olympus, Japan).

5.2.4 Light microscopy

Specimens of mango fruit skin containing lenticels ($n = 3$) were dissected from ripe 'B74' fruit and fixed in FAA (50 ml ethanol 95%, 5 ml glacial acetic acid, 10 ml formalin formaldehyde 37 - 40%, 35 ml distilled water). After dehydration through an increasing ethanol series of 70, 90 and 100% twice (45 min each change), samples were infiltrated with 100% xylene twice for 45 min each time. Samples were covered with paraffin wax (Leica Bio Systems, USA) at 60 °C twice for 45 min each time. Then samples were mounted with paraffin wax at 60 °C and embedded on a Microm EC 350-2 embedding station (Thermo Fisher Scientific Inc., New Zealand). Serial 5 µm-thick sections were cut on a Leica RM 2245 microtome (Leica, Germany). They were de-waxed three times in 100% xylene (RCI Labscan, Thailand) for 2 min at each step and washed in a 100, 100, 90 and 70 % ethanol series. The tissue sections were washed in running water for 2 min and distilled water for 30 s. They were stained with using 0.1% Toluidine Blue O (TBO) in 1% (w/v) borax. Sections were viewed and photographs were taken with an Olympus BX61 microscope (Olympus, Japan) equipped with a DP 70 camera (Olympus, Japan).

5.2.5 SBF-SEM

Fixation

Approximately 1 mm-thick slices of mango pericarp were excised using a scalpel blade (Swann-Morton®, England) and then fixed in 2.5% glutaraldehyde (ProScitech, Australia) in 0.1M sodium phosphate buffer (pH 6.8) containing 1% caffeine (Merck, Australia) for 2 h to preserve phenolics (Mueller and Greenwood, 1978).

Sample preparation (Feng et al., 2016)

The samples ($n = 2$) were washed three times for 5 min each in 1X phosphate buffered saline buffer (PBS). The samples were postfixed for 1 h in 2% osmium tetroxide/1.5% potassium ferric cyanide (Sigma Aldrich, USA) in aqueous solution and washed three times in ultra-high quality water (UHQ) for 5 min each. They were then transferred into 1% thiocarbonylhydrazide solution (Sigma, Australia) for 20 min and stirred occasionally. After washing again in UHQ water the samples were placed in 2% osmium tetroxide for 1 h, washed in UHQ water and placed overnight in a solution of 1% uranyl acetate. The samples were subsequently washed three times in UHQ water and transferred into a 0.66% lead aspartate solution (Sigma Aldrich, China) in a 60 °C oven for 60 min. The specimens were dehydrated in an ascending ethanol series (20%, 50%, 70%, 90%, 100% and 100 %) for 5 min each followed by 100% ice cold dry acetone for 10 min. After infiltration with

Durcupan™ resin (Sigma, USA) and acetone mixes (20%, 50% and 70%) for 2 h each, samples were held in 100% Durcupan overnight followed by another change of 100% Durcupan for 2 h. The samples in 100% Durcupan resin were polymerised in a 60 °C oven for 48 h. The samples were excised from the resin block using a halved stainless-steel shaver blade and mounted on small aluminium pins using conductive epoxy glue. To give extra conductivity to the samples, silver paint was placed around them. The samples were examined using a Zeiss Sigma SEM with Gatan 3View 2XP System operated at 1.5 kV and a magnification of 400 and an aperture of 30 µm. Approximate numbers of 2,000 and 3,260 sections, respectively, were imaged for non-coloured and discoloured lenticels cut with 100 nm slice thickness. The images were each comprised of 4000 x 4000 pixels taken of the sample block-face exposed after each sectioning. The reconstruction of data sets from serial images was conducted in order to observe multiple position and angular views of lenticels. Reconstruction of the image stack into a 3D data set was completed using IMOD software (University of Colorado, USA).

5.3 Results

5.3.1 Binocular microscope observations

On ‘B74’ mango fruit, LD appeared as dark-brown to black dots on the fruit skin (Figure 5.1). The general features of non-coloured and discoloured lenticels on ‘B74’ mangoes are shown in Figure 5.1A-B. Non-coloured lenticels on the surface of mango fruit appeared as small, round spots surrounded by a white corona (Figure 5.1C). Discoloured lenticels consisted of a small round to cross-shaped centre surrounded by a white corona and then a dark brown to black region that gradually dispersed into the mango tissue (Figure 5.1D). No such bordered dark-brown centre was found in non-coloured lenticels.

5.3.2 Free-hand section observations

‘R2E2’ mango fruit (Figure 5.2G, H) appeared to have relatively larger and more open lenticels than those in ‘Kensington Pride’ (Figure 5.2E, F). Moreover, lenticels typically had an irregular vase or bowl-shaped structure cavity. A waxy cuticle covered the full extent of the pore on the corona part of the lenticel. The major difference between non-coloured and discoloured lenticels was the bordered dark-brown centre surrounding the corona. In cross sections, discoloured tissue was mainly manifested in the sub-lenticellular cell layers beneath the cuticle and the sub-epidermal layers of pericarp (Figure 5.2B, D, F, H). In non-discoloured lenticels, there was no such browning of these cells surrounding the cavity (Figure 5.2A, C, E, G). However, morphometric data for

lenticels in sections cut by hand were not made because sections to a side of a cavity did not include the complete opening.

5.3.3 Light microscopy observations

In fixed and embedded discoloured lenticels of 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' mango fruits, a green colour region of TBO stained materials presented in the sub-lenticellular cell layers beneath the epidermis (Figure 5.3). The sub-lenticellular cells surrounding the lenticel cavity were smaller than the other cells of the exocarp. In non-coloured lenticels, there was no evidence of TBO-stained material which would otherwise indicate deposition of phenolics (Figure 5.3 A, C, E, G). Phenolic compounds heavily stained by TBO (i.e. green colour/s vs. purple colour/s) were visible in the sub-lenticellular cell walls surrounding the cavity of discoloured lenticels (Figure 5.3 B, D, F, H).

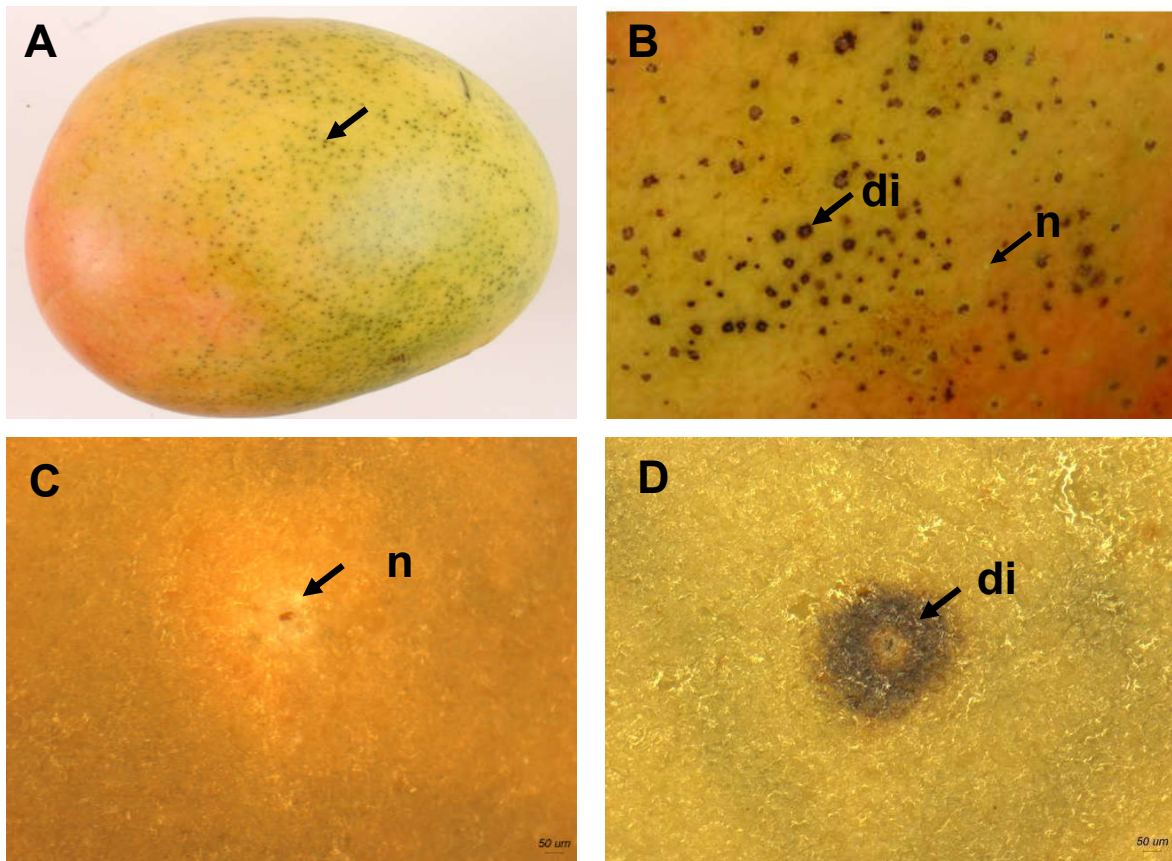


Figure 5.1 Symptom of LD on 'B74' mango fruit. (A) External feature of LD on fruit. (B) Arrows point (panel B) to each of characteristic non-coloured (n) and discoloured (d) lenticels. (C) Arrow point to non-coloured lenticel (B) Arrow point to discoloured lenticel. Scale bars = C, D, 50 μm . n, non-coloured lenticel; di, discoloured lenticel.

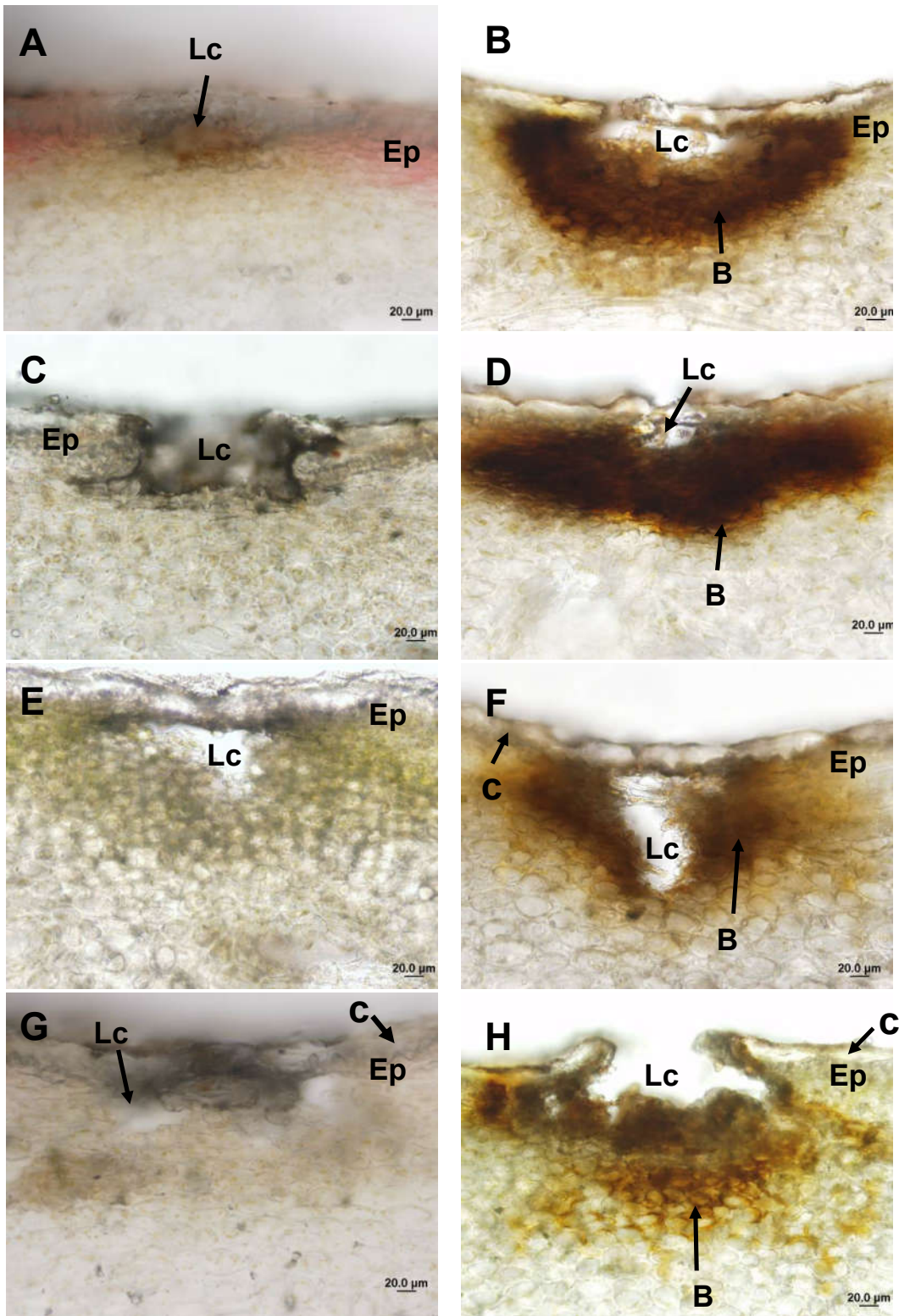


Figure 5.2 Light photomicrographs of lenticels on ‘B74’ (A, B), ‘Honey Gold’ (C, D), ‘Kensington Pride’ (E, F) and ‘R2E2’ (G, H) mango fruit. (A, C, E and G) Transverse sections (TS) showing no browning in non-coloured ‘control’ lenticels. (B, D, F and H) TS showing browning in discoloured lenticels. c, cuticle; Ep, epidermal cells; Lc, lenticel cavity; B, browning. (A-H) Scale bars = 20 µm.

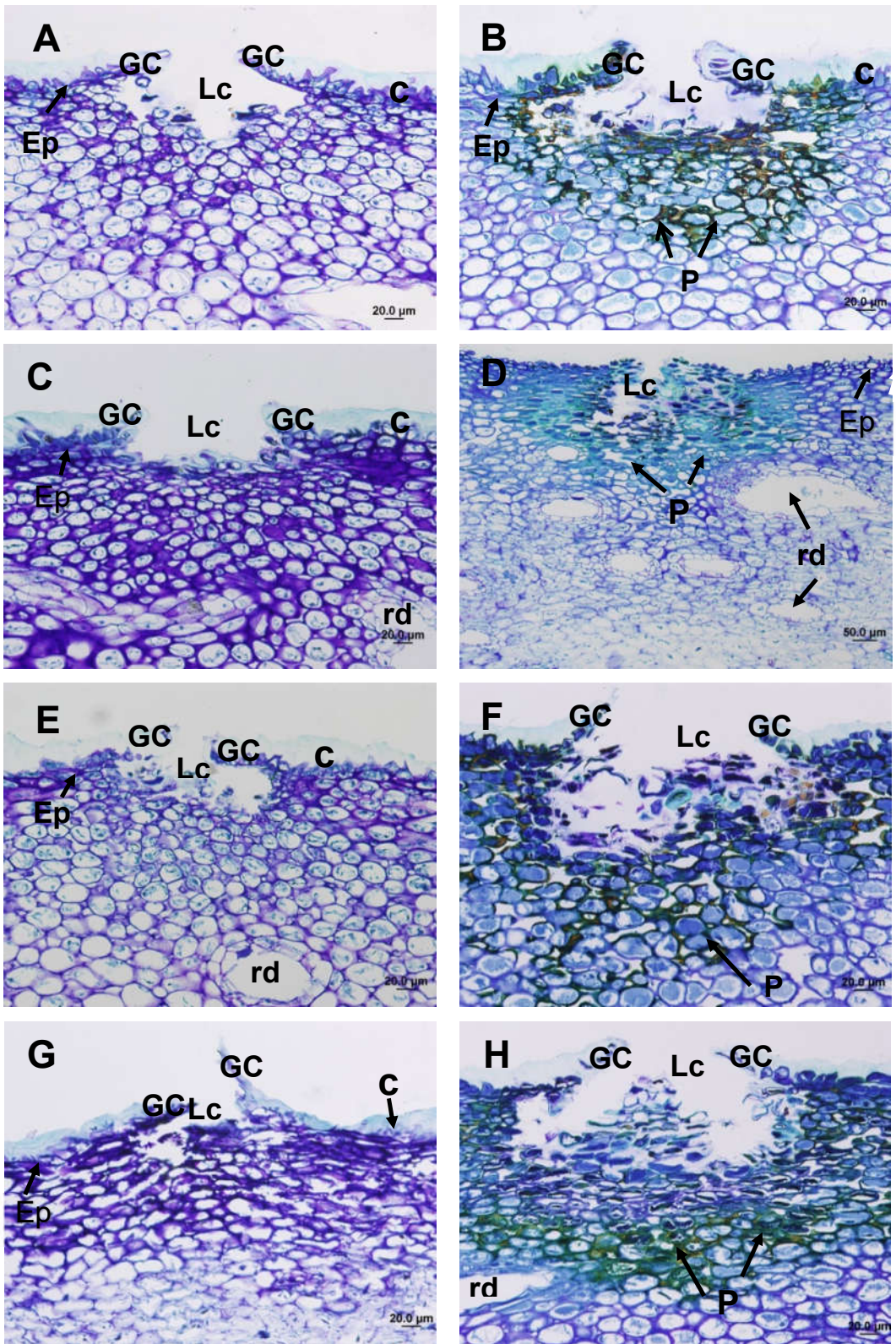


Figure 5.3 Light microscope images of lenticels on ‘B74’ (A, B), ‘Honey Gold’ (C, D), ‘Kensington Pride’ (E, F) and ‘R2E2’ (G, H) mango fruit. The transverse 5 μm -thick sections were examined using the paraffin wax embedding method and stained with metachromatic Toluidine Blue O. (A, C,

E and G) TS showing phenolics are absent as purple staining colour in non-coloured lenticels. (B, D, F and H) TS showing phenolics are present as green staining colour in discoloured lenticels. c, cuticle; Ep, epidermal cells; GC, stomatal guard cells; P, phenolics; rd, resin duct; Lc, lenticel cavity. Scale bars = A-C, E-H, 20 μm ; D, 50 μm .

5.3.4 SBF-SEM observations

SBF-SEM study of non-coloured versus discoloured 'B74' mango fruit lenticels was undertaken to explore their 3D architecture. That is, SBF-SEM was used to better understand and potentially differentiate non-coloured and discoloured complete architecture of lenticels at the cellular level. By reconstruction of data sets from serial images it is possible to show multiple positions and angular views of lenticels.

Some individual sections illustrated detailed ultrastructural information. The electron-dense granular layer above the cuticle in Figure 5.4 is conductive silver paint as applied to the samples to reduce charging potential artifacts. The technique enabled clear visualisation of exterior (Figure 5.4 A-D), central (Figure 5.4 E-F) and periphery (Figure 5.4 G-H) regions of the lenticels. At a cellular level, the discoloured lenticel showed extensive accumulation of electron-dense materials in hypodermis under the cuticle and sub-lenticellular cells surrounding the cavity. The electron-dense materials are most likely polymerised phenolics (Du Plooy et al., 2006; Ramsey and Berlin, 1976).

SBF-SEM imaging involved relatively massive data collection for 3D space capturing complete or almost complete lenticels at relatively high resolution. The image stacks acquired for 'B74' mango fruit consisted of 2,000 continuous aligned sections for a non-coloured lenticel (Movie 5.1a-b) and 3,259 sections for a discoloured lenticel (Movie 5.2). The image stacks illustrated the changes from the outer cell layers to inner cell layers surrounding the lenticel cavity of lenticels.

In Movie 5.1a-b, image stacks consisting of 2,000 aligned sections illustrating the structural features of a non-coloured lenticel of 'B74' mango fruit are presented. (Electronic files 'Movie 5.1a non-coloured lenticel stack' and 'Movie 5.1b non-coloured lenticel stack' attached in the external hard drive). Viewing the SBF-SEM sequence, a progressive change was distinguished in the interior of non-coloured lenticel due to degenerated guard cells and cuticle rupture into the disclosed cavity (in Movie 5.1 and 5.2). In Movie 5.2, the image stack consisting of 3,259 aligned sections illustrates the structural features of a discoloured lenticel of 'B74' mango fruit. (Electronic file 'Movie 5.2 discoloured lenticel stack' attached in the external hard drive). The stack started with a section at

the outermost edge of the cavity, and contiguous sections were recorded every 100 nm from this point.

In Movie 5.3, a reconstruction of a data set from serial block-face images of a non-coloured lenticel of 'B74' mango fruit illustrates the structure of the non-coloured lenticel from different angles. (Electronic file 'Movie 5.3 non-coloured lenticel' attached in the external hard drive). In Movie 5.4, a reconstruction of a data set from serial block-face images of a discoloured lenticel of 'B74' mango fruit illustrates the structure of the discoloured lenticel from different angles (Electronic file 'Movie 5.4 discoloured lenticel' attached in the external hard drive).

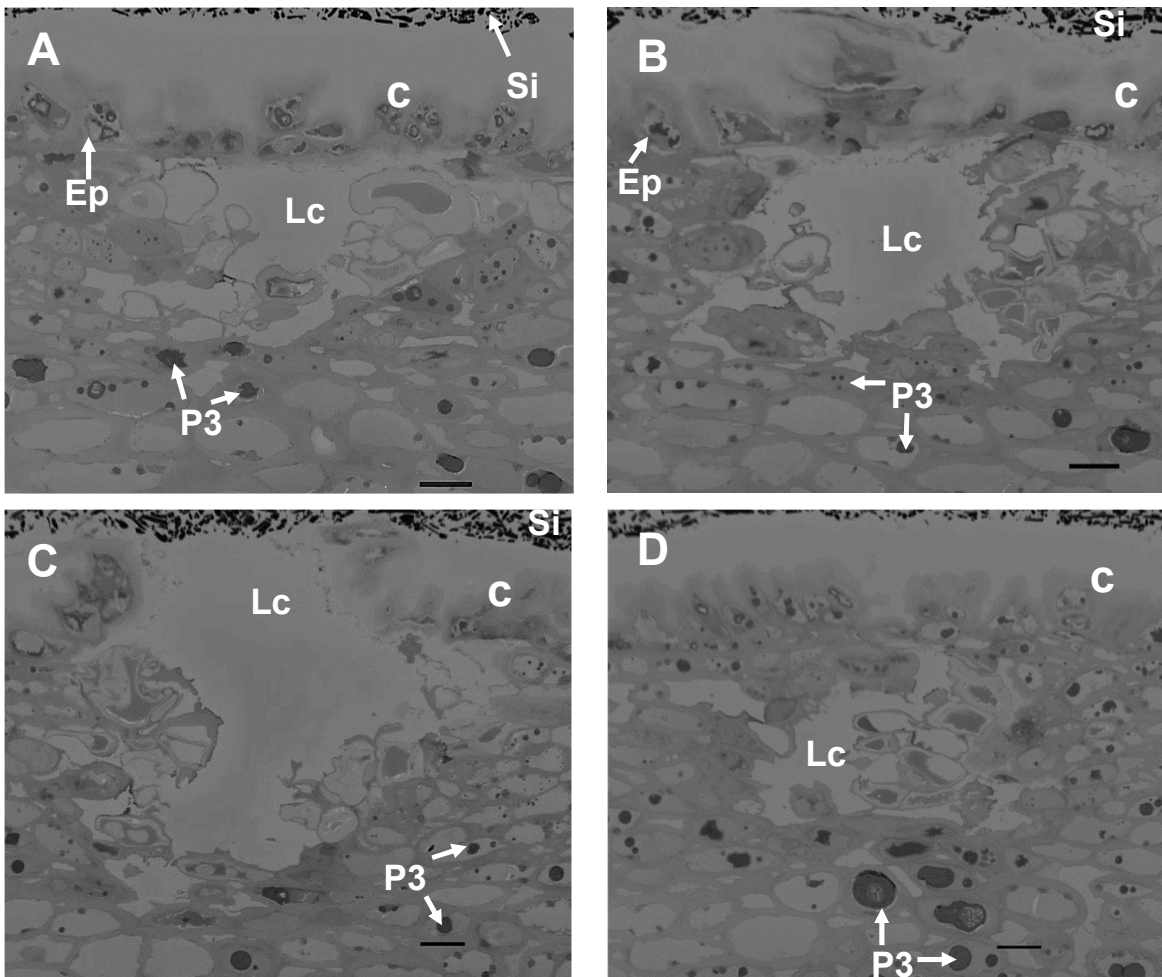


Figure 5.4. Four image slices from 3D datasets of non-coloured lenticel of 'B74' mango fruit. Images A, B, C and D are sections 1, 216, 725 and 1501, respectively, of a total stack of 2,000 sections at 100 nm thickness. These images of a non-coloured lenticel show minimal evidence of electron-dense material accumulation in cells. c, cuticle; Ep, epidermal cells; Lc, lenticel cavity; P3, the third type of phenolics; Si, silver conductive paint layer. Scale bars = 25 μ m.

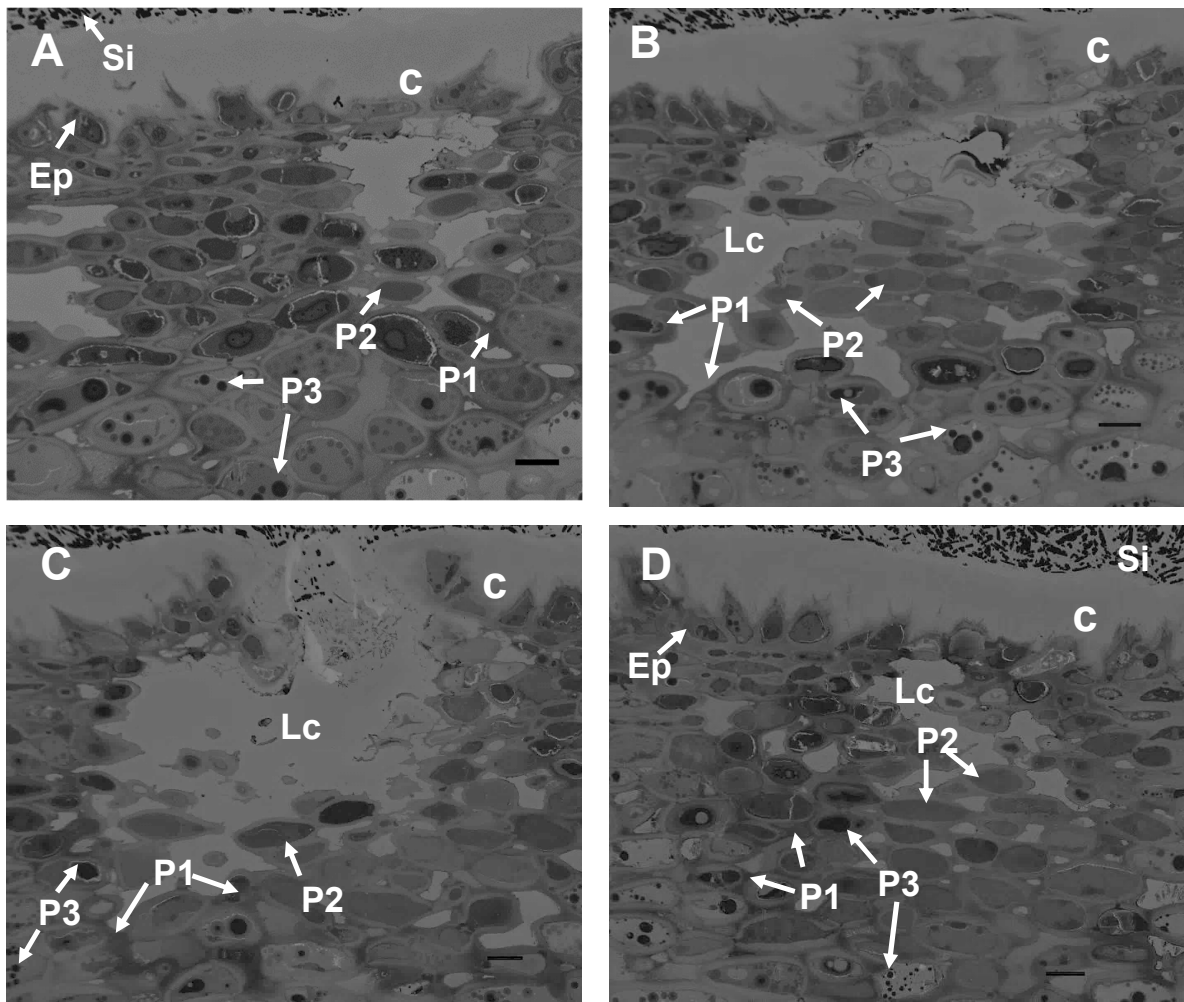


Figure 5.5. Four image slices from 3D datasets of discoloured lenticel of ‘B74’ mango fruit. Images A, B, C and D are sections 776, 1,121, 1680 and 2,800, respectively, of a total stack of 3,259 sections at 100 nm thickness. These images of a discoloured lenticel illustrate the evidence of pronounced electron-dense material accumulation in cells. c, cuticle; Ep, epidermal cells; Lc, lenticel cavity; P1 - P3, three types of phenolics; Si, silver conductive paint layer. Scale bars = 25 μm .

LD was observed from different angles to reflect the slice-to-slice variability and reveal the disparity of actual spatial structure arrangement between non-coloured (Movie 5.3) and discoloured lenticels (Movie 5.4). In the discoloured lenticel, phenolic deposition was more prevalent and occurred as three types (Figure 5.5, 5.6). Firstly, the appearance of electron-dense materials was observed in the cell wall. The second type of deposition was characterised by phenolics completely filling the cytoplasm of sub-lenticellular cells next to the cavity. Thirdly, there were further, apparently different, areas of differing electron density in the cell cytoplasm of whole tissue sections at up to 20 μm in diameter which most likely represented a discrete deposit of phenolics.

The first and second types of polymerised electron-dense phenolic depositions were not observed in the non-coloured lenticel (Figure 5.4, 5.6).

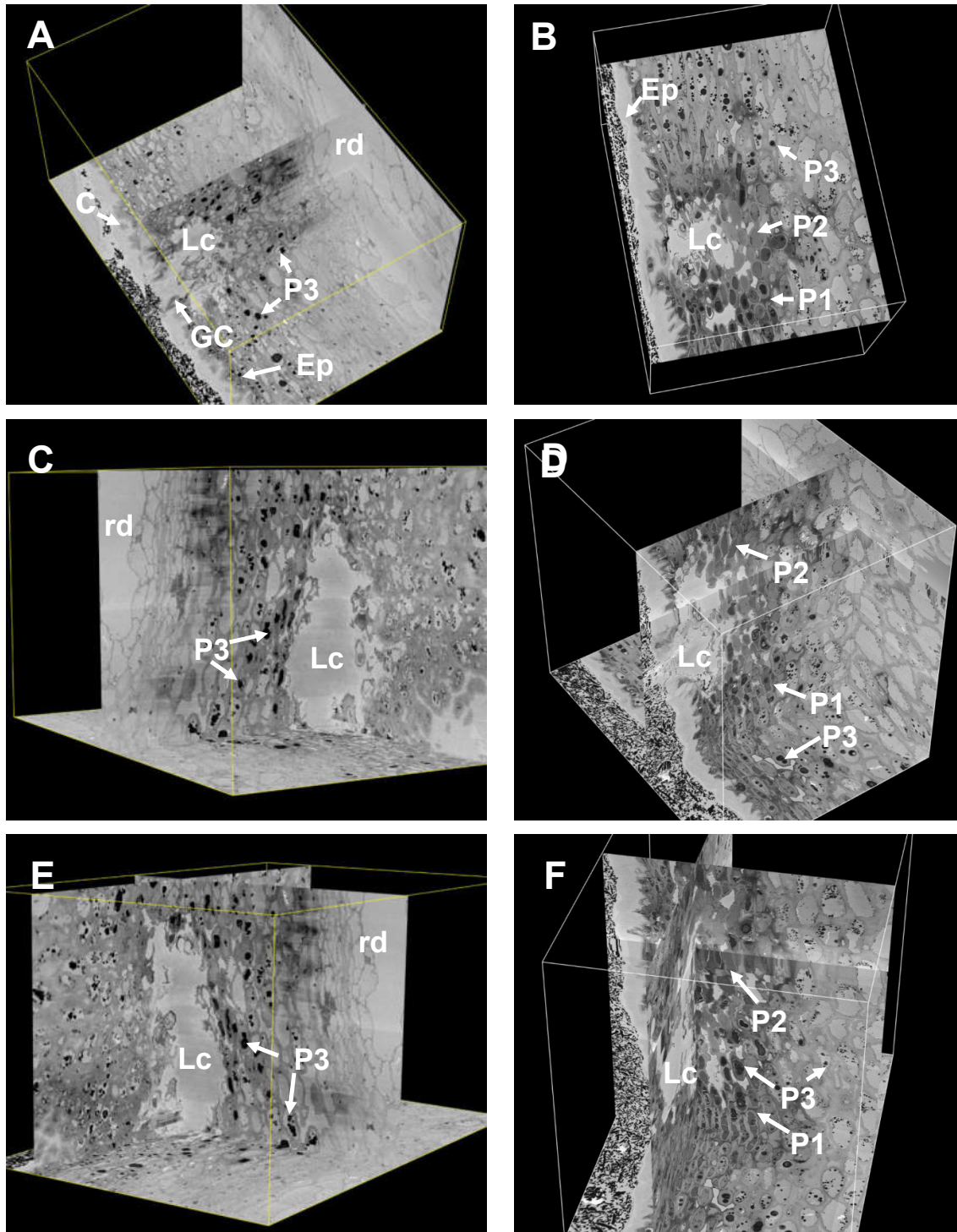


Figure 5.6. Images taken from SBF-SEM movie sequences of non-coloured and discoloured of 'B74' mango fruit lenticels. (A, C, E) Images showing minimal evidence of electron-dense material in a non-coloured lenticel. (B, D, F) Images showing evidence of more extensive electron-dense

material in a discoloured lenticel. c, cuticle; Ep, epidermal cells; GC, stomatal guard cells; Lc, lenticel cavity; P1 - P3, three types of phenolics; rd, resin duct.

5.4 Discussion

LD is a physicochemical process characterised by darkened tissues around the cavities of lenticels. LD can also be evident as a brown to black-coloured blemish surrounding lenticels (Du Plooy et al., 2006). LD can reduce fruit visual appeal and lead to loss of market confidence, including consumer appeal (Self et al., 2006).

In the present study, no marked difference in the morphology of lenticels between the 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' cultivars were discerned. Nevertheless, there was in the anatomy of individual lenticels in all cultivars (Figure 5.3). LM imaging showed that the shape of lenticels was typically irregularly oval-shaped (Figure 5.3). LD was evident as browning of sub-lenticellular cells surrounding the lenticel cavity (Figure 5.2). Based on the evidence of phenolics heavily stained by TBO (i.e. green colour/s vs. purple colour/s), this browning was associated with polymerised phenolic deposition in sub-lenticellular cells surrounding the lenticel cavity (Figure 5.3). Bezuidenhout et al. (2005) observed that such discolouration in the lenticel of 'Tommy Atkins' mango fruit was due to pigment accumulation in the vacuoles of sub-lenticellular cells. Du Plooy et al. (2009) considered that the blackening development of lenticels was due to vacuolar deposition of phenolics. In the current research, the location of the phenolics within the cells could not be clearly observed under LM. Further examination by SBF-SEM was carried out to observe the intracellular partitioning of phenolics.

Resin ducts were visible close to both discoloured and non-coloured lenticels (Figure 5.4). This suggests that proximity of canals close to lenticels is not likely to be a factor in the development of LD in 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' cultivars. This is in contrast to the previous suggestion by Bezuidenhout et al. (2005) that for 'Tommy Atkins' and 'Keitt' cultivars a relationship between resin ducts and LD may exist. Tamjinda et al. (1992) reported that 'Falan' mango cultivar did not develop LD due to the presence of a cork cambium. Bezuidenhout et al. (2005) indicated some mango cultivars were more susceptible to LD, a possible result of the absence of a cork cambium and cork cells in lenticels. The cambium was not observed in 'B74', 'Honey Gold', 'Kensington Pride' and 'R2E2' mango samples, which means that these cultivars are more likely to get LD.

SBF-SEM made it possible to derive 3D anatomy at the ultrastructure level of mango fruit skin lenticels. This structure was the same for both coloured and non-discoloured lenticels, with a similar change observed in the discoloured lenticel (in Movie 5.1 and 5.2). Single 50 nm sections observed in the TEM only showed a part of lenticel structure. SBF-SEM image stacks provided additional information to differentiate between the architecture of non-coloured (in Movie 5.3) and discoloured lenticels (in Movie 5.4) at the cellular level.

In general, both non-coloured and discoloured lenticels of 'B74' mango fruit did not vary significantly in their structure (Figure 5.6). The lenticels had an irregular oval-like shape. On the outer surface of the lenticel, a waxy cuticle covered the epidermis, penetrating into the exposed cavity without the waxy cuticle of inner lenticel. Discolouration of the lenticel was apparent beneath the cuticle in the sub-lenticellular cells of the pericarp (Figure 5.3 - 5.7). Electron-dense phenolic materials were found in the cell wall and cytoplasm of sub-lenticellular cells surrounding the cavity (Figure 5.7). These electron-dense phenolic materials were more prevalent in the discoloured lenticel than in non-coloured lenticel.

SBF-SEM provided detailed ultra-structure of LD. Three different types of phenolic deposition could be identified in the tissues of a discoloured lenticel (Figure 5.5, 5.7). The first type was deposition in the cell wall of sub-lenticellular cells surrounding the cavity. The second type was distinguished by phenolics completely filling the cytoplasm of sub-lenticellular cells next to the cavity. Thirdly, dark granules of various sizes were distributed randomly in the vacuoles of sub-lenticellular cells in the region adjacent to the lenticel cavity. However, only the third type of phenolic deposition was observed in the non-coloured lenticel (Figure 5.4). Bezuidenhout et al. (2005) reported LD only associated with phenolic deposition in the vacuolar contents of sub-lenticellular cells surrounding the lenticel cavity.

SBF-SEM has been used to study 3D structure of the thylakoids of *Arabidopsis thaliana* *Nicotiana tabacum* and *Spinacia oleracea* (Austin and Staehelin, 2011) and the meristematic cell of *Arabidopsis* root (Kittelmann et al., 2016). In the present study, this technique is applied for the first time to study mango LD. It was beneficial to reveal aspects of the 3D nature of the lenticels. In this context, the method was superior to LM and traditional SEM. Using TEM, Du Plooy et al. (2006) associated LD with electron-dense deposition spreading from the cell wall (i.e. apoplast) towards the cytoplasm (i.e. symplast) of the cells.

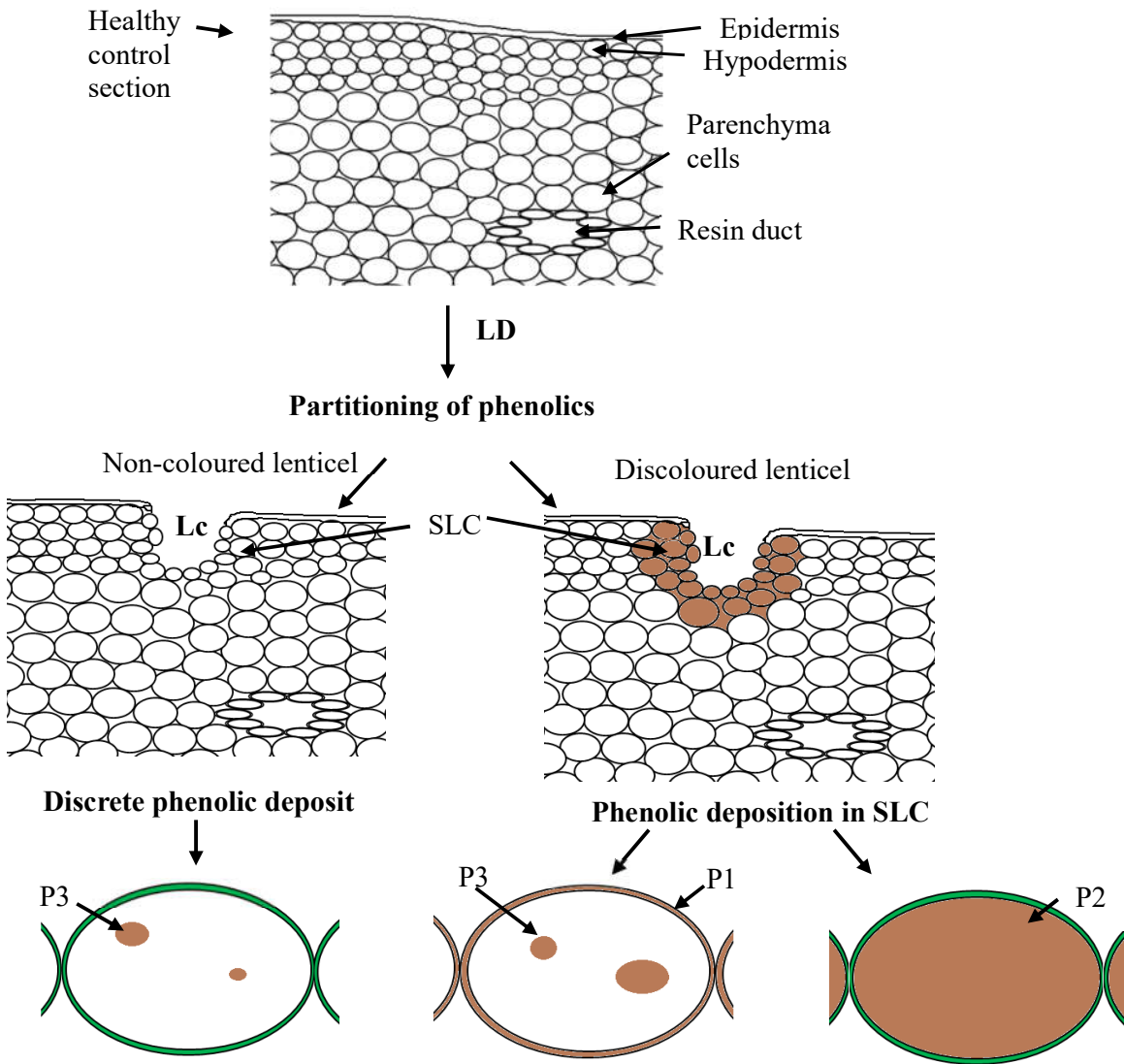


Figure 5.7. A comparative diagrammatic model of LD in mango fruit is based on current findings (this chapter) and published work (Bezuidenhout et al., 2005; Du Plooy et al., 2009; Du Plooy et al., 2006). LD is visible as browning in the sub-lenticellular cells surrounding the lenticel cavity. This browning was associated with phenolic deposition in the sub-lenticellular cells surrounding the cavity. There was three different types of phenolic deposition as polymerised electron-dense phenolic depositions in the cell walls and cytoplasm and a discrete deposit of phenolics in the cytoplasm of sub-lenticellular cells. Lc, lenticel cavity; LD, lenticel discolouration; P1 - P3, three types of phenolics; SLC, the sub-lenticellular cells.

5.5 Conclusion

This study characterised the ultrastructural differences of LD in mango fruit. Correlative imaging of LD with LM, and SBF-SEM were employed to study the structure of lenticels at different scales. SBF-SEM in particular revealed something of the 3D structure of both discoloured and non-discoloured lenticels. This approach showed relatively detailed ultrastructural changes in the partitioning of phenolics as associated with LD. Namely, polymerised electron-dense phenolic depositions in the cell wall and cytoplasm of sub-lenticellular cells surrounding the cavity were revealed for the first time. This work has progressed knowledge regarding the functional microstructure of LD. In due course, it will contribute to greater understanding of the mechanism of LD.

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CHAPTER 6. DIURNAL HARVEST CYCLE AND SAP COMPONENTS AFFECT THE DEVELOPMENT OF UNDER-SKIN BROWNING IN ‘HONEY GOLD’ MANGO FRUIT

Abstract

Under-skin browning (USB) is a physiological disorder that affects harvested ‘Honey Gold’ mango fruit. USB is thought to be associated with the sap release from resin canals (laticifers) within the exocarp. USB symptoms can develop in response to physical stress (e.g. abrasion) and low temperature exposure (e.g. < 10 - 12 °C) during postharvest handling. A postulated effect of harvest time over the diurnal cycle regarding the propensity of fruit to develop USB was evaluated. This was tested by lightly abrading fruit from over the diurnal cycle to simulate vibration damage in transport. The fruit were then held at 10 - 12 °C for 6 d in line with commercial handling. In complementary work, sap extracts were applied to skin abrasion points on additional fruit. Naturally expressing USB was compared with induced USB symptoms at the cellular level. Fruit harvested at 1000 h, 1400 h and 1800 h showed 3 to 5-fold higher USB incidence levels than did those picked at 2200 h, 0200 h and 0600 h. The concentrations of key aroma volatile compounds (2-carene, 3-carene, α -terpinene, *p*-cymene, limonene, α -terpinolene) in the fruit sap were significantly higher ($P \leq 0.05$) at 1400 h as compared to other harvest times. Treatment with spurt sap collected from fruit picked at 1400 h caused up to 15-fold higher browning incidence and 29-fold higher browning severity as compared to treatment with spurt sap from fruit picked at 0600 h. Fruit harvested in the afternoon were more prone to developing USB than those picked at night and early morning. The diurnal variation in sensitivity was attributed to changes in the concentration of fruit sap volatiles. Night and early morning harvesting evidently afforded an opportunity to reduce the incidence and severity of USB on ‘Honey Gold’ mangoes. To this end, ‘Honey Gold’ growers have changed their harvest practices and enhanced the quality of fruit on offer to consumers.

6.1 Introduction

The ‘Honey Gold’ mango (*Mangifera indica* L.) is a relatively new Australian cultivar. It has juicy, fibre-free flesh and a pleasant flavour (Marques et al., 2012). However, an unsightly physiological skin disorder termed ‘Under-Skin Browning’ (USB) can cause economic loss in the marketplace due to poor fruit appearance (Hofman et al., 2010b). USB is visible beneath the epidermis as diffuse discoloured areas. There is, however, no damage to the flesh (Marques et al., 2012). Sometimes the skin and cuticle can also take on an opaque appearance. ‘Honey Gold’ mango is most susceptible

among the common Australian mango cultivars to USB. The physicochemical mechanisms of USB and its control are largely unknown. They evidently reflect discolouration of latex vessels and surrounding cells (Marques et al., 2012). Delayed or slow cooling of fruit before or after packing can reduce the incidence of USB. This is especially so if combined with soft packaging options, such as use of foam liners that may reduce vibration damage during transport (Marques et al., 2016; Marques et al., 2012).

Mango fruit and stems, including pedicle and peduncle, are characterised by an internal longitudinal multilayered network of branching resin ducts (Joel, 1980). The extensive laticifer system consists of sap that stores and can emit resinous secretions (Joel and Fahn, 1980). Turgor pressure in the ducts is maintained because the sap contains large amounts of non-dialysable and non-starchy carbohydrate (John et al., 2003). These osmolytic compounds allow high water status to be maintained in the laticifers, even under tree water deficit stress conditions (Pongsomboon et al., 1991). Thus, sap or latex in the ducts is generally under high turgor pressure while the fruit is connected to the peduncle. When the peduncle is broken, the resin ducts are severed and sap spurts and / or oozes out.

Visually, mango sap is a viscous clear or slightly milky liquid. It typically separates into two phases, an oily yellow-brown non-aqueous phase and a milky viscous aqueous phase (Loveys et al., 1992). The non-aqueous phase contains mono-terpenes (John et al., 1999) and alk(en)ylresorcinols (Hassan et al., 2009). The aqueous phase has high polyphenol oxidase (PPO) and peroxidase (POD) activities (John et al., 2003).

Contact of the fruit surface with the sap exudate can lead to skin injury termed 'sapburn' (Loveys et al., 1992). De-sapping of mangoes in lime and Mango Wash[®], a commercial detergent combining alkaline salts, phosphates and a biodegradable surfactant, is an important approach to reduce the incidence of sapburn (Amin et al., 2008). John et al. (2002) reported that treatment of 'Badami', 'Malgoa', 'Mallika' and 'Totapuri' mango fruit with either whole sap or the non-aqueous phase caused sapburn. No skin injury was associated with treatment of fruit with the aqueous phase. The sapburn is likely due to terpenoids in sap and PPO and POD in the peel (John et al., 2002). The sap terpenoid composition varies considerably among different mango cultivars (John et al., 1999; Loveys et al., 1992). Thus, it seems likely that a range of terpenoids may be responsible for sapburn in different mango cultivars. For example, limonene, ocimene, β -myrcene and α -pinene caused sapburn in Indian mango cultivars (John et al., 2002). Terpinolene was strongly associated with sapburn in 'Kensington Pride' fruit (Loveys et al., 1992).

Differences in sapburn on fruit harvested at different times of the day have been noted. They were suggested to be due to varying sensitivity of the mango fruit skin (Maqbool et al., 2007). The severity of sapburn injury was reported to be considerably less for ‘Samar Bahisht Chaunsa’ fruit harvested in the morning (e.g. 7 AM) as compared to those harvested other times of the day. It was highest among fruit collected at noon (12 PM) (Amin et al., 2008).

In the present research, we investigated the effects of diurnal fruit harvest time and sap components on the propensity of ‘Honey Gold’ mango fruit to develop USB. We hypothesised that ‘Honey Gold’ mango fruit harvested in the morning and night would be more resistant to USB than those harvested in the afternoon. Moreover, we considered that this response may be related to varying characteristics of the sap at different harvest times. To test that, we harvested fruit at several times, determined USB susceptibility, and quantified the concentration of aroma volatiles in the sap from the diurnal harvest cycle by GC-MS. We also examined the anatomy of USB and induced USB symptoms. In general, it was proposed that better understanding of the mechanisms involved in the putative diurnal effect, including any differences in sap composition, may provide deeper insight into measures to minimise USB incidence in commercial consignments.

6.2 Materials and methods

6.2.1 Experiment 1: Effect of diurnal harvest cycle on USB development

‘Honey Gold’ mango fruit of uniform size were harvested at commercial maturity (dry matter content of $16.8 \pm 1.0\%$) from a commercial orchard near Katherine, Northern Territory, Australia (14.28 °S, 132.16 °E). Trees were grown under standard commercial practices (Johnson and Hofman, 2009; Kernot et al., 1999). Field temperature and relative humidity during the harvest periods were recorded using a Tinytag Ultra 2 logger (Hasting Data Loggers, Australia).

The fruit ($n = 80$) were harvested every 4 - 6 h in the 2013/2014 season (viz., 1000, 1400, 1800, 2400, 0600 and 1400 h) and every 4 h in the 2014/2015 season (viz. 0600, 1000, 1400, 1800, 2200 and 0200 h) fruiting seasons. Fruit with 2-5 cm-long pedicels attached were carefully transported to a nearby packing shed. On a de-sapping rack (Figure 6.1A), the pedicel was broken off at the abscission zone and fruit inverted to allow sap to drain over 4 - 5 min. Where required, spurt sap (0600 and 1400 h) was collected from extra fruit (60 fruit) in the first 10 - 15 s after stem removal for experiment 2. Ooze sap was collected from then on. The sap was collected into glass vials. The sap from 20 fruit was collected for each of four replications comprising a total of 80 fruit per harvest time. The vial opening was then covered with a piece of aluminium foil and closed with an

aluminium screw-on cap. Sap samples were kept on dry ice (BOC, Australia) in a foam container. They were transported by car (ca. 22 °C) to the airport and then air-freighted from the Northern Territory to Brisbane, Queensland within 1.5 d of harvest. They were then transported in an air-conditioned (ca. 22 °C) vehicle to the nearby postharvest laboratory in Brisbane. The collected sap was then stored at -20 °C pending analysis. The non-aqueous and aqueous phases of sap samples were analysed as described in sections 3.2.4, 3.2.6 and 3.2.7 (Chapter 3). Concentrations of eight volatiles in the non-aqueous and aqueous phases were referenced to standard curves and calculated from the peak area ratios for the unlabelled and labelled compounds versus the concentration ratio. The concentrations of volatiles, including hexanal, 2-carene, 3-carene, α -terpinene, p-cymene, limonene, α -terpinolene and ethyl octanoate, were expressed as $\mu\text{g}/\text{kg}$.

After de-sapping, fruit susceptibility to USB was determined by a standard USB test developed for this cultivar. The test consisted of abrading the fruit for 2 s at each of four sub-sample locations around the largest fruit circumference using a small orbital finishing sander (280 W, Ozito Industries Pty Ltd, VIC, Australia) at a speed setting of '5' on a scale of '1 - 6' and 80 grit sandpaper (Trojan, NSW, Australia). About 110 g pressure was applied to the sanding disc (Figure 6.1B). All fruit were abraded within 1 h of harvest. However, half were placed in a cold room at 12 ± 1 °C after 1 d ('1 d delay'). The other half was placed in the cold room within 2 h of abrasion ('no delay').

All the fruit from experiments 1 and 2 were kept at 10 ± 1 °C for a further 5 d and then road-freighted under commercial conditions in a standard 20-pallet 40-foot refrigerated trailer at about 14 °C to Wamuran in Queensland, a distance of approx. 3,100 km from the farm. From Wamuran, the fruit were then transported in an air-conditioned vehicle at ca. 22 °C to the Eco-Science Precinct, Department of Agriculture and Fisheries building in Brisbane, approx. 70 km from Wamuran, and ripened in a ripening room at 20 °C and 90 - 100% RH.

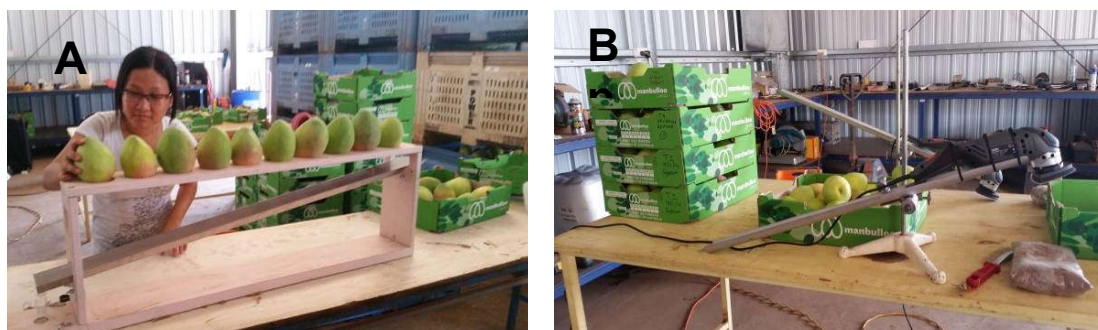


Figure 6.1 Collecting sap on a de-sapping rack (A) and USB abrasion test as applied with an orbital finishing sander (B).

6.2.2 Experiment 2: Effect of sap components on USB development

To test the hypothesis that USB diurnal responses were at least partly due to sap characteristics, either whole sap or its component spurt or ooze sap were collected from additional 'Honey Gold' fruit harvested in the morning (0600 h, n = 40) and afternoon (1400 h, n = 40) in the 2014/2015 season, as described above. An aliquot of 0.1 ml of either spurt or ooze sap, of whole sap or of distilled water was placed onto the abraded surface of morning- (0600 h) and afternoon-harvested (1400 h) fruit as mentioned in section 6.2.1 in experiment 1. To affirm effects of main oil fraction ingredients in mango fruit sap, 0.1 ml of pure terpinolene (Sigma-Aldrich), limonene (Aldrich), 2-carene (Aldrich) and distilled water were placed onto 1 cm² rectangular filter papers. The filter papers were then covered with pieces of aluminium foil (~ 35 - 40 cm²) with plastic tape to reduce vaporisation. The fruit were then held at 12 ± 1 °C for 6 d, road-freighted to Brisbane under commercial conditions as described above and ripened in a ripening room at 20 °C and 90 - 100% RH before being assessed for damage areas around abrasion sites, as described below.

6.2.3 Assessment of USB, induced USB symptoms, and sapburn severity and incidence

The severity of USB, induced USB and sap burn around the abrasion site on each fruit was rated according to the following scale (Hofman et al., 2010a): 0 = nil; 1 = < 3% (1 cm²) of skin surface affected; 2 = ~ 3% (1 - 3 cm²); 3 = ~ 10% (3 - 12 cm²); 4 = 10% - 25% (12 - 25 cm²); and 5 = > 25% of skin surface affected. Placing sap onto abraded fruit often resulted in slight brown-coloured damage to the surrounding skin. This symptom is referred to as sapburn. Injection of terpinolene and limonene as described in section 6.2.2 caused significant brown-coloured damage to the surrounding skin, similar to natural USB symptoms. This symptom is referred to as induced USB symptoms.

The incidence of USB within each replication was calculated as the proportion (%) of fruit or abrasion sites that developed USB relative to the total number of fruit or abrasion sites. The severity of USB lesions not associated with abrasion treatment (i.e., those which developed on other areas of the fruit) were also recorded. The severity of USB and induced USB symptoms induced by terpinolene and limonene and also of sapburn were calculated as the average severity rating of those fruit as affected by USB, induced USB or sapburn symptoms in each treatment, excluding the non-affected fruit.

6.2.4 Determination of sap volume and composition

The proportional volumes of whole sap, non-aqueous and aqueous sap phases from the diurnal trials in 2013/2014 and 2014/2015 fruiting seasons were measured using a graduated cylinder after the phases had separated. The non-aqueous and aqueous phases of sap collected each harvest time were separated at 3000 rpm using a 5810 R centrifuge (Eppendorf, Germany) for 10 min. Aliquots of 0.1 g of whole sap or 0.03 g of non-aqueous phase were mixed in 10 ml of distilled water using a ball mill MM400 (Retsch GmbH, Germany) for 30s, diluted a further 50 times with distilled water, and then diluted a further either 50 times or 25 times with distilled water for the whole sap and non-aqueous phase, respectively. The non-aqueous and aqueous phases of sap samples were then analysed for aroma volatile quantification by using stable isotope dilution analysis (SIDA) in conjunction with headspace (HS) solid-phase microextraction (SPME) coupled with gas-chromatography mass spectrometry (GCMS) as described in sections 3.2.5, 3.2.6 and 3.2.7 (Chapter 3). The concentrations of eight volatiles in the mango samples were referenced to standard curves and calculated from the peak area ratios for the unlabelled and labelled compounds versus the concentration ratio. Concentrations of volatiles, including hexanal, 2-carene, 3-carene, α -terpinene, p-cymene, limonene, α -terpinolene and ethyl octanoate, were expressed as $\mu\text{g}/\text{kg}$.

6.2.5 Anatomy of natural USB, induced USB symptoms and sapburn

Fruit tissues ($n = 3$) with and without skin browning symptoms were prepared for light microscopy. USB and sapburn on 'Honey Gold' fruit were compared and contrasted at the cellular level. Free-hand sections were cut through the excised tissues after Ruzin (1999). The tissue sections were transferred into a drop of distilled water on a glass microscope slide and covered with a glass cover slip. The hand sections were viewed and photographed with an Olympus BX61 LM equipped with a DP 70 camera (Olympus, Japan).

6.2.6 Experimental design and statistical analysis

In experiment 1, a factorial design was used [six harvest times x two (time) delay treatments] with four replications (rows). Ten individual fruit from 10 trees per replication comprising four sub-samples per fruit per harvest time per time delay treatment were used for USB induced by the abrasion test in the diurnal harvest effect. Four replications with 20 individual fruit from 10 trees per replication per harvest time were used for sap volume and composition. In experiment 2, seven fruit per replicates comprising two sub-samples per fruit were used for sap and chemical injection.

Statistical analyses were performed using Genstat 14 (VSN International Ltd., UK). USB incidence was analysed using a Generalised Linear Model (GLM) with a binomial distribution and logit link. Where treatment interactions were significant, pairwise comparisons were made using Fishers protected least significant difference (LSD) procedure at $P = 0.05$. Back-transformed means are presented. The effects of harvest time and delay treatments were included. Analyses on average severity were performed using an unbalanced analysis of variance (ANOVA). In order to determine the overall average USB severity rating, the first results (USB incidence) were combined with the second results (average severity of affected fruit with USB) and t-tests were used to find the pairwise comparisons between treatments.

The General Analysis of Variance model was used to analyse the data on severity and browning incidence by chemical and sap injection. This model was also used for field temperature, relative humidity, the volume of sap and the proportion of sap phases.

The One-Way Analysis of Variance model was applied for volatiles concentrations. In all trials, the least significant difference (LSD) procedure at $P = 0.05$ was used to test for differences between treatment means.

6.3 Results

6.3.1 Experiment 1: Effect of diurnal harvest cycle on USB development

6.3.1.1 Field temperature and relative humidity

In the 2013/2014 season, field temperatures during harvest increased from 30 °C at 1000 h to almost 36 °C at 1400 h, then decreased to about 24 °C by 2400 h, and then increased to 37 °C at 1400 h on the second day (Figure 6.2A). RH was lowest at 1400 h for both harvest days at 37 and 38%, respectively (Figure 6.2B). As the temperature decreased, the relative humidity increased, reaching 100% at 2400 h and 0600 h.

Similar trends were observed in the 2014/2015 season (Figure 6.3A). Field temperature increased from 20 °C at 0600 h to almost 40 °C at 1400 h and then decreased to about 27 °C by 2200 h. RH was highest at 0600 h (91%) and lowest at 1400 h (37%), and then increased to about 66% by 0200 h (Figure 6.3B).

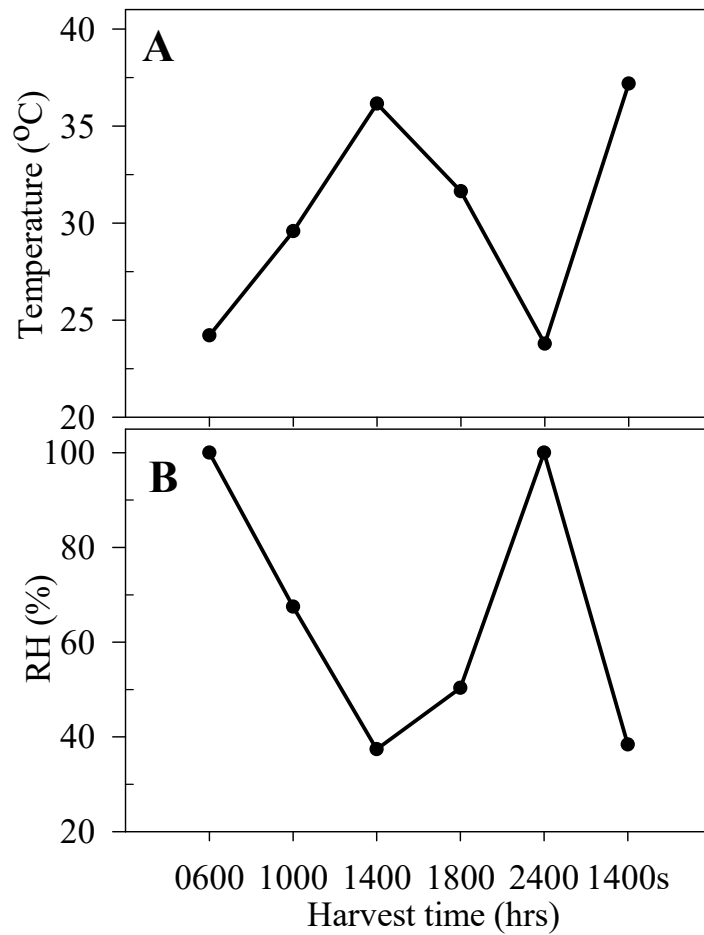


Figure 6.2 Field temperatures (A) and relative humidity (B) over a diurnal harvest cycle in the 2013/2014 season. Field temperature and relative humidity during the harvest periods were recorded using a Tinytag Ultra 2 logger.

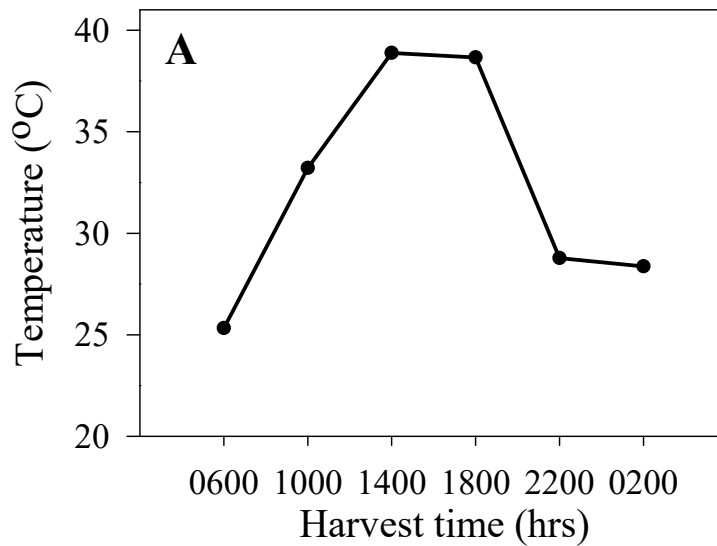


Figure 6.3 Field temperatures (A) and RH (B) over a diurnal harvest cycle in the 2014/2015 season.

Field temperature and relative humidity during the harvest periods were recorded using a Tinytag Ultra 2 logger.

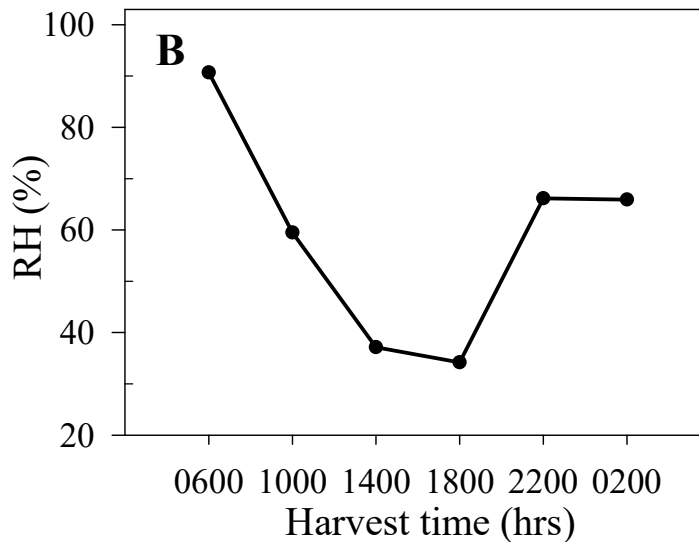


Figure 6.3 (Continued).

6.3.1.2 Diurnal harvest effect

In the 2013/2014 season, USB incidence was highest on fruit harvested at 1400 h and 1800 h for both harvest days (Figure 6.4A). Similarly, fruit harvested at 1400 h and 1800 h showed higher USB severity than for all other harvest times (Figure 6.4B). However, there was no significant interaction between harvest time and delay ($P>0.05$) on USB incidence and severity at abraded sites.

The results in 2014/2015 were similar regarding USB incidence (Figure 6.5A). It was significantly different ($P\leq 0.05$) between harvest times (Figure 6.5A, 6). USB incidence was lowest in fruit harvested at 2200, 0200 and 0600 h and the highest in fruit harvested at 1400 h and 1800 h. There was no significant ($P>0.05$) difference in USB incidence between fruit placed in the cold room immediately after abrasion and those that were held for 1 d before cooling. Opposite patterns were observed for the average severity of USB (Figure 6.5B). That is, there was significant interaction between harvest time and delay ($P\leq 0.05$) on USB severity at abraded sites. There was little diurnal effect on USB severity if fruit were placed in the cold room 24 h after abrasion.

USB lesions were also observed on areas of the skin that had not been abraded, presumably as a result of physical damage during handling and transport. These USB lesions from non-abraded regions would likely reflect natural USB occurring under commercial conditions. The results for

2014/2015 indicated that the incidence of fruit with commercially significant USB not caused by artificial abrasion was highest in fruit harvested at 1000 h and 1400 h as compared with other harvest times (Figure 6.7A). Delaying for 24 h before cooling increased the incidence and severity in fruit harvested at 1000 h and 1400 h and there was a significant difference ($P \leq 0.05$) in USB incidence and severity between no delay and a 1 d delay with fruit harvested at other times (Figure 6.7). USB incidence at 1400 h in no delay fruit was significantly ($P \leq 0.05$) higher than other harvest times. These results generally reflect those using the USB test in that USB incidence was significantly ($P \leq 0.05$) higher than at 1400 h as compared to other harvest times. Similar patterns for the fruit at 1400 h were observed for the average severity of USB as shown in Figure 6.7B.

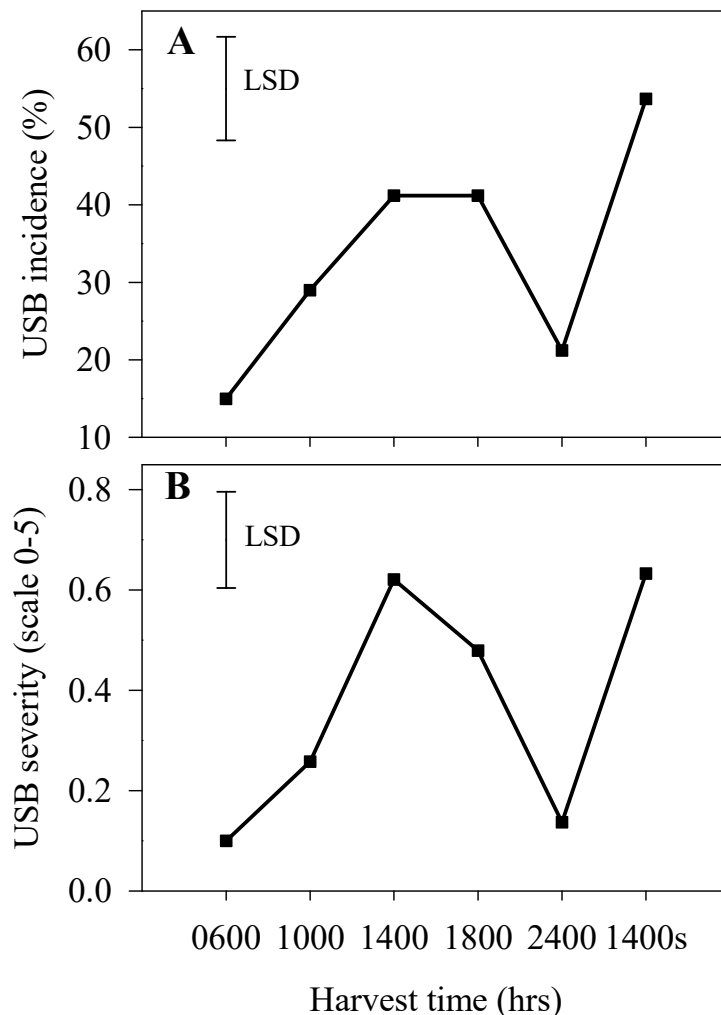


Figure 6.4 Under skin browning (USB) induced by the abrasion test in 2013/2014. (A) The incidence of USB from the diurnal harvest cycle. (B) The average severity of USB from the diurnal harvest cycle. The LSD ($P = 0.05$) bars are for comparison between harvest times. If the difference between means is less than the vertical bar, then there is no statistically significant difference

between those means ($P > 0.05$). n.s. indicates no significant difference ($P > 0.05$). $n = 4$ replications each comprised of 10 individual fruit on each of which four sub-samples were taken.

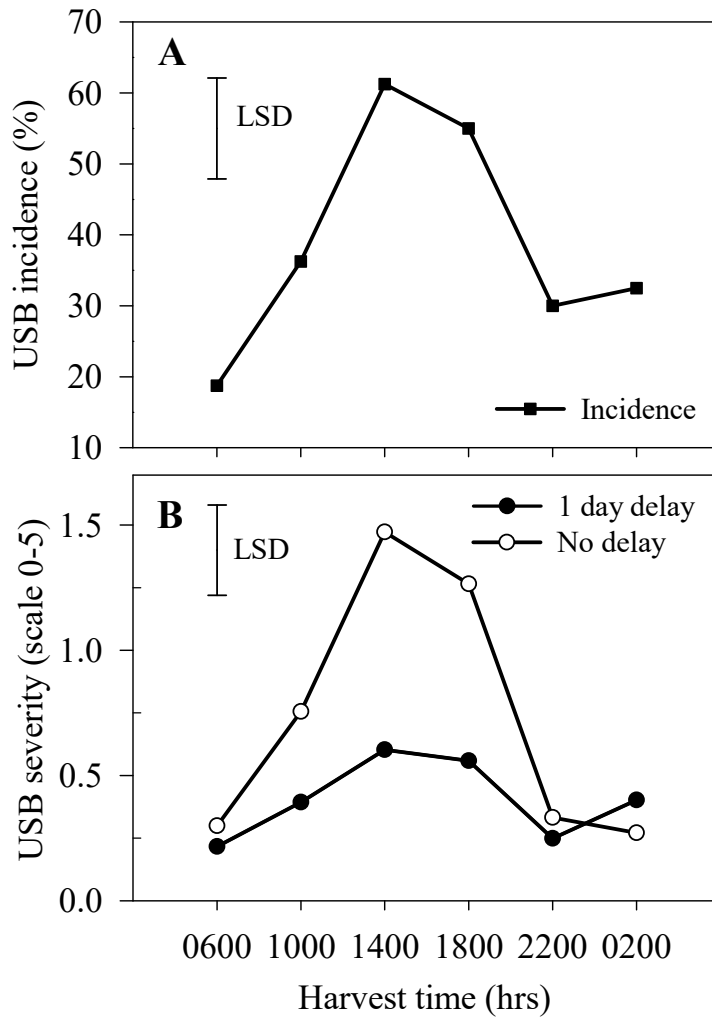


Figure 6.5 Under skin browning (USB) induced by the abrasion test in 2014/2015. (A) The incidence of USB from the diurnal harvest cycle. (B) The average severity of USB from the diurnal harvest cycle. The LSD ($P = 0.05$) bar on graph A is for comparison between harvest time and on graph B for the interaction between harvest time and delay treatments. Fruit were placed in the cold room after 1 d of abrasion (termed ‘1 d delay’) and fruit were placed in the cold room within 2 h of abrasion (termed ‘no delay’). If the difference between means is less than the vertical bar, then there is no statistically significant difference between those means ($P > 0.05$). $n = 4$ replications each comprised of 10 individual fruit on each of which four sub-samples were taken.



Figure 6.6. USB associated with the abrasion test on fruit harvested at 1400 h (A) and 2200 h (B).

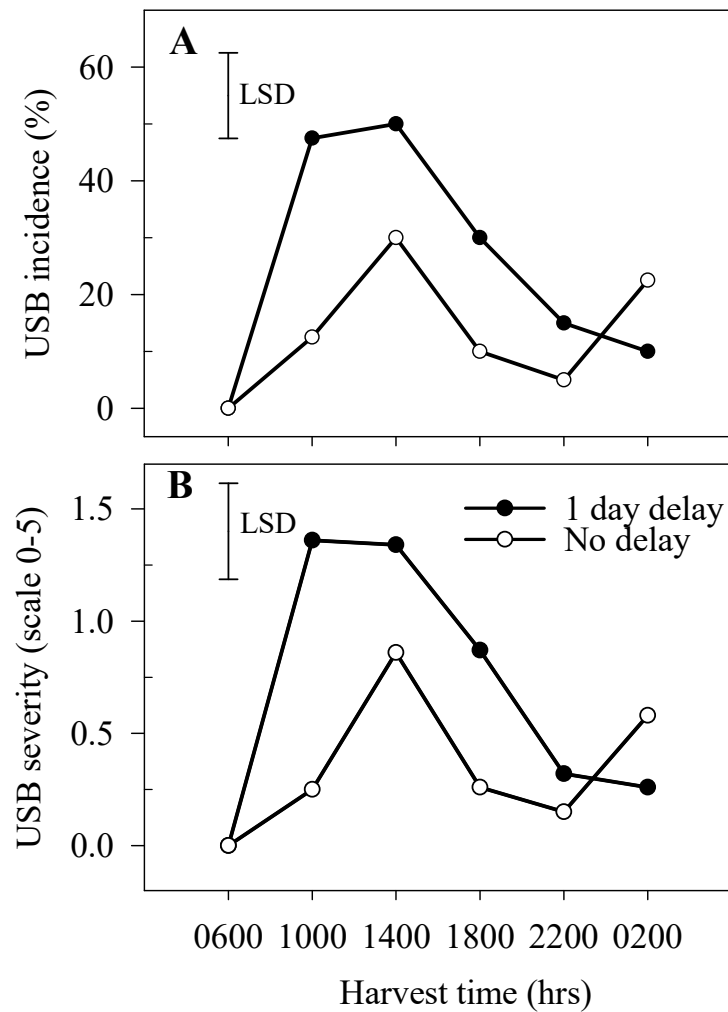


Figure 6.7 USB not associated with the abrasion test (i.e. natural USB) in 2014/2015. (A) The incidence of USB over the diurnal harvest cycle. (B) The average severity of USB over the diurnal harvest cycle. The LSD ($P = 0.05$) bars are for the interaction between harvest time and delay treatments. Fruit were either placed in the cold room after 1 d of abrasion ('1 d delay') or within 2 h

of abrasion ('no delay'). If the difference between means is less than the vertical bar, then there is no statistically significant difference between those means ($P > 0.05$). $n = 4$ replications each comprised of 10 individual fruit on each of which four sub-samples were taken.

6.3.1.3 Sap components over the diurnal harvest cycle

In the 2013/2014 season, the volume of whole sap collected decreased significantly ($P \leq 0.05$) from 0.7 ml/fruit at 1000 h to 0.4 ml/fruit at 1400 h, then increased to 1 ml/fruit by 0600 h and then declined to 0.3 ml/fruit at 1400 h on the second day (Figure 6.8A). The volume of the non-aqueous and aqueous phases showed similar trends over the diurnal harvest cycle (Figures 6.8B and 6.9C). The volumes of the non-aqueous and aqueous phases were lowest in fruit harvested at 1400 h on both days. There were no significant differences ($P > 0.05$) in the relative proportions of the non-aqueous and aqueous phases between 1000 h and 1800 h on the first day harvest (Figure 6.8D). However, the relative proportion of the non-aqueous phase tended to be 1.7-fold higher and that of the aqueous phase was lower at 1400 h on the second day (Figure 6.8D).

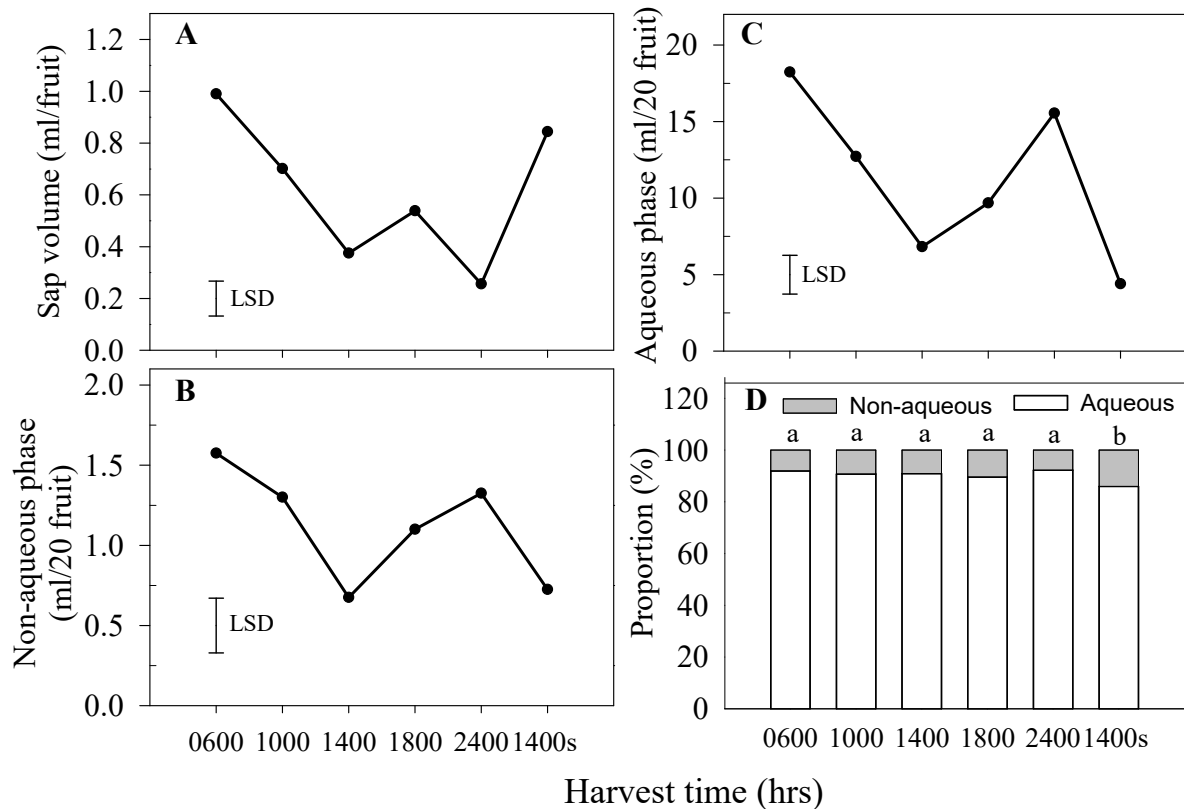


Figure 6.8 Changes in the volume of whole sap (A), non-aqueous phase (B), aqueous phase (C) and the relative proportions of non-aqueous phase and aqueous phase (E) over the diurnal harvest cycle in the 2013/2014 season. The LSD ($P = 0.05$) bars are for comparison of means between harvest times. $n = 4$ replications.

Similar results were found in the 2014/2015 season. The volume of whole sap decreased considerably from approximately 0.9 ml/fruit at 0600 h to around 0.3 ml/fruit at 1400 h and then increased to about 0.7 ml/fruit by 0200 h (Figure 6.9A). The volumes of the non-aqueous and aqueous phase were significantly ($P \leq 0.05$) lower at 1400 h and 1800 h than other harvest times (Figure 6.9B). However, the diurnal harvest cycle did not significantly ($P > 0.05$) differentially affect the relative proportions of non-aqueous and aqueous sap phases (Figure 6.9D).

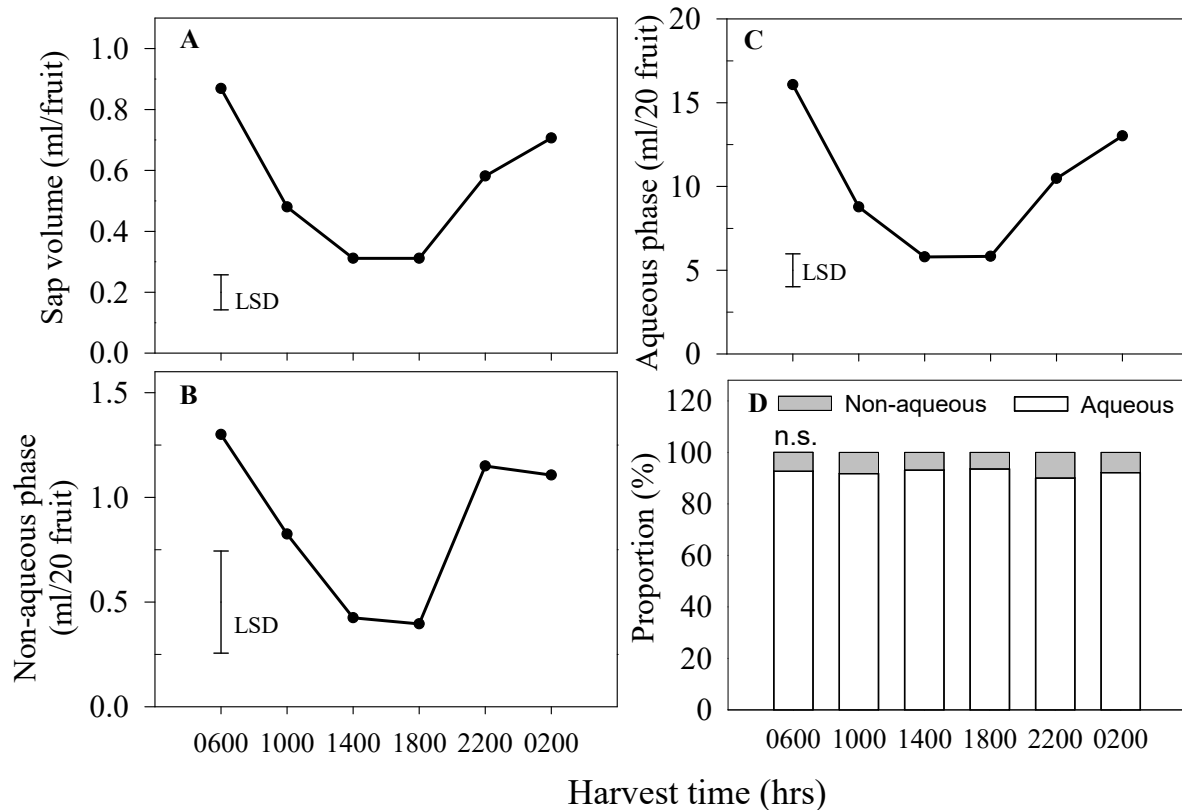


Figure 6.9 Changes in the volume of whole sap (A), non-aqueous phase (B), aqueous phase (C) and the relative proportions of non-aqueous phase and aqueous phase (E) over the diurnal harvest cycle in the 2014/2015 season. The LSD ($P = 0.05$) bars are for comparison of means between harvest times. n.s. indicates no significant difference ($P > 0.05$). $n = 4$ replications.

6.3.1.4 Sap aroma volatiles over the diurnal harvest cycle

In the 2013/2014 fruiting season, 2-carene, 3-carene, *p*-cymene, α -terpinene, limonene and α -terpinolene concentrations were significantly ($P \leq 0.05$) higher in the whole sap from fruit harvested at 1400 h from the second harvest day as compared to all other harvest times (Figure 6.10). α -Terpinolene was the dominant volatile compound as compared with the other compounds in the whole sap, being 178 mg/g at 1400 h on the second day.

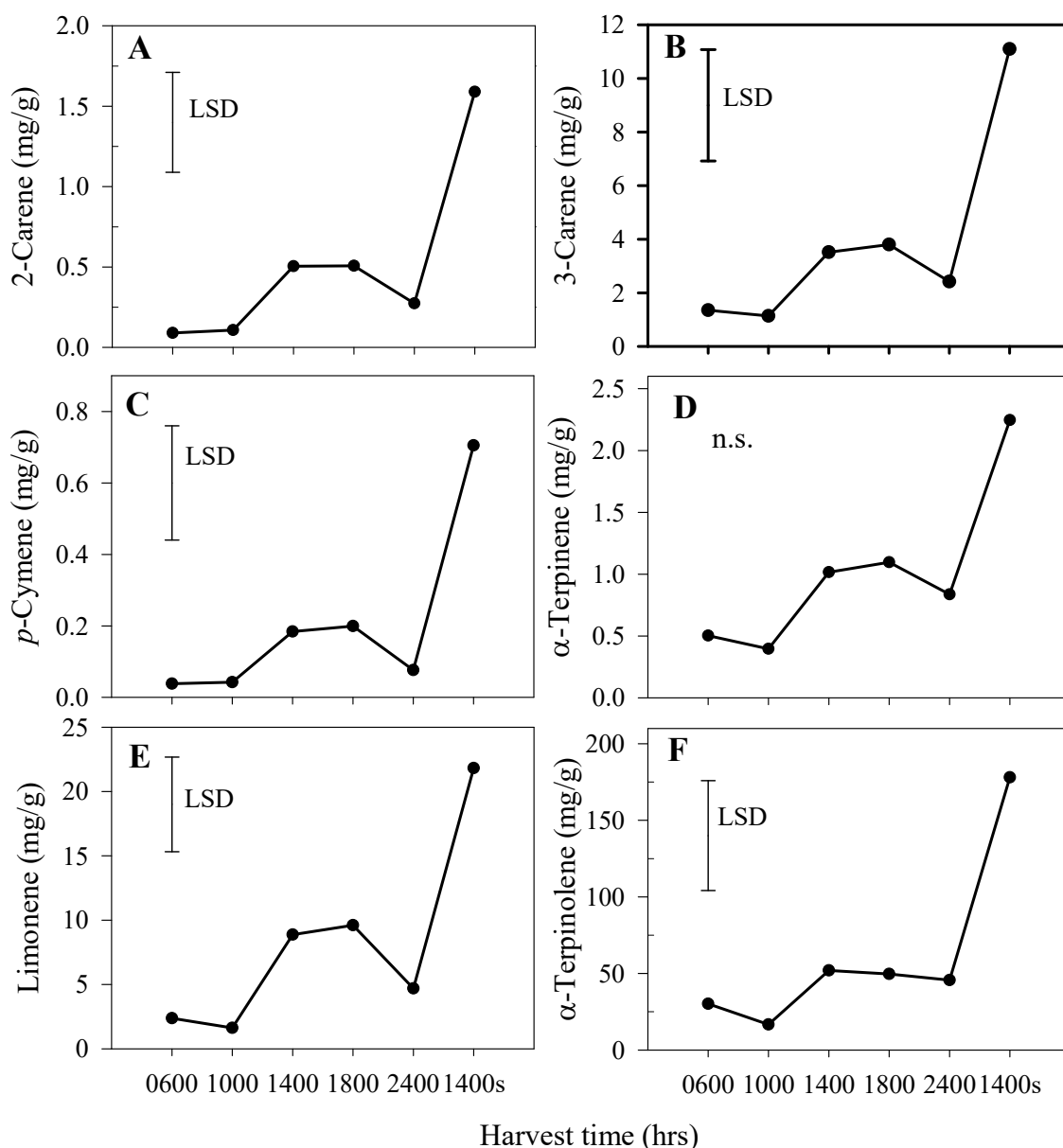


Figure 6.10 Changes in the concentrations of aroma volatiles, including 2-carene (A), 3-carene (B), *p*-cymene (C), α -terpinene (D), limonene (E) and α -terpinolene (F), in the whole sap over the diurnal harvest cycle in the 2013/2014 season. The LSD ($P = 0.05$) bars are for comparison of means between harvest times. n.s. indicates no significant difference ($P > 0.05$). $n = 4$ sap replications plus two laboratory replicates per replication.

In the non-aqueous phase, the concentrations of 2-carene, 3-carene, α -terpinene, and limonene were not significantly ($P > 0.05$) different at each harvest time (Figure 6.11). On the other hand, *p*-cymene and α -terpinolene were highest at 1400 h and lowest at 2400 h and 0600 h. Furthermore, the concentrations of the six volatile compounds mentioned above in the non-aqueous sap phase were

markedly higher than in the aqueous phase. For example, the non-aqueous phase of sap collected from fruit at 1400 h on the second day had more than a 1000-fold higher concentration of α -terpinolene than the aqueous sap phase.

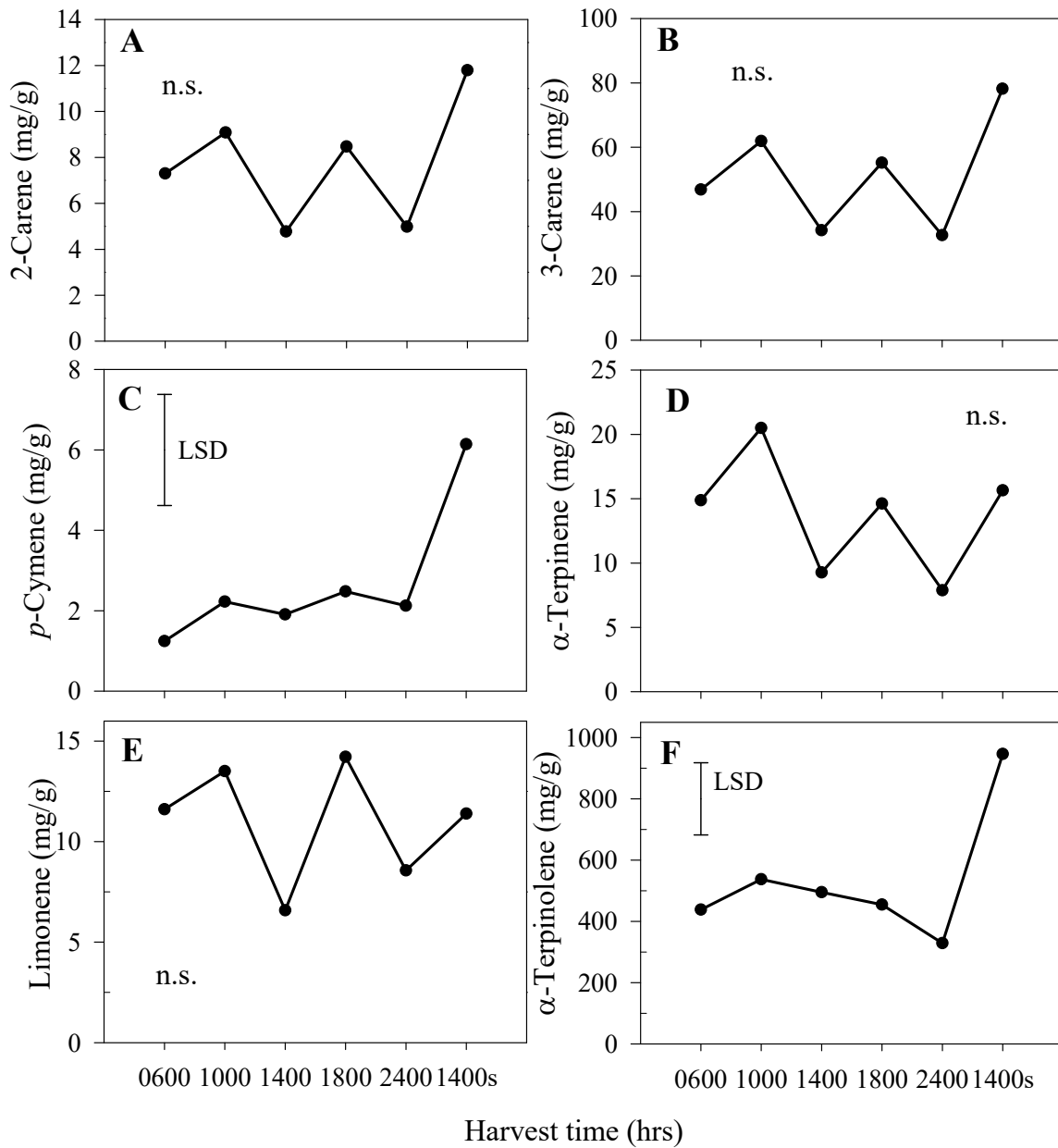


Figure 6.11 Changes in the concentrations of aroma volatiles, including 2-carene (A), 3-carene (B), *p*-cymene (C), α -terpinene (D), limonene (E) and α -terpinolene (F), in the non-aqueous phase over the diurnal harvest cycle in the 2013/2014 season. The LSD ($P = 0.05$) bars are for comparison of means between harvest times. n.s. indicates no significant difference ($P > 0.05$). $n = 4$ sap replications plus two laboratory replicates per replication.

In the aqueous phase, the concentrations of 2-carene, 3-carene, *p*-cymene, α -terpinene, limonene and α -terpinolene in samples collected between 1000 h and 1400 h were significantly ($P \leq 0.05$) higher than those from other harvest times as shown in Figure 6.12.

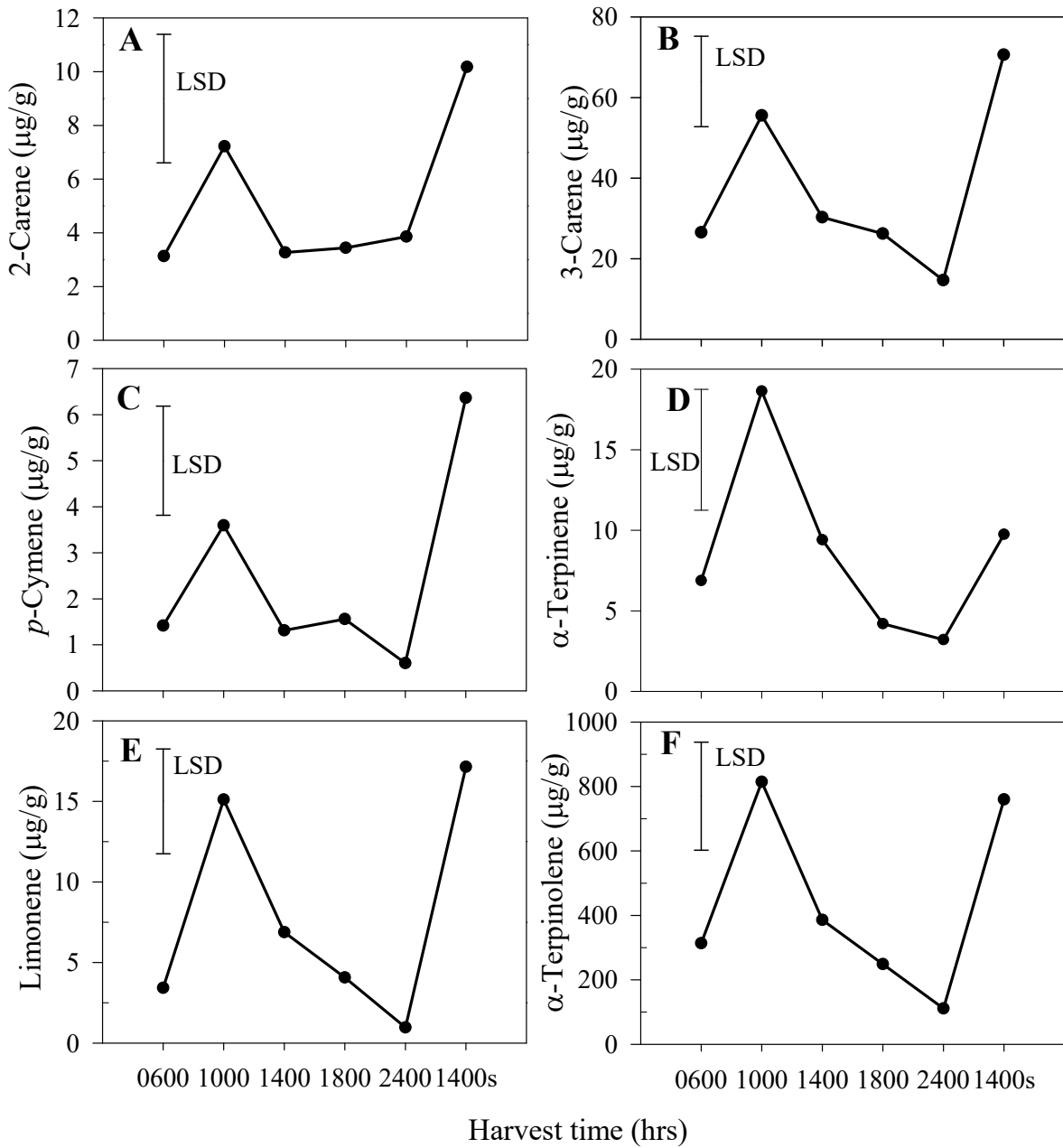


Figure 6.12 Changes in the concentration of aroma volatiles of the aqueous phase including 2-carene (A), 3-carene (B), *p*-cymene (C), α -terpinene (D), limonene (E) and α -terpinolene (F) over the diurnal harvest cycle in the 2013/2014 season. The LSD ($P = 0.05$) bars are for comparison of means between harvest times. $n = 4$ sap replications plus two laboratory replicates per replication.

The general pattern of volatile concentrations was similar in the 2014/2015 season (Figure 6.13). In whole sap, concentrations of 2-carene, 3-carene, *p*-cymene, α -terpinene, limonene and α -terpinolene at 0600 h increased considerably to a peak at 1400 h and then reduced dramatically by 2200 h (Figure 6.13). The whole sap obtained at 1400 h had the highest concentrations of these compounds, with dominance of α -terpinolene at approximately 68.7 mg/g. The whole sap at between 2200 h and 1000 h had the lowest concentration of these compounds.

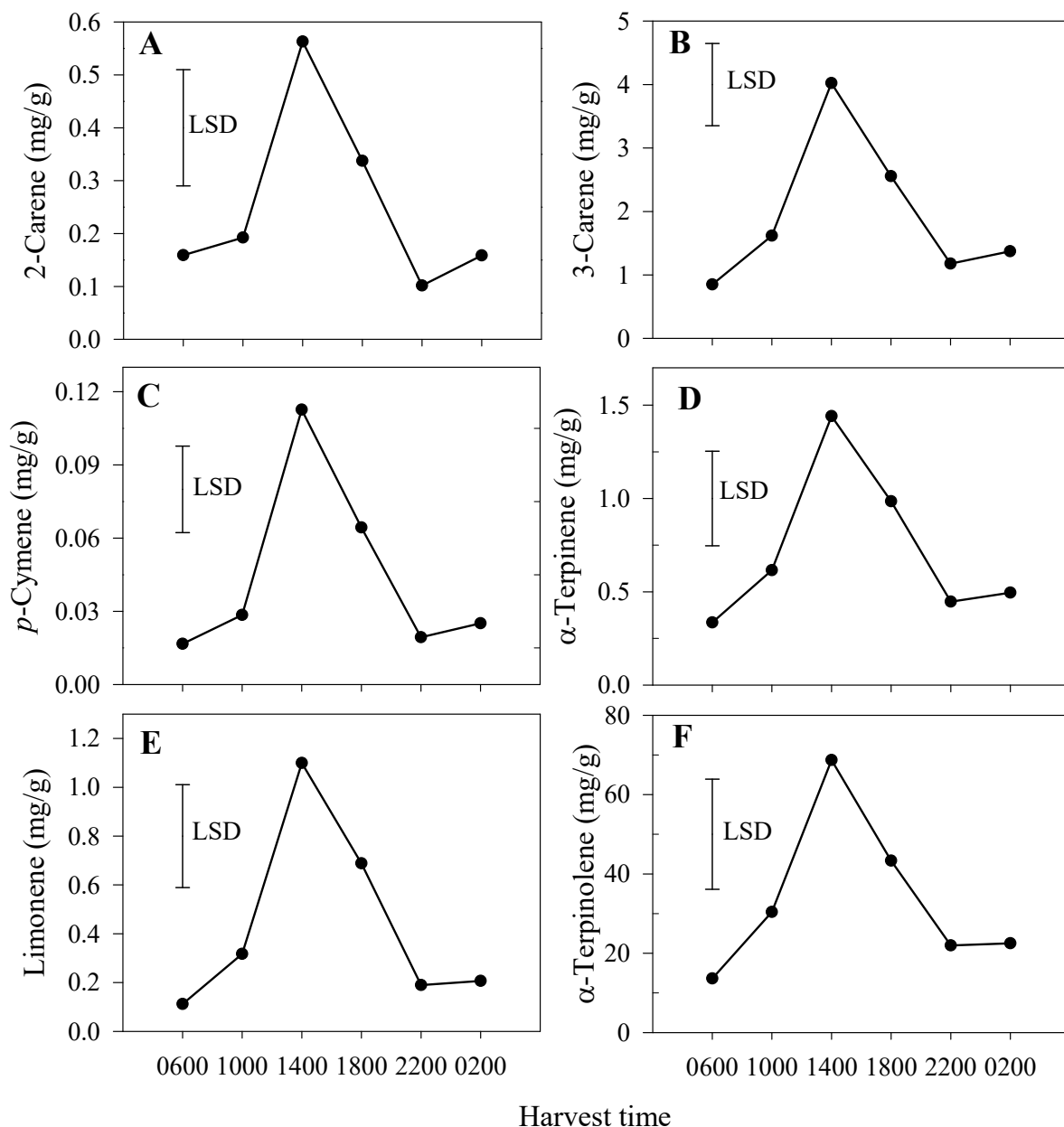


Figure 6.13 Changes in the concentrations of aroma volatiles of the whole sap, including 2-carene (A), 3-carene (B), *p*-cymene (C), α -terpinene (D), limonene (E) and α -terpinolene (F), over the

diurnal harvest cycle in the 2014/2015 season. The LSD ($P = 0.05$) bars are for comparison of means between harvest times. $n = 4$ sap replications plus two laboratory replicates per replication.

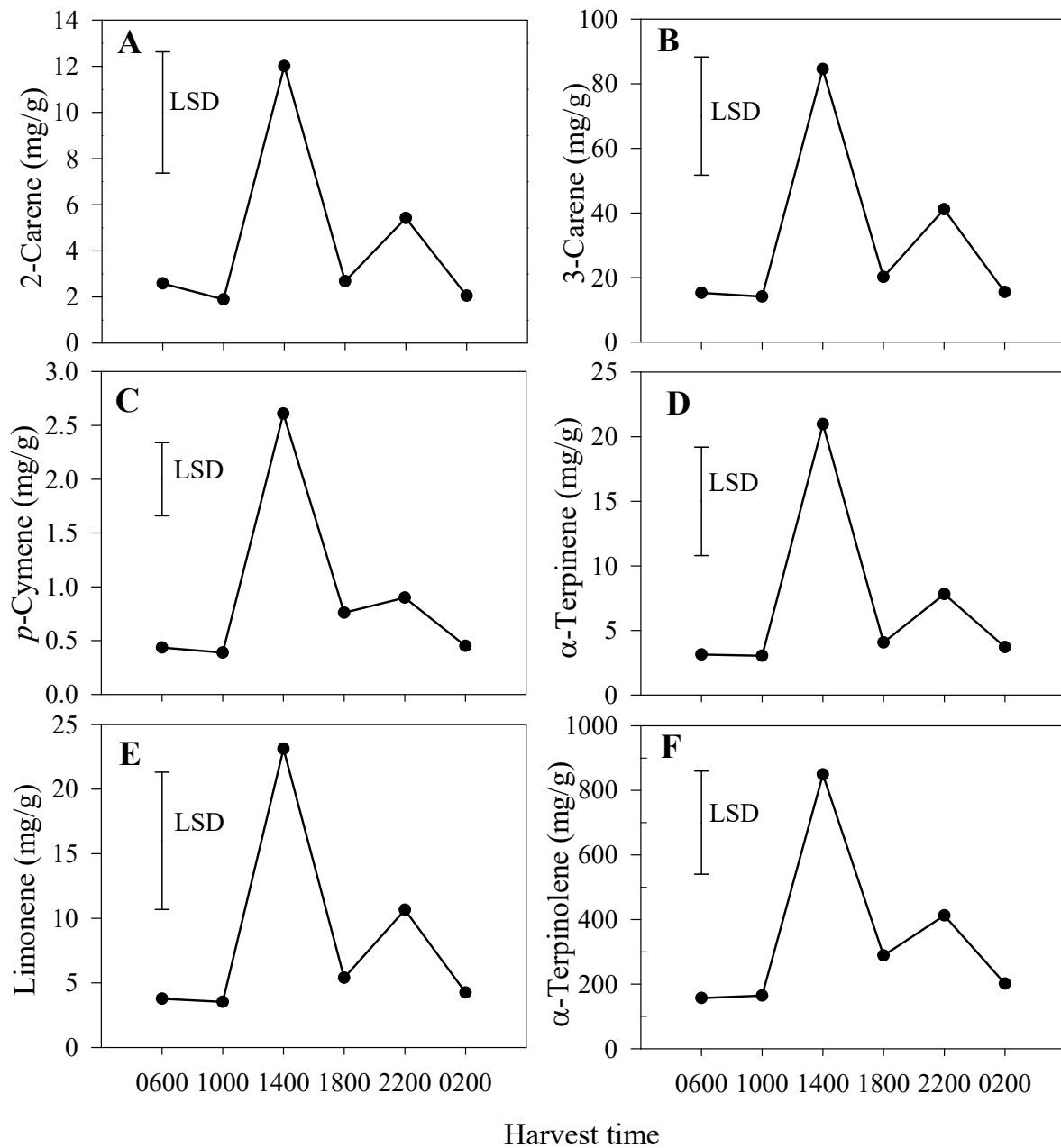


Figure 6.14 Changes in the concentrations of aroma volatiles of the non-aqueous phase, including 2-carene (A), 3-carene (B), *p*-cymene (C), α -terpinene (D), limonene (E) and α -terpinolene (F), over the diurnal harvest cycle in the 2014/2015 season. The LSD ($P = 0.05$) bars are for comparison of means between harvest times. $n = 4$ sap replications plus two laboratory replicates per replication.

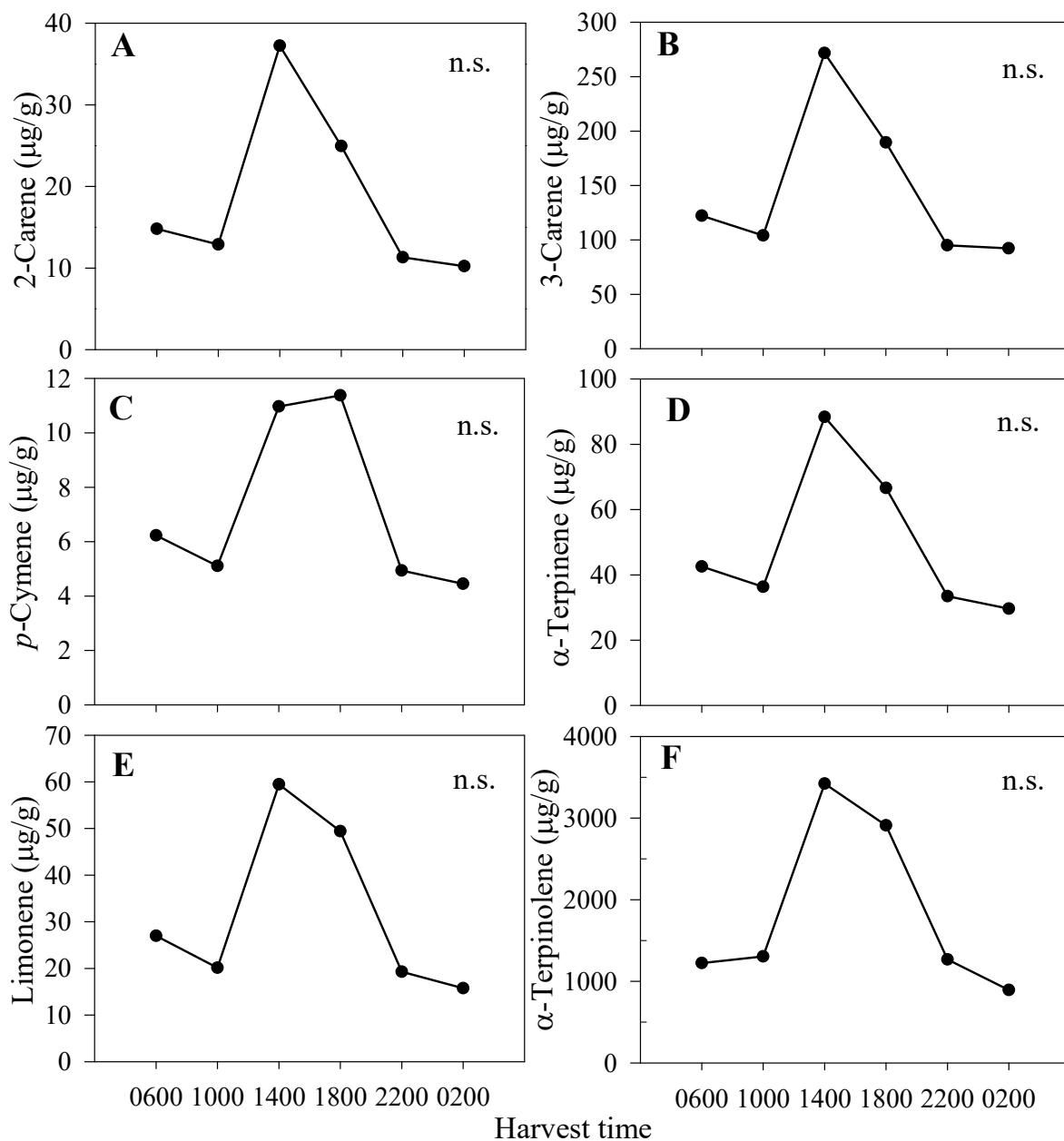


Figure 6.15 Changes in the concentration of aroma volatiles of the aqueous phase, including 2-carene (A), 3-carene (B), *p*-cymene (C), α -terpinene (D), limonene (E) and α -terpinolene (F), over the diurnal harvest cycle in the 2014/2015 season. The LSD ($P = 0.05$) bars are for comparison of means between harvest times. $n = 4$ sap replications plus two laboratory replicates per replication.

The non-aqueous phase had the greatest concentration of the six volatile compounds, whereas the aqueous phase had the least (Figure 6.14). In the non-aqueous phase sap, the highest concentrations of 2-carene, 3-carene, *p*-cymene, α -terpinene, limonene and α -terpinolene were at 1400 h. The lowest levels were at between 0200 h and 1000 h. However, the concentrations of these volatiles in

the aqueous phase sap were not significantly ($P>0.05$) different over the diurnal harvest cycle (Figure 6.15). Hexanal and ethyl octanoate were not detected in the ‘Honey Gold’ mango sap.

6.3.2 Experiment 2: Effect of sap components on USB incidence

Placing 0.1 ml sap or the main ingredients of oily fraction, including terpinolene and limonene, onto fruit could elicit brown-coloured damage to the surrounding skin (Table 6.1). The symptoms were generally similar, but not identical, to USB. They illustrated the potential for sap to cause significant damage.

Table 6.1 Effects of sap components on the response of fruit harvested at different times on USB development. To test the hypothesis that USB diurnal responses were at least partly due to sap characteristics, an aliquot of 0.1 ml of either spurt or ooze sap, of whole sap from additional ‘Honey Gold’ fruit harvested in the morning (0600 h) and afternoon (1400 h) in the 2014/2015 season or of distilled water was placed onto the abraded surface of morning- (0600 h) and afternoon-harvested (1400 h) fruit. The fruit were then held at 12 ± 1 °C for 6 d, road-freighted to Brisbane under commercial conditions in a standard 20-pallet 40-foot refrigerated trailer at about 14 °C to Wamuran in Queensland. Then the fruit were transported in an air-conditioned vehicle at ca. 22 °C to the lab in Brisbane and ripened in a ripening room at 20 °C and 90 - 100% RH.

Treatment	Browning incidence (%)	Browning severity
Water	0	0.0 a
Applied to the morning harvested fruit		
Afternoon sap	50	2.7 c
Afternoon spurt sap	57	1.8 b
Afternoon ooze sap	0	0.0 a
Applied to the afternoon harvested fruit		
Morning spurt sap	7	0.1 a
Morning ooze sap	0	0.0 a
Afternoon sap	100	2.2 b
Afternoon spurt sap	100	2.9 c
Afternoon ooze sap	0	0.0 a

Means within assessed parameter columns with different lower case letters differ significantly ($P\leq 0.05$) by Fisher's protected LSD. $n = 7$ individual fruit replicates each comprised of two sub-samples per fruit.

The average results for browning incidence and severity are stated (Table 6.1). Neither water nor the ooze sap obtained from morning- or afternoon-harvested fruit resulted in sap burn injury when placed on the fruit. However, whole sap and spurt sap from afternoon-harvested fruit resulted in considerable browning to all the fruit; viz. 100% browning incidence. The spurt sap from morning-harvested fruit resulted in less damage. In addition, the incidence data for applying afternoon spurt sap showed a higher proportion of treated sites with skin damage on fruit harvested in the afternoon as compared to morning-harvested fruit.

Treatment of fruit skin with 2-carene resulted in less skin browning (viz., 7% of incidence) as compared to those with terpinolene and limonene (viz., 100% incidence). Topical applications of terpinolene and limonene caused similar degrees of induced USB symptoms on all fruit (Table 6.2). The symptoms from induced USB by terpinolene and limonene were similar, but not identical, to natural USB (Figure 6.16). They illustrate the potential for sap to cause damage. There was no evidence of differing efficacy between these two sap components.

Table 6.2 Effect of main oil fraction ingredients including terpinoline, 2-carene and limonene to induce USB symptoms in ‘Honey Gold’ mango fruit skin. An aliquot of 0.1 ml of pure terpinolene, limonene, 2-carene and distilled water were placed onto abraded surface of morning- (0600 h) and afternoon-harvested (1400 h) fruit. The fruit were then held at 12 ± 1 °C for 6 d, road-freighted to Brisbane under commercial conditions in a standard 20-pallet 40-foot refrigerated trailer at about 14 °C to Wamuran in Queensland. Then the fruit were transported in an air-conditioned vehicle at ca. 22 °C to the Eco-Science Precinct, Department of Agriculture and Fisheries and ripened in a ripening room at 20 °C and 90 - 100% RH.

Treatment	Induced USB incidence (%)	Induced USB severity
Water	0	0.0 a
Pure 2-carene	7	0.1 a
Pure Limonene	100	4.0 b
Pure terpinolene	100	4.0 b

Means within assessed parameter columns with different lower case letters differ significantly ($P \leq 0.05$) by Fisher's protected LSD. $n = 7$ individual fruit replicates each comprised of two sub-samples per fruit.

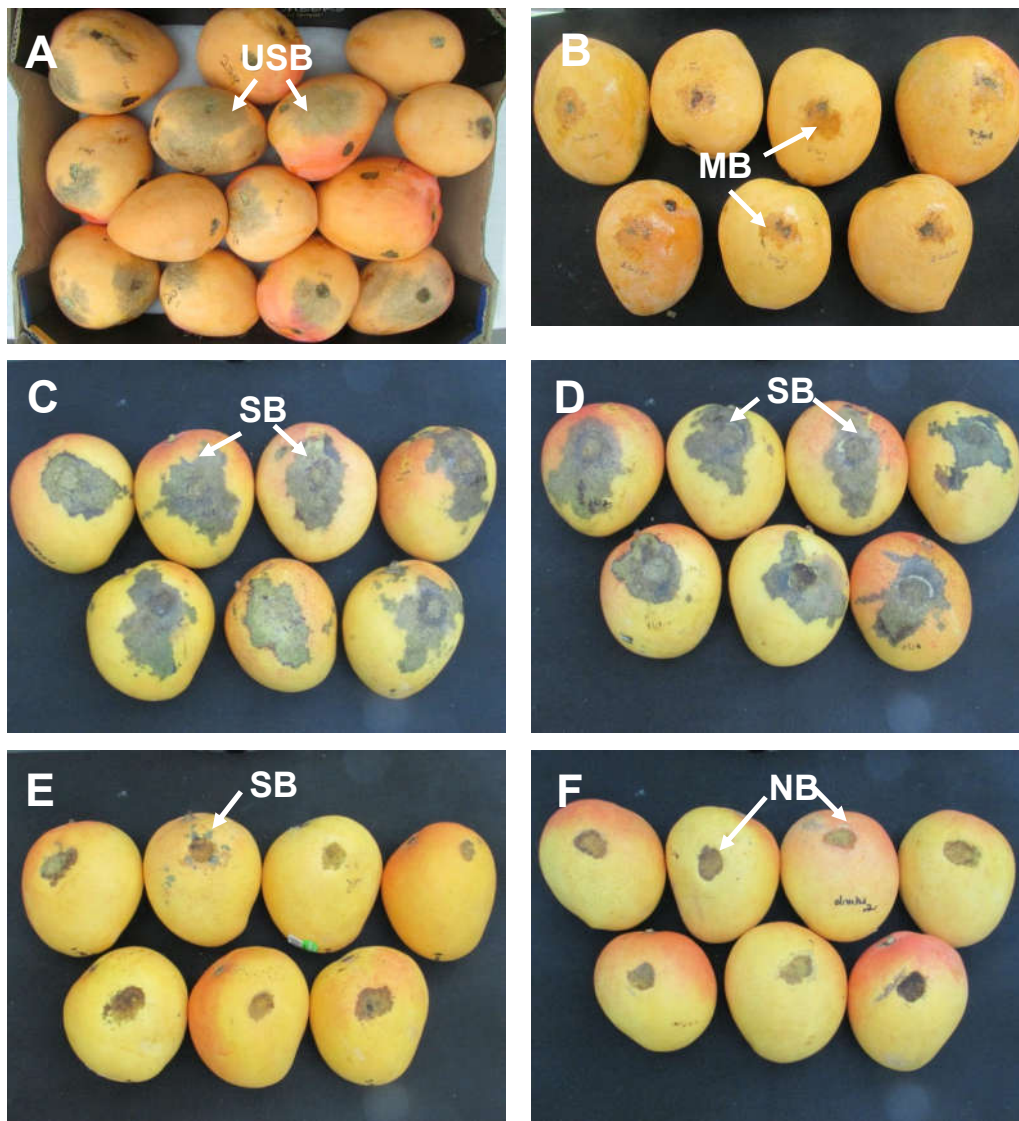


Figure 6.16 Symptoms of USB in the afternoon-harvested (1400 h) fruit caused by abrasion test as applied with an orbital finishing sander (A), sap-burn browning due to topical sap application (B), induced USB symptoms by topical terpinolene (C), limonene (D) and 2-carene (E) applications, and control sample treated with distilled water (F). MB, mild skin browning; NB, no browning; SB, severe skin browning; USB, under-skin browning.

6.3.3 Anatomical observations

Natural USB symptoms were localised to parenchyma tissues under the epidermis and were not associated with the cuticle (Figure 6.17A). There was no browning of the cuticle through to the fruit surface and as comprised of the waxy cuticle and epidermal and hypodermal cell layers. Natural USB-affected tissues showed dark brown parenchyma cells around the epithelial cells that line resin ducts (Figure 6.17B).

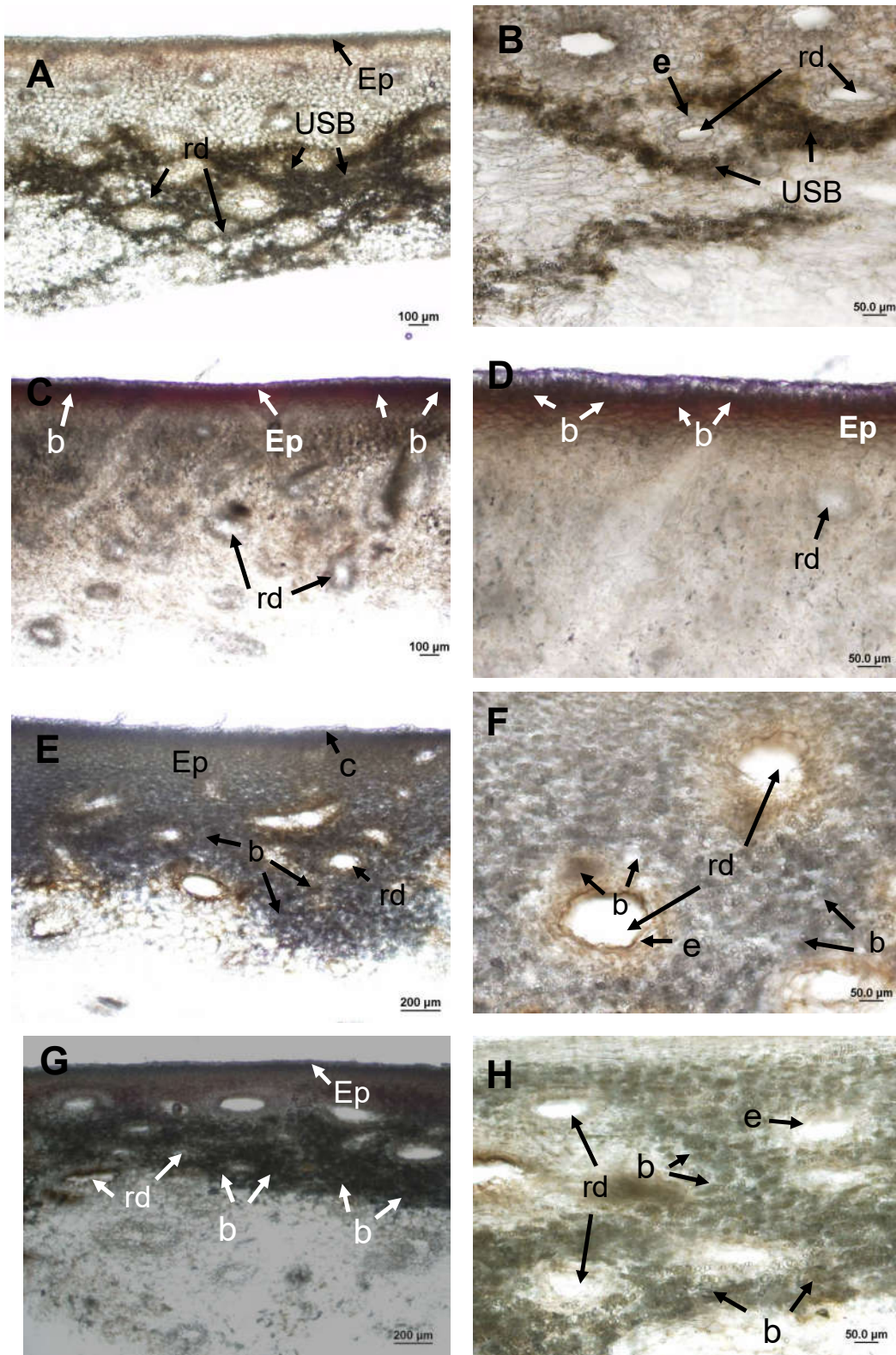


Figure 6.17 Hand sections of natural USB (A, B), browning due to topical spurt sap application (C, D), induced USB symptoms by topical terpinolene (E, F) and limonene (G, H) versus control (distilled water) applications (I, J) in 'Honey Gold' mango fruit. (A) Transverse section (TS)

through USB-affected skin tissue showing browning beneath the epidermis. (B) TS through USB-affected skin tissue showing dark-brown cells surrounding resin ducts. (C, D) TS through browning due to topical spurt sap application showed mild browning was appeared slightly in the epidermis. (E) TS through induced USB-affected skin tissue by topical terpinolene showing browning beneath the epidermis. (F) TS through induced USB by topical terpinolene showing dark-brown cells surrounding resin ducts. (G) TS through induced USB-affected skin tissue by topical limonene showing browning beneath the epidermis. (H) TS through induced USB by topical limonene showing dark-brown cells surrounding resin ducts. (I) TS through control tissue showing no browning beneath the epidermis. (J) TS through control tissue showing no browning around resin ducts. Scale bars = A, C, 100 μm ; B, D, 20 μm . c, cuticle; rd, resin duct; Ep, epidermal cells; e, epithelial cells; USB, under-skin browning; b, browning; Scale bars = A, C, E, G, I, 100 μm ; B, D, F, H, J, 50 μm .

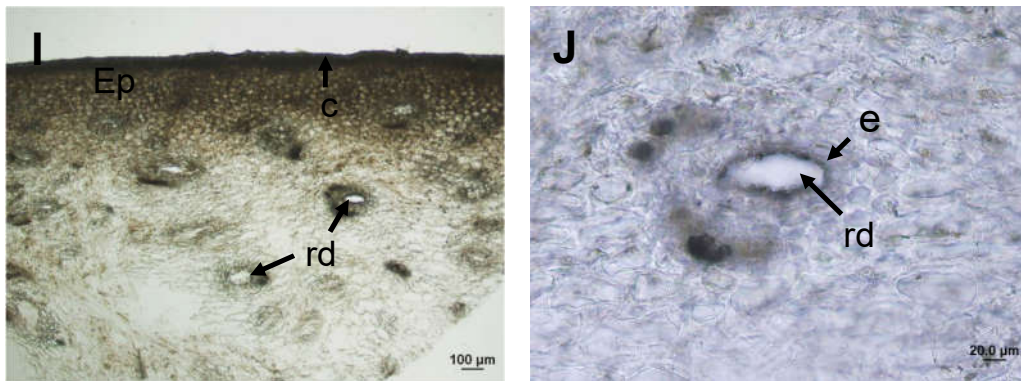


Figure 6.17 (Continued).

Topical sap injection caused mild browning which was appeared slightly in the epidermis (Figure 6.17C and D). No browning was observed in healthy fruit tissues as treated by topical water application (Figure 6.17 I and J). Induced USB symptoms as caused by the main non-aqueous fraction sap components terpinolene and limonene also showed distinct browning beneath the epidermal layer (Figure 6.17 E and J). Observation of cross sections through induced USB symptoms due to terpinolene and limonene showed similar dark brown parenchyma cells surrounding the epithelial cells as compared with natural USB symptoms (Figure 6.17 F and H). While the induced USB symptoms by terpinolene and limonene were not identical to natural USB, the potential for sap components to cause damage was clearly demonstrated.

6.4 Discussion

There was a strong association between harvest time and USB incidence and severity. ‘Honey Gold’ fruit harvested at night and in the early morning were less sensitive to USB as induced by a ‘standardised test’ comprised of light skin abrasion followed by storage at 10 - 12 °C for 6 d before fruit ripening and assessment. The results affirmed a strong consistent effect of harvest time. ‘Honey Gold’ mango fruit harvested at 1400 h and 1800 h under high temperature (37 - 40 °C) and low RH (38 - 40%) developed much higher USB incidence (i.e. 50 - 70%) and severity (i.e. 5 to 8-fold higher) as compared to fruit harvested at either 2200 h or 0200 h or 0600 h. Thus, ‘Honey Gold’ fruit harvested at night and in the early morning are ‘commercially’ less susceptible to USB.

The higher USB incidence in the afternoon harvest could be a stress response related to physical damage (viz., abrasion) and temporal harvest time (viz. concentrated volatile concentrations). In the experiments, natural USB was observed on areas of the fruit that had not been abraded. It is likely that physical damage during road freight from the Northern Territory to south-east Queensland was contributory. Natural USB incidence and severity on these non-abraded areas was characteristically less on fruit harvested at night and in the early morning as compared with those harvested in the afternoon (Figure 6.7). Marques et al. (2012) similarly determined that USB often appeared around visible physical injury sites on fruit, such as those from prolonged transport (Marques et al., 2012). Likewise, the severity of sap burn injury was higher on fruit harvested at noon and in the afternoon than on those harvested at other times (Amin et al., 2008). Thus, afternoon-harvest combined with physical damage probably involving disruption of cellular integrity upon abrasion leads to more USB damage.

Based on largely anecdotal evidence, it was hypothesised that the incidence and severity of USB on ‘Honey Gold’ mango were at least partly due to effects of sap. It was considered likely that differences in USB incidence and severity of mango fruit across the diurnal harvest cycle could be associated with putative differences in sensitivity of the mango fruit skin and / or the changes in composition of potentially phytotoxic sap. Sap collected from afternoon-harvested fruit proved to cause more damage to abraded skin than sap from morning-harvested fruit, which actually caused no damage (Table 6.1). It was possible that higher concentrations of phytotoxic components were present in afternoon sap. Moreover, it was clear that spurt sap and the non-aqueous sap phase (oil fraction) was responsible for the damage. Thus, afternoon sap, especially afternoon spurt sap evidently contributes to the higher sensitivity of afternoon-harvested fruit to USB. This conclusion is in general agreement with sap burn studies, which showed that skin damage was mainly due to the upper non-aqueous phase (Loveys et al., 1992). Similarly, Maqbool et al. (2007) reported that

spurt sap exuded within first 15 s after removing the pedicel of fruit was the most injurious to the skin of fruit.

The phytotoxic response is evidently related to individual sap components, especially concentrations of volatiles including terpinolene and limonene. Topical application to the fruit skin of terpinolene and limonene, both major components of the oil fraction, resulted in USB-like browning damage. In contrast, topical applications of carene and distilled water did not (Table 6.2). While the symptoms were not identical to USB (Figure 6.16), the potential for sap components to cause damage was clearly demonstrated. Terpinolene and sap upper phase also caused tissue damage in 'Kensington' mangoes, with associated subsequent enzymic browning (Loveys et al., 1992). Anatomically, both natural (Figure 6.17 A and B) and induced USB symptoms (Figure 6.17 E, F, G and H) featured browning around the epithelial cells that lined resin ducts, while sap injection gave slight browning in the epidermis (Figure 6.17 C and D). Terpenoid components of spurt sap are no doubt mainly responsible for the damage manifested as browning. Further work is required to characterise the anatomy of natural versus induced USB symptoms, such as are caused by sap volatiles components, and the enzymology of the browning process.

Volumes of 'Honey Gold' whole sap ranged from 0.3 to 0.9 ml/fruit over the diurnal harvest cycle. By comparison, O'Hare (1994) estimated sap volumes exuded from 'Kensington Pride' fruit of around 1.67 ml/fruit. The volumes of the non-aqueous and aqueous phases at 1400 h and 1800 h were considerably ($P \leq 0.05$) lower than were those collected at other harvest times. In an earlier study, Maqbool et al. (2007) also reported lower sap volumes related to afternoon harvest. However, there was no significant difference ($P > 0.05$) in relative proportions of non-aqueous and aqueous sap phases over the diurnal harvest cycle (Figure 6.9). This means that both phases became more concentrated at the same time.

Harvest time also affected the concentrations of aroma volatile compounds in mango fruit sap. Key aroma volatile compounds, including 2-carene, 3-carene, α -terpinene, *p*-cymene, limonene and α -terpinolene were more concentrated in sap from fruit at 1400 h as compared with sap from early morning and night harvests (Figures 6.10 and 6.13). Terpinolene was the most abundant compound in the whole sap and non-aqueous phase of 'Honey Gold' fruit. Terpinolene in non-aqueous sap was 4-6 times higher in fruit harvested in the afternoon than in those picked at other harvest times (Figure 6.14). This 'peaking' was associated with the higher USB susceptibility of fruit harvested at 1400 h. Under high temperatures and low RH, it is likely that all cells of the afternoon-harvested fruit decreased in water content (Link et al., 1998; Schroeder and Wieland, 1956). This response would concentrate aroma compounds within the sap. The apoplasmic water potential gradient is

correlated with the laticifer and leaf turgor pressure in water-stressed plants (Downton, 1981; Milburn et al., 1990). Reduced sap flow into the tomato fruit is related to water potential gradient between fruit and stem (Johnson et al., 1992). Thus, harvesting in the afternoon is likely to increase ‘Honey Gold’ fruit susceptibility to USB because of higher phytotoxic volatile components especially in the non-aqueous phase of the fruit sap (Figure 6.18).

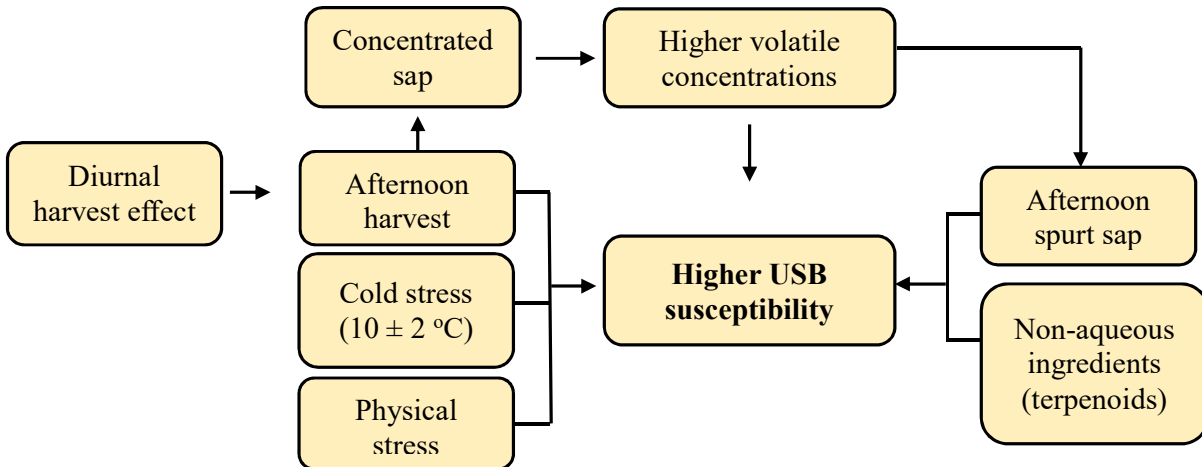


Figure 6.18 A model representing the effects of diurnal harvest cycle and sap components on USB development in ‘Honey Gold’ mango fruit. The arrows mean ‘related with’.

6.5 Conclusion

This study demonstrated that harvesting at night or early morning increases ‘Honey Gold’ fruit resistance to USB. Delaying for 24 h before cooling enhanced the incidence and severity of normal USB in the afternoon-harvested fruit. One of the contributing factors towards more USB in afternoon-harvested fruit is a concomitant increase in “aggressive” volatile components in sap from these fruit. It is clear that harvest time is an important quality enhancer for ‘Honey Gold’ mango fruit. Overall, these results support practise change from conventional afternoon to night and early morning harvesting of ‘Honey Gold’ mango fruit to reduce the incidence and severity of USB. Added benefits from night and early morning harvesting include less physiological stress on mango pickers because of relatively lower field heat temperatures and lessened fruit respiration rates by virtue of lower ambient fruit temperatures. As night and early morning harvesting offers a tremendous opportunity to prevent USB on fruit and improve the quality of ‘Honey Gold’ mango in coming seasons, the grower would be encouraged to adopt this practice.

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CHAPTER 7. COMPARATIVE ANATOMY AND ENZYMATIC BROWNING OF UNDER-SKIN BROWNING AND RESIN CANAL DISORDER

Abstract

Under-skin browning (USB) and resin canal disorder (RCD) are two fruit browning disorders of concern to the Australian mango industry. USB is a sub-surface spreading grey-brown lesion that may only become evident in the marketplace. RCD is a dark brown discolouration of the finely branched pattern of ramifying sub-surface resin canals. These two browning disorders were compared and contrasted at the macroscopic and microscopic levels. USB symptoms were evident as dark-brown cells in the tissue surrounding the resin ducts. In contrast, RCD was characterised by browning of the lumen of resin ducts. Accordingly, USB and RCD involve distinctly different cell biology processes at cell and tissue levels as evidence by means of light microscopy and transmission electron microscopy. Five different stains including iodine - potassium iodide, Sudan IV, Sudan Black, Vanillin - HCl and Toluidine Blue O were used to characterise the histochemistry of these disorders. Naturally occurring USB was distinguished by starch retention and phenolic deposition in cell layers surrounding the epithelial cells of resin ducts. However, only starch retention was observed in tissues from fruit in which USB had been induced by treatment with terpinolene and limonene. USB was evidently related to host defence against stress. RCD was distinguished by deposition of polymerised phenolics inside laticifer lumens. PPO and POD activities were associated with both natural USB and RCD browning. However, POD was located differently in both natural USB and RCD browning. Nevertheless, POD was not detected in tissue prints from fruit in which USB had been induced by terpinolene and limonene. The comparative anatomy and enzymatic expression provides better understanding of browning mechanism of USB and RCD expression. This knowledge would contribute to management of these disorders.

7.1 Introduction

USB is visible through the surface of 'Honey Gold' mango fruit. It occurs under the epidermis as a grey-brown 'bruise-like' injury with no damage to the flesh (Hofman et al., 2010b). The defect is not usually obvious at harvest. Rather, visual symptoms become pronounced as the fruit ripen. Expression of symptoms is reportedly associated with starch retention in affected tissues (Marques et al. 2012). The mechanism of and controls for USB development are unclear. USB incidence and severity can be diminished by delayed or slow cooling of fruit to $\geq 14 - 16$ °C before or after packing (Hofman et al., 2010b). USB often appears around visible physical injury sites on fruit,

such as those that result from vibration in prolonged transport (Marques et al., 2012). Thus, USB development is related to mechanical damage after harvest in combination with low temperatures at just below 14 - 16 °C.

The occurrence of RCD has seemingly been on the rise in the domestic Australian mango industry in recent seasons. The disorder primarily affects the 'Kensington Pride' cultivar and involves discolouration of the finely branched network of sub-cuticle resin canals (Holmes, 1999). It usually expresses itself on the distal tip and /or around the mid-region of the fruit (Holmes, 1999). Causal factors and mechanisms of symptom expression are as yet unknown (Macnish et al., 2014). Unlike with USB, there is no evidence of suppression of starch hydrolysis (Holmes, 1999).

Mangoes contain a branching network of resin ducts distributed throughout fruit (Joel, 1980). This extensive duct system stores or emits resinous secretions (Joel and Fahn, 1980). Turgor pressure in the ducts is maintained due to the presence of large amounts of non-dialysable and non-starchy carbohydrate in sap (John et al., 2003). Sap in the fruit ducts is under high turgor pressure and when the pedicel is removed, sap initially spurts and then oozes out from the severed surface.

Mango sap includes two phases; an oily yellow-brown non-aqueous phase and a milky aqueous phase (Loveys et al., 1992). Mono-terpenes (John et al., 1999) and alk(en)ylresorcinols are the main constituents of the non-aqueous phase (Hassan et al., 2009). The aqueous phase contains high amounts of polyphenol oxidase (PPO) and peroxidase (POD) (John et al., 2003). The non-aqueous phase, which is abundant with terpenoids, has been proposed to contribute to plant defence against insect attacks, such as those from fruit flies (Joel, 1980) and prevent the fruit from bacterial and fungal infection (Negi et al., 2002).

Browning processes in fruit typically involve PPO and / or POD enzymes oxidising phenolic substrates with the presence of oxygen (Franck et al., 2007; Jiang et al., 2004). Anthocyanins are substrates in the oxidation process for PPO in litchi browning (Jiang et al., 2004). PPOs are mainly present in vacuoles and plastids (Murata et al., 1997). PODs are mostly located in chloroplasts (Nakano and Asada, 1981), mitochondria (Prasad et al., 1995; Toivonen and Brummell, 2008) and plant cell plasma membranes (Vianello and Macri, 1991). PPO and POD are active in mango skin and sap (John et al., 2002; Robinson et al., 1993). Robinson et al. (1993) reported that PPO in the skin of 'Kensington' mango fruit was responsible for the browning associated with sapburn injury. John et al. (2002) showed that PPO and POD activities in the skin of mango fruit were associated with sap-burn injury.

There is relatively limited information on the histochemistry and enzymatic browning of USB and RCD disorders. USB and RCD are hypothesised to be enzymatic browning processes which are related to PPO and POD activities. The aim of this study was to gain more understanding of the comparative cellular biology of USB and RCD symptom expression in Australian mango cultivars at cell and tissue levels, including in relation to PPO and POD activities. In this chapter, the anatomy and histochemistry of USB and RCD were compared by means of binocular microscope observation, free-hand sectioning, light microscopy (LM) and transmission electron microscopy (TEM). Further anatomical comparison of natural USB and artificially induced USB was examined using LM and TEM. Localisation of PPO and POD in natural USB, artificially induced USB-affected tissue and RCD was determined using tissue printing. A better understanding of the underlying biology of USB and RCD should help inform and improve management of these physiological disorders.

7.2 Materials and methods

7.2.1 Materials

‘Honey Gold’ (dry matter content of $16.9 \pm 1.4\%$) and ‘Kensington Pride’ (dry matter content of $16.8 \pm 1\%$) mango fruit were harvested at commercial maturity from orchards near Katherine and Darwin (14.28°S , 132.16°E), respectively, in the Northern Territory, Australia. ‘Honey Gold’ fruit were de-stemmed in a solution containing 2.5 g/l Mango Wash[®] (Septone, ITW AAMTech, Australia) for 2 min. They were then taken to a nearby commercial packing house and run over a pack line under standard conditions, including fungicide treatment (Sportak[®], a.i. prochloraz, Bayer Crop Science, Australia), brushing, drying and sorting (Hofman et al., 2010a). Next the fruit were graded for uniform quality and size as per commercial procedures. The fruit were packed as a single layer in cardboard trays with semi-rigid plastic inserts. ‘Kensington Pride’ fruit were picked from the tree and de-stemmed without standard commercial harvest practices. They were packed directly into cardboard trays. All fruit were transported by car (ca. 22°C) to the Darwin airport and then air-freighted from the Northern Territory to Brisbane, Queensland within 1.5 d of harvest. They were then transported in an air-conditioned (ca. 22°C) vehicle to the nearby postharvest laboratories in Brisbane or to Gatton for postharvest treatments.

7.2.2 Treatments

At the laboratory, trays containing ‘Honey Gold’ fruit were randomised in cardboard trays and placed on an EQ 21857 vibration table (RL Windsor & Son Pty Ltd., Australia) in a cold room at 12

± 1 °C. The fruit were exposed to 12 Hz vibration for 12 h at 12 °C and 90 - 100% RH. They were then removed from the table and held at 12 ± 1 °C for 6 d. Thereafter, the fruit were maintained at 20 °C and 90 - 100% RH until ripe. These treatments are to simulate commercial handling and transport. Skin tissues displaying USB symptoms were excised for anatomical study. Additional 'Honey Gold' fruit for the control treatment were maintained at 12 °C without vibration for 6.5 d and ripened at 20 °C and 90 - 100% RH until ripe without vibration treatment. The 'Kensington Pride' fruit were also maintained under standard shelf life conditions at 20 °C and 90 - 100% RH. Flesh and skin tissues from fruit exhibiting RCD symptoms were collected at firm (at hand firmness scale 1) and ripe stages for anatomical study.

7.2.3 Comparative anatomy of USB and RCD

7.2.3.1 Binocular microscope observation

Rectangle skin tissues ~ 2 cm width, ~ 3 - 4 cm length displaying USB and RCD symptoms were removed using a sharp knife. Briefly, a cross section through a USB lesion was excised with a single-edge stainless-steel blade (Proscitech, Australia). For tissues affected by RCD ~ 3 - 4 mm depth, the underlying pulp under the skin was removed using its blunt edge of blade. These symptomatic tissues were photographed using a digital camera (Canon DOS40D, Canon Inc., Japan), and with a SZH10 research stereo microscope (Olympus, Japan) equipped with Micro Publisher 3.3 RTV camera (Q-Imaging, Germany).

7.2.3.2 Free-hand sectioning

Fruit tissues ($n = 5$) with and without skin browning symptoms were processed for light microscopy. USB on 'Honey Gold' fruit and RCD on 'Kensington Pride' fruit were compared and contrasted at the cellular level. Free-hand sections were cut through the excised tissues following the methods of Ruzin (1999a). The tissue sections were transferred onto a drop of distilled water on a glass microscope slide, and covered with a glass cover slip. The hand sections were viewed and photographed with an Olympus BX61 LM equipped with a DP 70 camera (Olympus, Japan).

In order to better understand the histology, free-hand sections were stained with five different stains:

- Iodine - potassium iodide (Biolab Australia Ltd, Australia) to stain starch after Ruzin (1999b): Sections were stained for 5 min with 1 g potassium iodine and 1 g iodine in 100 ml distilled water.

- Sudan Black (Sigma, USA) to detect lipids after Ruzin (1999b): Sections were immersed in 50% ethanol for a few seconds. They were then incubated for 5 - 10 min in freshly prepared Sudan Black dye solution of 0.07 g Sudan Black in 100 ml 70% ethanol. The sections were rinsed with 50% ethanol for 1 min.
- Sudan IV (Proscitech, Australia) to stain ethanol-soluble lipids after Ruzin (1999b): The procedure was as above for Sudan Black.
- Vanillin - HCl to stain condensed tannins after Vermerris and Nicholson (2006): Sections were immersed in a saturated solution of vanillin (Sigma Aldrich, China) in 90% ethanol for 15 - 30 min. They were then placed onto a microscope slide and one drop of 9N HCl (Ajax Chemicals, Australia) was added. The section was immediately viewed under the microscope.

Stained free-hand sections were viewed and photographed with an Olympus BX61 LM equipped with a DP 70 camera (Olympus, Japan).

7.2.3.3 LM

Tissues displaying USB and RCD symptoms were fixed in FAA solution comprised of 50 ml 95% ethanol, 5 ml glacial acetic acid, 10 ml 37 - 40% formalin formaldehyde and 35 ml distilled water. After dehydration through an increasing ethanol series of 70, 90 and 100% twice (45 min each change), samples were infiltrated with 100% xylene twice for 45 min each time. Samples were covered with paraffin wax (Leica Bio Systems, USA) at 60 °C twice for 45 min each time. Then samples were mounted with paraffin wax at 60 °C and embedded on Microm EC 350-2 embedding station (Thermo Fisher Scientific Inc., New Zealand). Serial sections 5 µm-thick sections were cut on a Leica RM 2245 semi-automated rotary microtome (Leica, Germany). They were de-waxed three times in 100% xylene (RCI Labscan, Thailand) for 2 min at each step and washed in a 100, 100, 90 and 70 % ethanol series. The tissue sections were washed in running water for 2 min and distilled water for 30 s. They were stained with using 0.1% Toluidine Blue O (TBO) in 1% (w/v) borax. Sections were viewed and photographs were taken with an Olympus BX61 automated microscope (Olympus, Japan) equipped with a DP 70 camera (Olympus, Japan).

7.2.3.4 TEM

Samples were prepared from thin slices of mango pericarp by using a scalpel blade (Swann-Morton®, England). They were fixed for 2 h in 2.5% glutaraldehyde (ProScitech, Australia) in 0.1M sodium phosphate buffer (pH 6.8) containing 1% caffeine (Merck, Germany) as described by

Mueller and Greenwood (1978). The fixed samples were washed for 1 h with four serial changes using the same 0.1M sodium phosphate buffer.

Further sample processing for TEM was conducted in a microwave processor equipped with a cooler (18 °C; Pelco Biowave® 34700 Microwave with SteadyTemp™ Cooler, Ted Pella Inc., USA). Fixed samples were washed two times with phosphate buffer (pH 6.8) and post-fixed twice serially in 1% (v/v) osmium tetroxide (ProSciTech) in 0.1 M sodium cacodylate buffer (CB; pH 7.2) for 6 min each in the microwave processor at 80 Watts and 74.5 kPa vacuum, followed by rinsing twice in ultrapure water. They were subsequently serially dehydrated using a 30%, 40%, 50%, 60%, 70%, 80%, 90% and twice in 100% ethanol series for 3 min per step (150 Watts, without vacuum). The dehydrated samples were infiltrated in LR White resin (Medium grade, ProSciTech) using a 1:3, 1:2, 1:1, 2:1, 3:1, 1:1 resin to ethanol and 100% resin series for 6 min in each step in the microwave processor (150 Watts, vacuum). Samples were changed to pure resin and rotated overnight in a BenchRocker™ 3D rotator (Benchmark Scientific Inc., Taiwan) followed by 30 min of infiltration at 150 Watts under high vacuum in a microwave processor with pure resin. They were then embedded in pure LR White resin, placed into 0.4 ml capacity gelatin capsules and polymerized in an oven (Axycs, Australia) at 60 °C for 24 h.

Semi-thin and thin sections were cut on a Leica EM UC6 Ultracut ultramicrotome (Leica, Austria). Semi-thin sections at 0.5 µm were placed onto glass microscope slides and stained with 0.5% Toluidine Blue in 1% (w/v) borax (Sigma-Aldrich, Australia). The sections were observed with an Olympus BX61 LM equipped with a DP 70 camera (Olympus, Japan). Thin sections were cut using a Diamond knife (Diatome, Switzerland) and placed onto Formvar™ coated glow discharged copper grids (200 square mesh; ProSciTech). They were stained with 5% uranyl acetate in 50% ethanol and in lead citrate (Reynold's lead stain, ProSciTech) for 30 s each. The samples were observed in a JEM 1010 transmission electron microscope (JEOL, Japan) at 80 kV accelerating voltage. Images were captured on a SIS Veleta digital camera using iTEM® software (Olympus Soft Imaging Systems, Germany).

7.2.4 Effects of non-aqueous fraction of sap on USB development

Fruit tissues ($n = 3$) with and without natural USB and induced USB symptoms induced by terpinolene and limonene (Chapter 6) were also prepared for further anatomical study.

7.2.4.1 Further anatomical comparison by LM of natural USB and induced USB symptoms

The unstained free-hand sections were prepared as per section 7.2.3.2. As above, iodine - potassium iodide (IKI) solution was used to characterise starch retention.

7.2.4.2 Further anatomical comparison of natural USB and induced USB symptoms using TEM

Fruit tissues ($n = 3$) were prepared for TEM as per section 7.2.3.5.

7.2.5 Localisation of polyphenol oxidase (PPO) and peroxidase (POD) in USB and RCD browning

Detection of PPO within the mango peel was investigated using a tissue printing method described by Melberg et al. (2009). Exposed faces of mango peel were made with a single-edge stainless-steel blade (Proscitech, Australia). The exposed surface was pressed gently for 1 min onto water-moistened nitrocellulose filter paper (Whatman GmbH, Germany). The tissue prints were then dipped into the PPO substrate solution for 5 min. The PPO substrate solution was made using 1 mM catechol (Sigma, Australia), 1 mM 3-methyl-2-benzo-thiazolinone hydrazone (MBTH) (Sigma, Australia) and 50 mM ascorbic acid (BDH Chemicals, Australia) in 50 mM phosphate buffer (pH 6.5), containing 2% (v/v) N,N-dimethylformamide (DMF) (Sigma, Australia). The membranes were next imaged under a Leica EZ4HD LM with an integrated camera (Leica, Singapore). Pink or red pigment on the paper indicated the location of PPO activity.

POD detection within the mango peel was also determined following the methods of Melberg et al. (2009). Exposed faces of mango peel were made with a single-edge stainless-steel blade (Proscitech, Australia). The exposed surfaces were pressed onto water-moistened nitrocellulose paper (Whatman GmbH, Germany) for 1 min. Then, the prints on the nitrocellulose paper were incubated for 1 min in a solution of 1-Step™ chloronaphthol solution (Thermo Scientific, USA) containing 100 µl of 3% hydrogen peroxide (Fluka Analytical, Switzerland) for every 50 ml of solution. The membranes were imaged under Leica EZ4HD LM (Leica, Singapore). Blue pigment on the paper indicated the location of POD activity.

7.3 Results

7.3.1 Comparative anatomy of USB and RCD

7.3.1.1 Macroscopic observations

USB symptoms

USB is a visible diffuse browning symptom under the epidermis of the fruit surface (Figure 7.1). USB-affected areas are beneath the epidermal layer (Figure 7.3A).

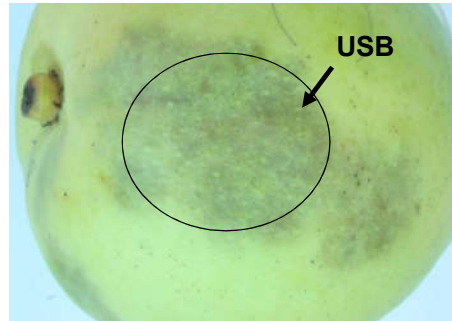


Figure 7.1 Natural USB symptoms on a 'Honey Gold' mango fruit.

RCD symptoms

RCD appears as a network of dark grey or brown branched black streaks and associated diffuse discolouration within the skin and pulp of mango fruit (Figure 7.2). Under the epidermis, white 'fibres' inside and along the brown canal may be exhibited (Figure 7.3B).

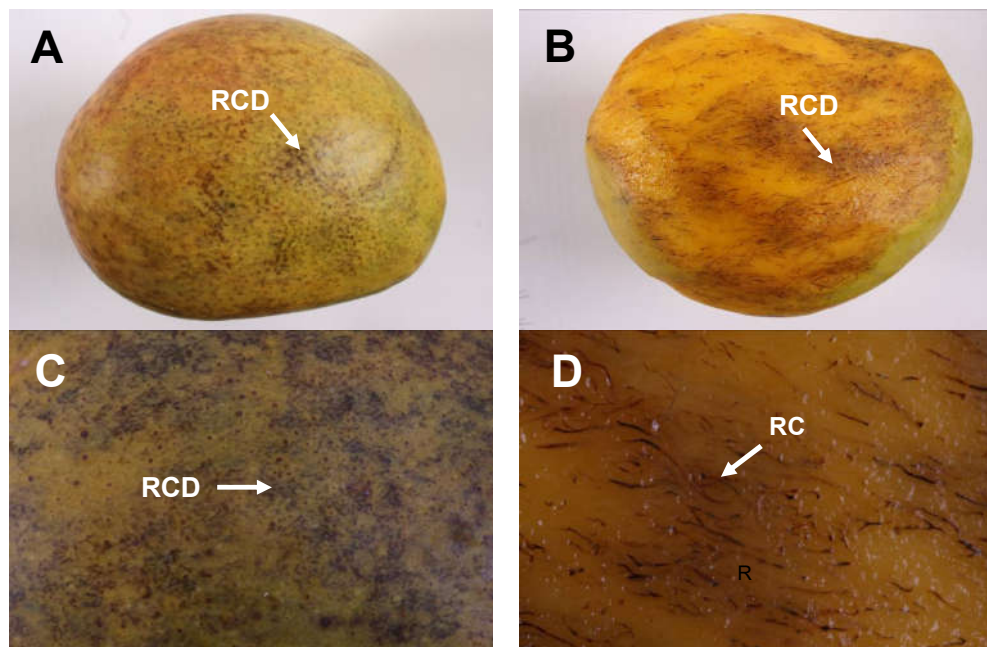


Figure 7.2 Symptoms of RCD on a 'Kensington Pride' mango fruit. (A, C) Dark traces of finely branched resin canals in the skin. (B, D) Discoloured resin canals (RC) in the outer flesh layers. RC, resin canal; RCD, resin canal disorder.

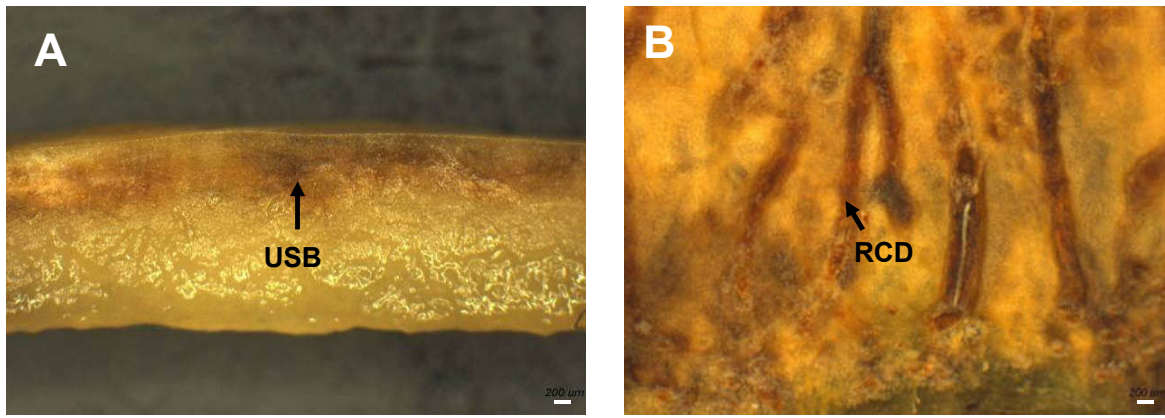


Figure 7.3 Comparative cross-sections of USB on ‘Honey Gold’ (A) and RCD on ‘Kensington Pride’ mango fruit (B). USB, under-skin browning; RCD, resin canal disorder. Scale bars = 200 µm.

7.3.1.2 Free-hand section observations

USB symptoms

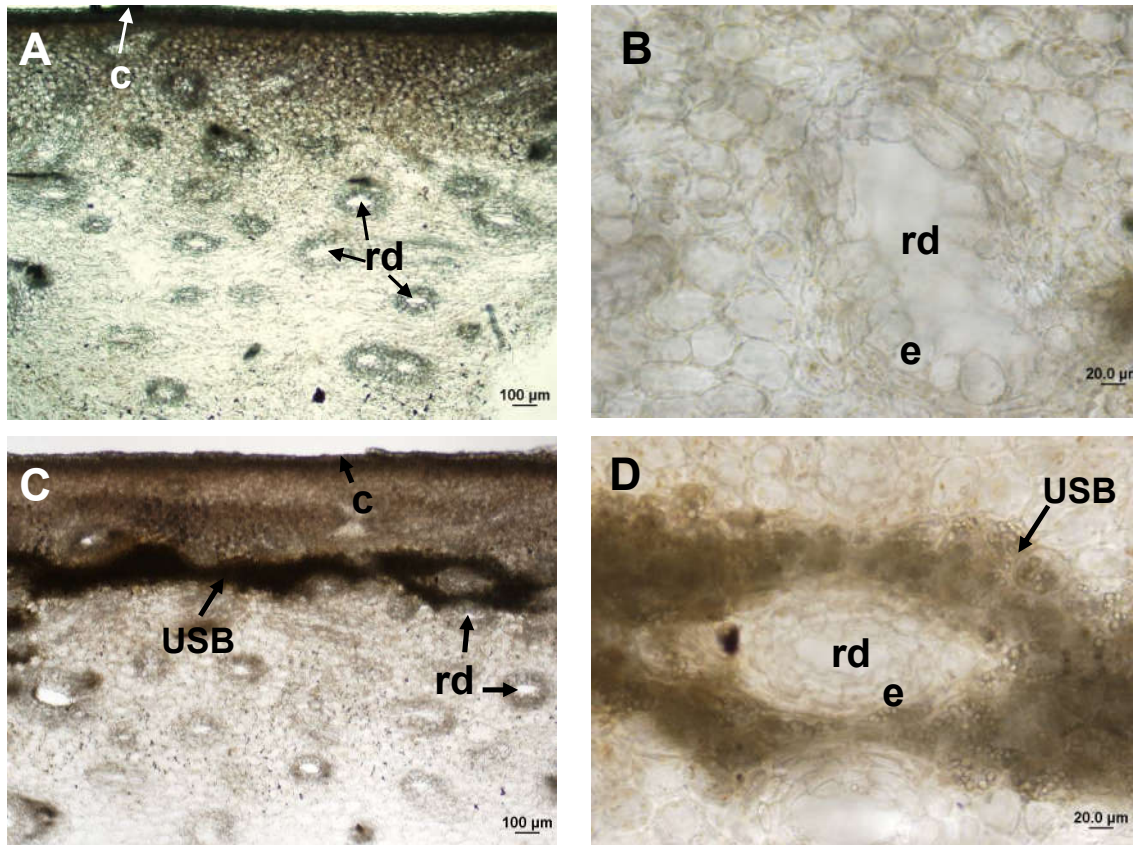


Figure 7.4 Hand sections of control and USB-affected tissues in ‘Honey Gold’ mango fruit skin. (A) Transverse section (TS) through control tissue showing no browning beneath the epidermis. (B) TS through control tissue showing no browning around resin ducts. (C) TS through USB-affected skin

tissue showing discolouration beneath the epidermis. (D) TS through dark-brown cells surrounding resin ducts. c, cuticle; rd, resin duct; e, epithelial cells; USB, under-skin browning. Scale bars = A, C, 100 μ m; B, D, 20 μ m.

In transverse section (TS), no browning was observed in sections through healthy, non-asymptomatic fruit tissues (Figure 7.4A and B). Cross sections through the fruit surface (Figure 7.4C) covering cuticle, epidermal and hypodermal cell layers in a severely USB-damaged area showed that symptoms were localised to tissues under the epidermis. Discolouration was not associated with the cuticle. USB-affected areas beneath the epidermal layer were dark brown to black and spread along resin ducts. A TS through USB-affected tissues showed dark brown cells immediately around epithelial cells that lined resin ducts (Figure 7.4D).

RCD symptoms

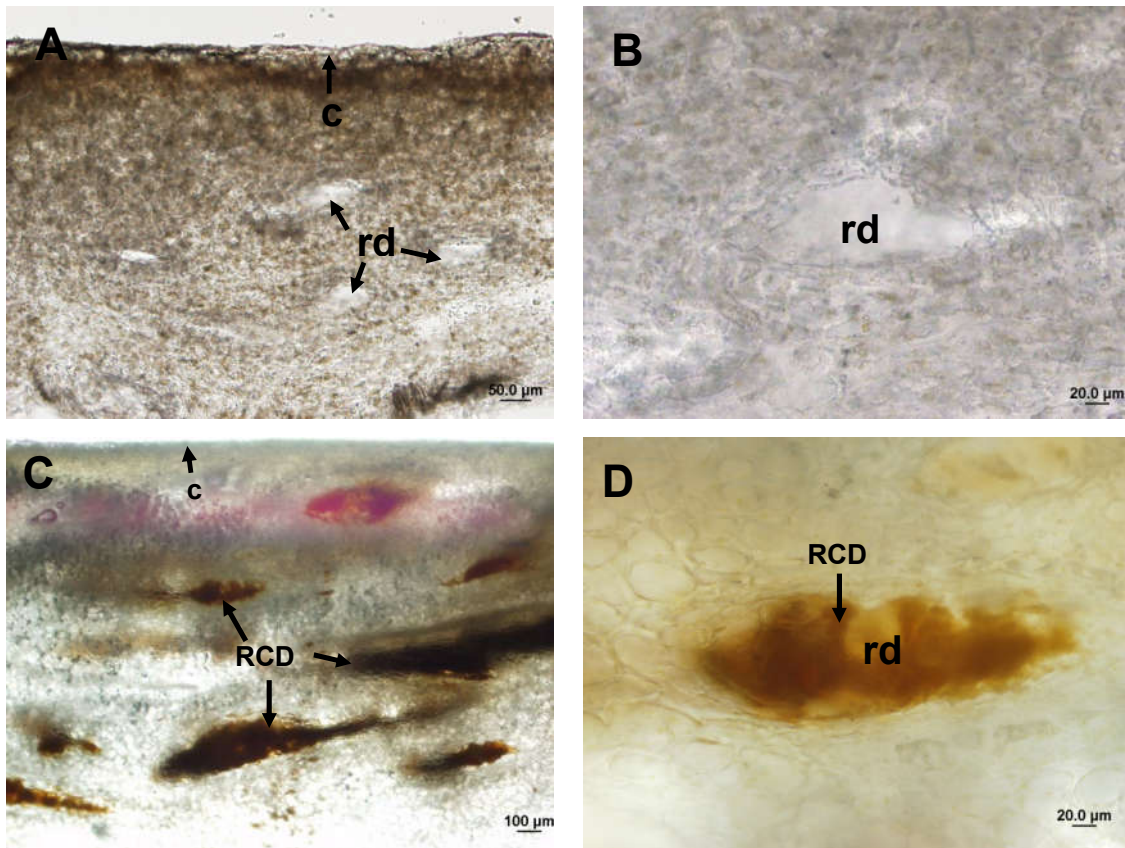


Figure 7.5 Hand sections of control and RCD-affected tissues in ‘Kensington Pride’ mango fruit skin. (A) Transverse section (TS) through control tissue showing no browning beneath the epidermis. (B) TS through control tissue showing no browning of the lumen of resin ducts. (C) TS through RCD-affected tissue showing discolouration beneath the skin. (D) TS through RCD-

affected tissues showing browning localised to the lumen of resin ducts. c, cuticle; rd, resin duct; RCD, resin canal disorder. Scale bars = A, 50 μm ; B, D, 20 μm ; C, 100 μm .

There was no browning of the cuticle in sections of RCD-affected tissues (Figure 7.5 C). RCD-affected areas were beneath the skin. Affected areas extended over much (e.g. > 90 %) of the surface of the fruit. Beneath the epidermis, many brown branched canals were visible in RCD-affected regions. The colour of canals exhibiting RCD symptoms was more brown than black. In TS, the browning was confined to the lumen of resin ducts of affected tissue (Figure 7.5D). Asymptomatic control fruit showed no such damage (Figure 7.5A and B).

7.3.1.3 Histochemical observations

USB symptoms

The granules were strongly distributed and concentrated inside the cells immediately surrounding the epithelial cells of resin ducts by TBO (Figure 7.6B). As shown by starch iodine staining, there was retention of starch granules within sections of USB-damaged fruit cells (Figure 7.7B). No granules were observed in control samples (Figure 7.6A) such that no starch granules were present in cells surrounding resin duct epithelial cells (Figure 7.7A).

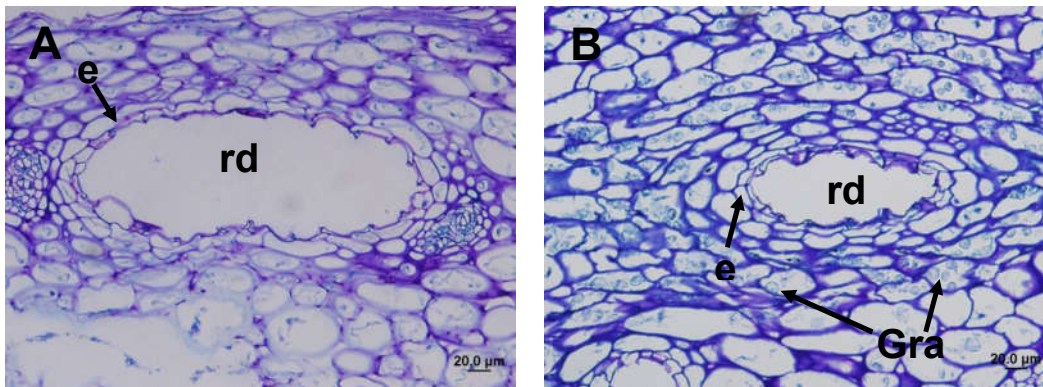


Figure 7.6 Paraffin wax embedded sections of control and USB-affected tissues in ‘Honey Gold’ mango fruit skin as stained by TBO. (A) Stained TS through a resin duct in a control tissue showing wherein no granules are present in epithelial cells that line the resin ducts. (B) Stained TS through a resin duct in USB-affected skin tissue showing granules inside the cells immediately surrounding the epithelial cells of resin ducts. rd, resin duct; e, epithelial cells; Gra, granules. Scale bars = A, B, 20 μm .

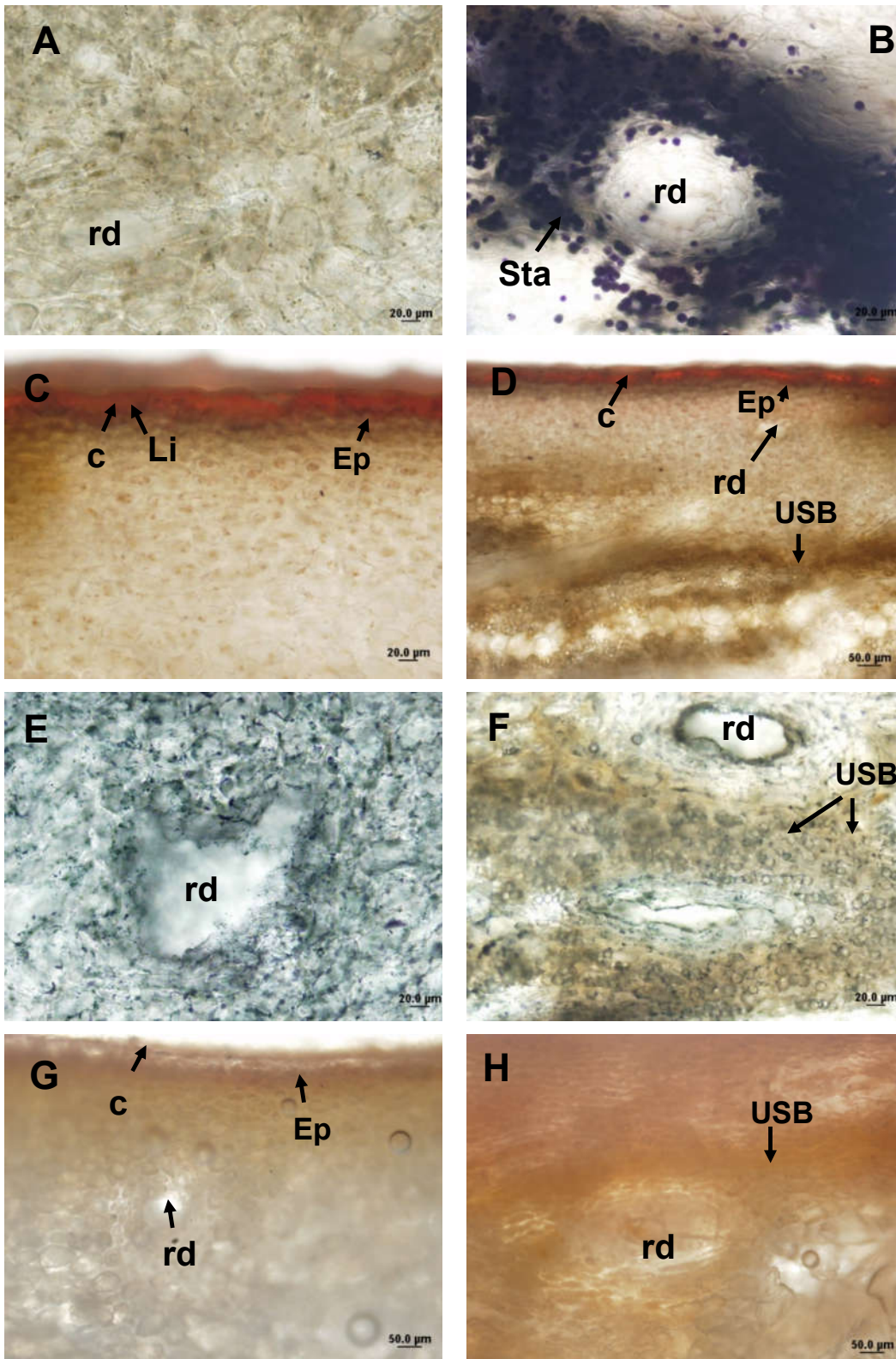


Figure 7.7 Histochemical images of control (A, C, E and G) and USB-affected tissues (B, D, F and H) in ‘Honey Gold’ mango fruit skin. (A, B) Staining of starch using IKI. (A) IKI stained control tissue showing no starch granules in cells surrounding the epithelial cells that bound the resin duct.

(B) IKI stained USB-affected tissues showing starch granules stained purple in cells around the epithelial cells that line the resin duct. (C, D) Staining of cuticle or lipids using Sudan IV showing the cuticle stained red. The control and USB-affected sections did not otherwise stain with Sudan IV. (E, F) Staining of lipids using Sudan Black. Neither control nor USB-affected sections stained with Sudan Black. (G, H) Staining for phenolics using Vanillin - HCl. Control tissue sections did not stain with Vanillin - HCl. It is likely that USB-affected sections stained slightly with Vanillin - HCl. c, cuticle; e, epithelial cells; Ep, epidermis, Li, lipid; rd, resin duct; Sta, starch granules; USB, under-skin browning. Scale bars = A, B, C, E, F, 20 μm ; D, G, H, 50 μm .

For tissues treated with Sudan IV, an apparent presence of lipids as stained red was common for the cuticle in both control and USB-affected regions (Figure 7.7C, D). Similarly, control and USB-affected tissue samples did not differentially stain with either Sudan Black (Figure 7.6E-F). This suggests that potential deposition of defence-associated lipids, like suberin, cutin, and tannins were absent. Control tissues did not stain with Vanillin - HCl (Figure 7.6G). In contrast, USB-affected tissues stained slightly with Vanillin - HCl (Figure 7.6H). However, the staining was not very clear. These observations suggest further anatomical observation using TEM should be conducted.

RCD symptoms

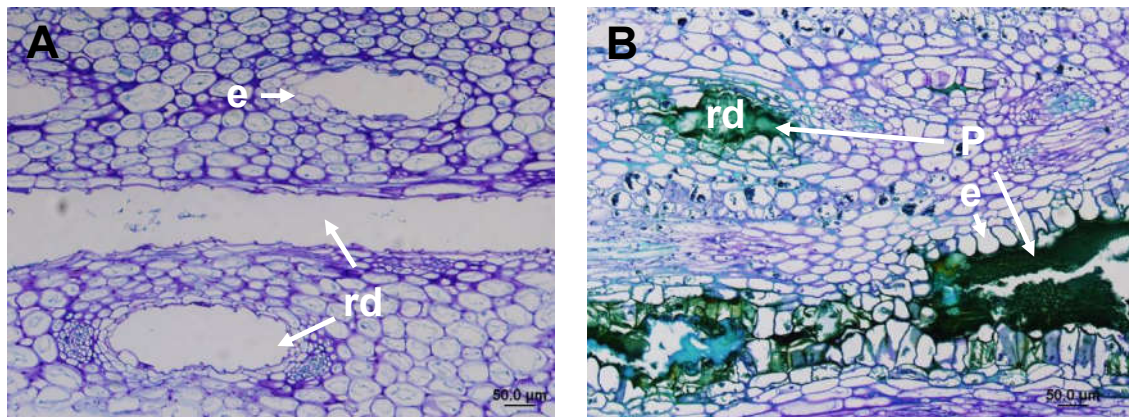


Figure 7.8 Paraffin wax embedded sections of control and RCD-affected tissues in 'Kensington Pride' mango fruit skin. (A) Stained TS through resin ducts of control tissue showing no phenolics inside the resin duct lumen. (B) Stained TS through RCD-affected tissues showing phenolics present inside the lumen of resin ducts. c, cuticle; e, epithelial cells; P, phenolics; rd, resin duct; RCD, resin canal disorder. Scale bars = A, B, 50 μm .

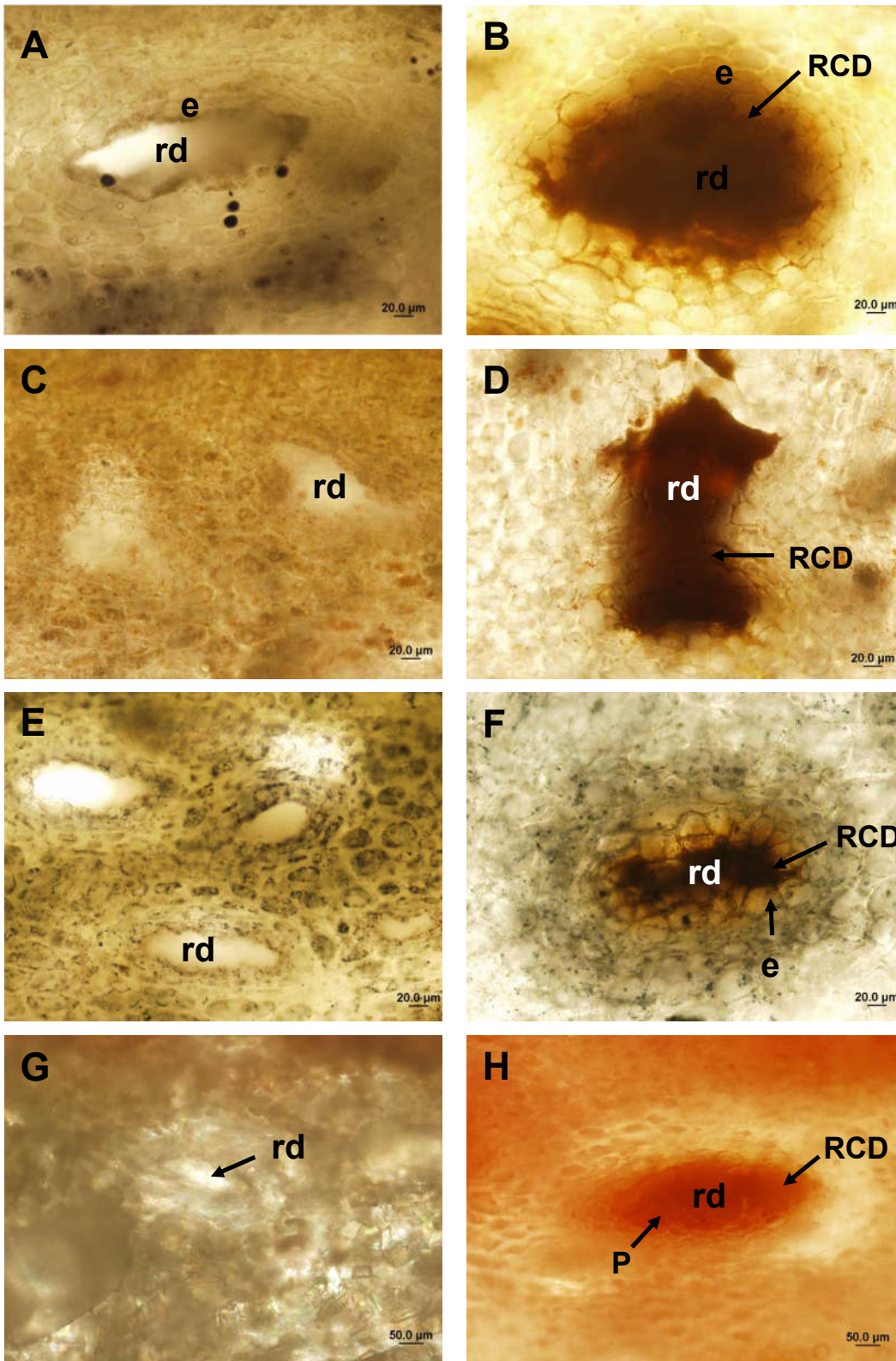


Figure 7.9 Histochemistry of control (A, C, E and G) and RCD-affected tissues (B, D, F and H) in 'Kensington Pride' mango fruit skin. (A, B) Staining of starch using IKI. The control and USB-affected sections did not stain with IKI. (C, D) Staining of cuticle or lipids using Sudan IV. The

cuticle stained red with Sudan IV. The control and USB-affected sections did not stain with Sudan IV. (E, F) Staining of lipids using Sudan Black. The control and USB-affected sections did not stain with Sudan Black. (G, H) Staining of phenolics using vanillin - HCl. (G) Vanillin - HCl stained control sample showing phenolics are absent in the resin duct lumen. (H) Vanillin - HCl staining of RCD-affected tissues showing phenolics present inside lumen of resin ducts. rd, resin duct; RCD, resin canal disorder; e, epithelial cells. Scale bars = A, B, C, E, F, 20 μm ; D, G, H, 50 μm .

In contrast with Vanillin - HCl, browned areas inside resin ducts of the RCD-affected area stained green by TBO. This observation indicates that phenolics were present inside the central cavity (lumen) of RCD-affected resin ducts (Figure 7.8B). TBO staining of the control tissue showed that the phenolics were, by contrast, absent in the resin duct lumen (Figure 7.8A).

Neither control nor RCD-affected sections stained in response to treatment with IKI such that there was no differential starch retention (Figure 7.9A, B). Similarly, the control and RCD-affected section did not stain with Sudan IV and Sudan Black, implying no lipids, such as suberin and cutin, in the tissue sections (Figure 7.9C-F). The lumen of resin ducts in the control sample showed no red colour when stained with the Vanillin - HCl reagent (Figure 7.9G). This lack of histochemical staining suggested that there were no phenolics inside the lumen of control tissue sections. The lumen was intensively coloured red by the Vanillin - HCl reagent, suggesting the presence of phenolics (tannins) inside the resin duct of RCD-affected regions (Figure 7.9H).

7.3.1.4 Transmission electron microscopy

USB symptoms

TEM imaging revealed the ultrastructure of unripe control fruit cells and their components, including cell wall, nucleus, cytoplasm and cell organelles (Figure 7.10A). There was deposition of starch in mango parenchyma cells of unripe fruit (Figure 7.10B). Nonetheless, there was no evidence of starch granules in ripe healthy control fruit (Figure 7.10C). Starch granules were, however, distributed in the cytoplasm of USB-affected cells (Figure 7.10D).

Relative to the control (Figure 7.10E), electron-dense material was observed in the cell wall of USB-affected cells (Figure 7.10D, F). The presence of electron-dense materials was also evident in the junction between two epithelial cells and the cell wall of epithelial cells in the USB-affected regions (Figure 7.11D, F). No such electron-dense materials were evident in control tissues (Figure 7.11C, E).

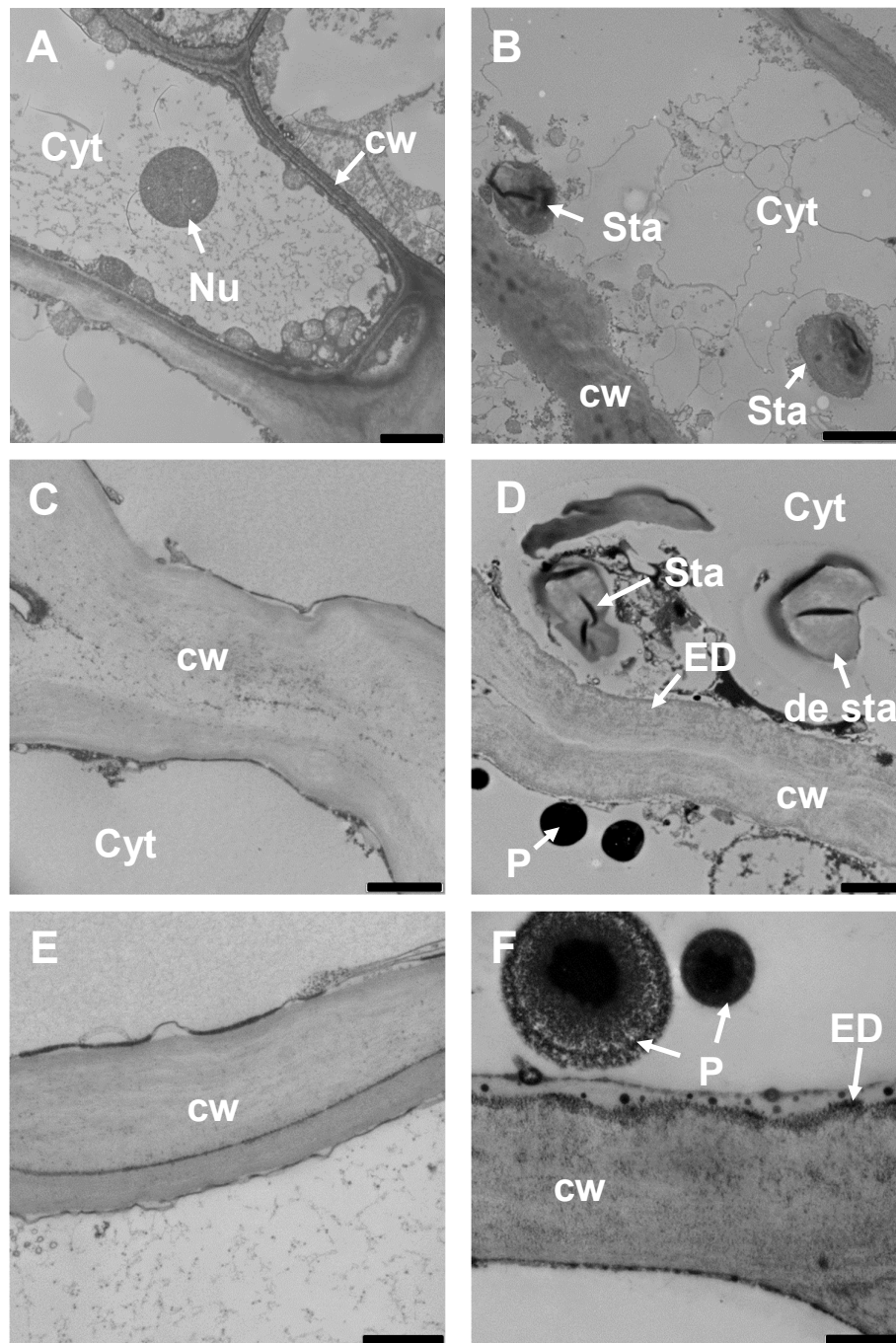


Figure 7.10 TEM of healthy green mature (A, B), ripe control (C, E) and ripe USB-affected cells (D, F) in ‘Honey Gold’ mango fruit skin. (A) A normal cell showing organelles in the cytoplasm (B). A control cell wherein no starch granules are present in the cytoplasm. (C) A control cell wherein no starch retention and phenolic deposition are present. (D) Starch retention and phenolic deposition present in USB-affected cell. Starch granules were partly degraded and present around epithelial cells. (E) No such electron-dense material in the cell wall in the control. (F) Electron-dense material evident in the cell wall of USB-affected cells. The phenolics were diffuse. cw, cell wall; c, cuticle; Cyt, cytoplasm; de sta, degraded starch; ED, electron-dense material; e, epithelial

cells; P, phenolics; rd, resin duct; Sta, starch granules. Scale bars = A, B, 5 μm ; C, D, 2 μm ; E, F, 1 μm .

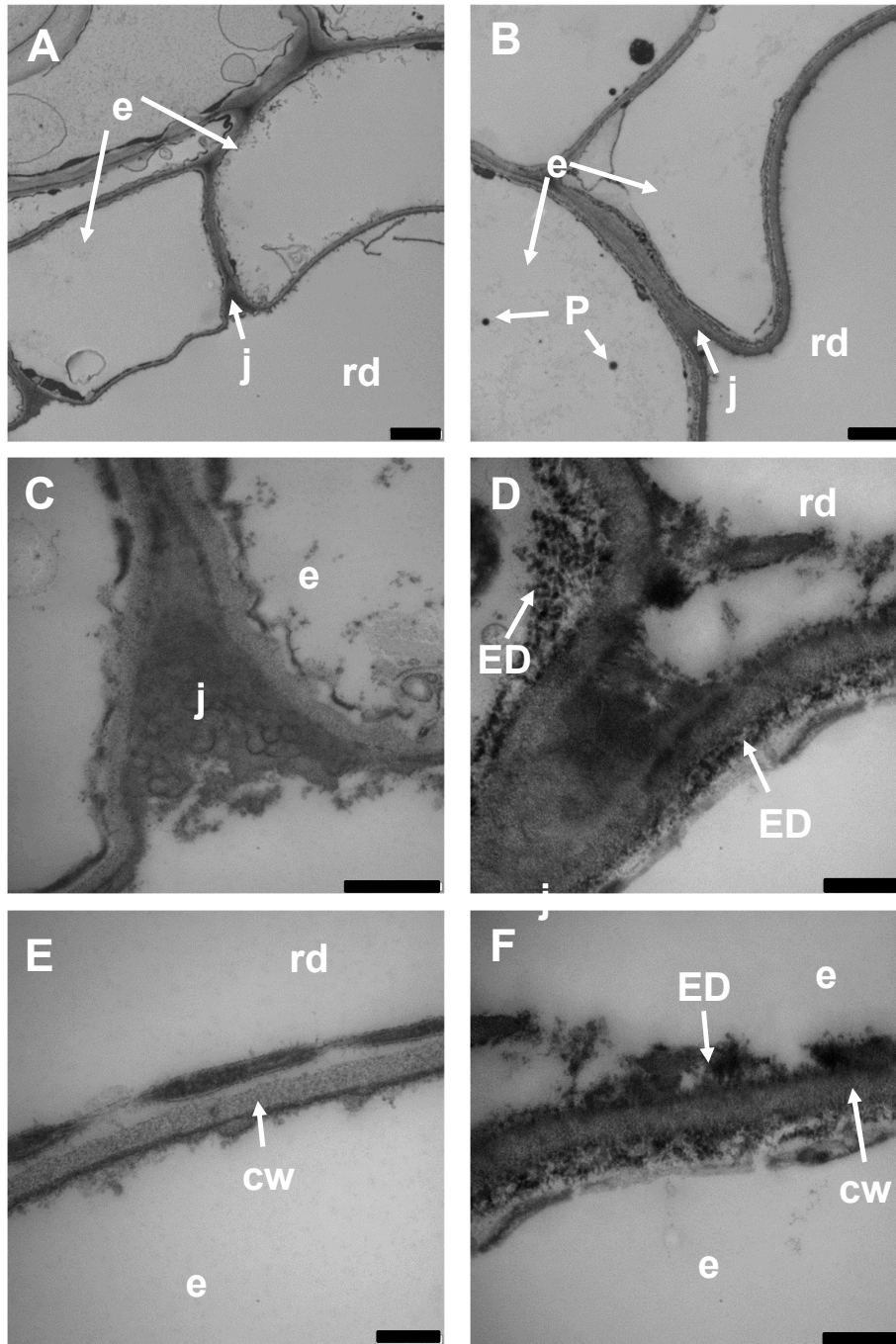


Figure 7.11 TEM of resin ducts of ripe control (A, C and E) and ripe USB-affected regions (B, D and F) in 'Honey Gold' mango fruit skin. (A) A control resin duct showing no phenolic deposition in the epithelial cells. (B) The resin duct of USB-affected region showing phenolic deposition in the epithelial cells. (C) No such electron-dense materials were found in the junction between two epithelial cells of control tissue sections (D) Electron-dense materials were present in the junctions

between two epithelial cells of USB-affected region. (E) No such electron-dense material was found in the cell wall of epithelial cells in the control tissue sections. (F) There was electron-dense material in the cell wall of epithelial cells in the USB-affected region. cw, cell wall; ED, electron-dense material; e, epithelial cells; j, junction between two epithelial cells; P, phenolics; rd, resin duct. Scale bars = A, B, 2 μm ; C, D, F, 500 nm; E, 200 nm.

Phenolics were evident in cells in USB-affected regions. Epithelial cells surrounding resin ducts of healthy (Figure 7.11A) and USB-affected (Figure 7.11B) tissues were characterised. Phenolics appeared as bodies of various sizes from 0.1 to 1 μm in epithelial cells that lined the resin ducts of USB-affected regions (Figure 7.11B). Epithelial cells of healthy control tissue showed no such phenolic deposition (Figure 7.11A). The electron-dense caffeine-fixed phenolic deposits had a diffuse, granular shape (Figure 7.10D, F).

RCD symptoms

For tissues affected by RCD, electron-dense materials were evident inside the lumens of resin ducts (Figure 7.12B). Healthy asymptomatic fruit showed no such materials (Figure 7.12A, E). This suggested polymerised phenolic deposition inside the lumen in RCD-affected regions.

More electron-dense materials were observed in the epithelial cells of RCD-affected areas (Figure 7.12D) as compared to healthy control tissues (Figure 7.12C). Phenolic deposition inside the lumen of resin ducts took the form of a network of many globe-shaped granules (Figure 7.12F).

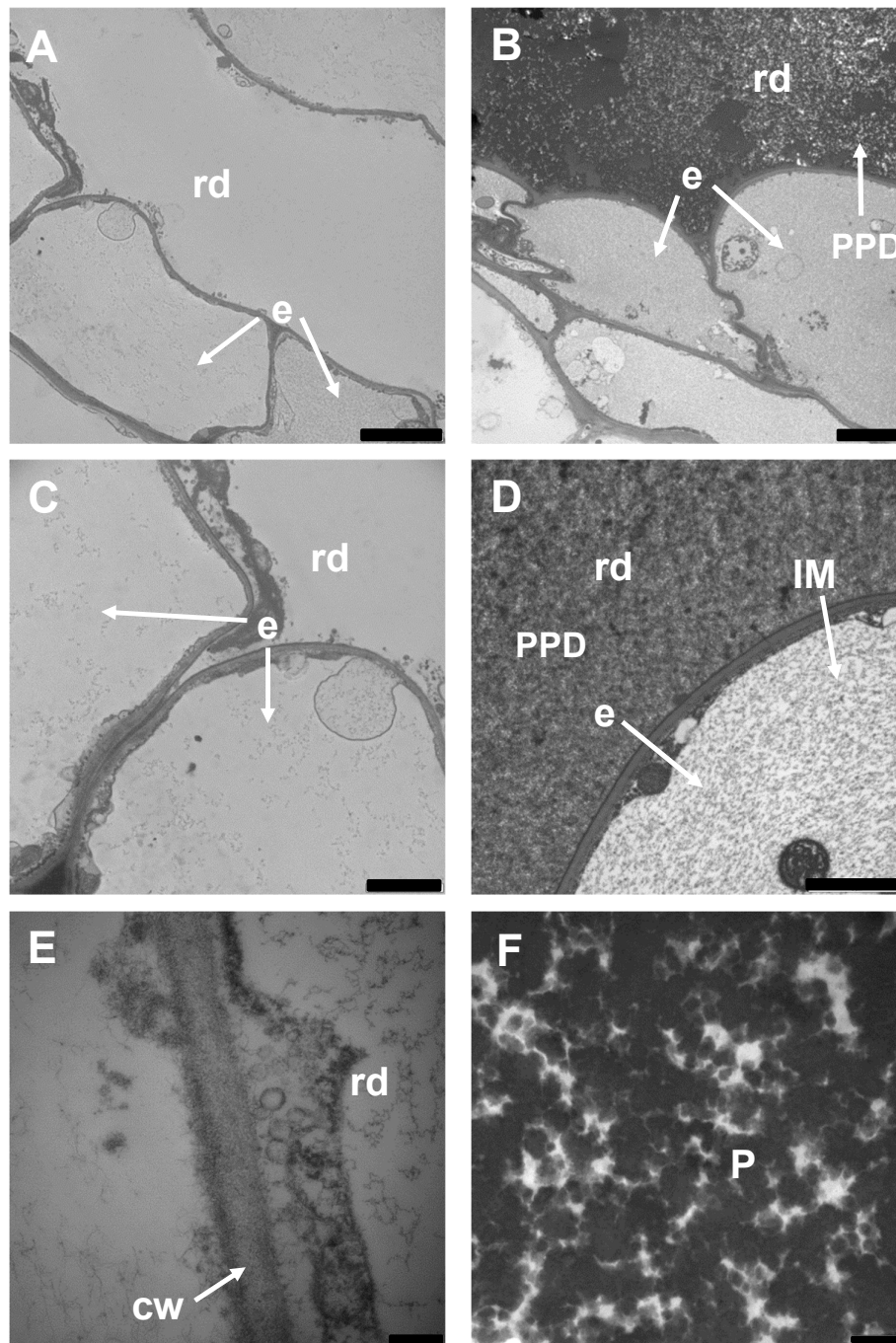


Figure 7.12 TEM of cells of control (A, C and E) and RCD-affected tissues (B, D and F) in ‘Kensington Pride’ mango fruit skin. (A) A normal tissue showing no phenolic deposition inside the lumen of resin ducts. (B) USB-affected region showing phenolic deposition inside the lumen of a resin duct. (C) A normal tissue showing no phenolic deposition inside the lumen of a resin duct and no dense materials in the junction of epithelial cells. (D) USB-affected region showing polymerised phenolic deposition inside the lumen and dense materials in the epithelial cells. (E) The control lumens showing phenolics were absent. (F) The lumen of the RCD-affected region showing polymerised phenolics was present. cw, cell wall; e, epithelial cells; IM, impenetrable materials; P,

phenolics; PPD, polymerised phenolic deposition; rd, resin duct. Scale bars = A, B, 5 μ m; C, D, 2 μ m; E, F, 200 nm.

7.3.2 Effects of the non-aqueous sap fraction on USB development

7.3.2.1 Further anatomical comparison of natural USB and induced USB symptoms by LM

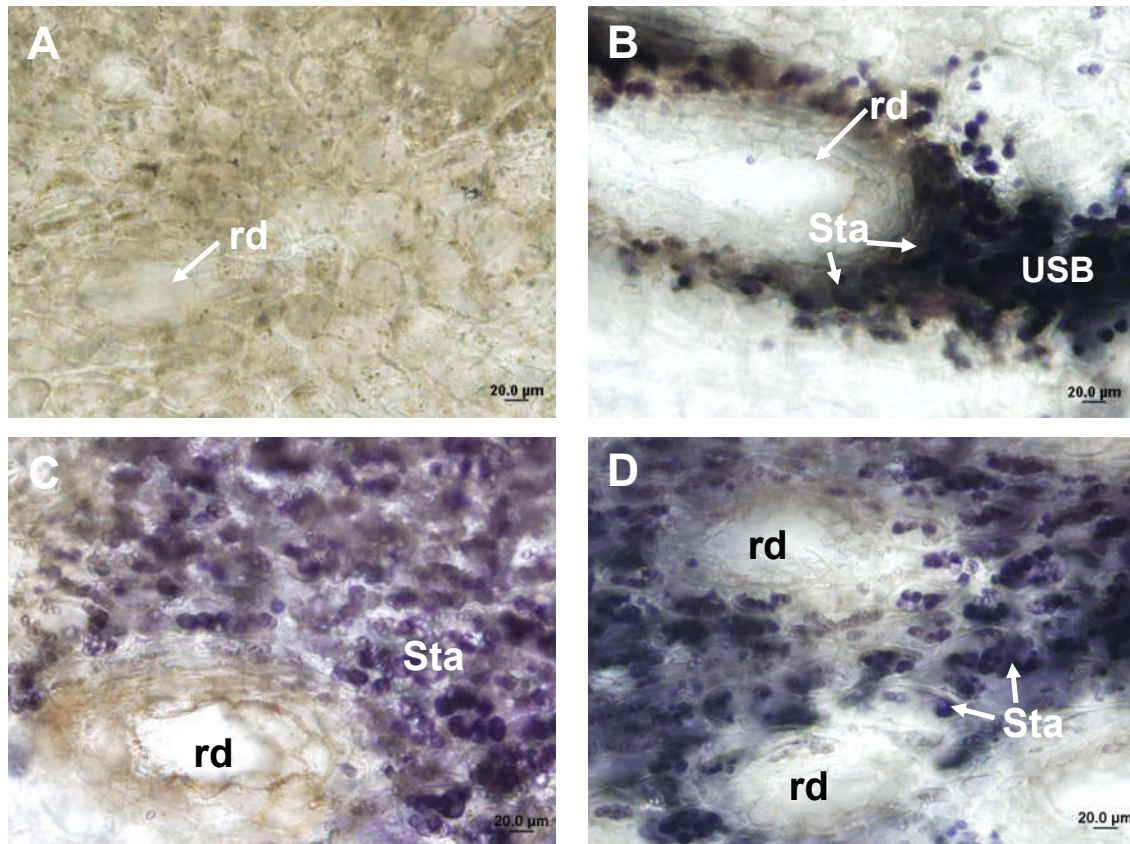


Figure 7.13 LM images of control (A), natural USB (B) and induced USB symptoms by terpinolene (C) and limonene (D) in ‘Honey Gold’ mango fruit skin stained by IKI. (A) IKI stained control showing no starch granules present surrounding resin duct epithelial cells. (B) IKI stained USB-affected tissues showing starch granules that stained purple in cells around resin duct epithelial cells. (C) IKI stained induced USB symptoms induced by terpinolene injection showing starch granules that stained purple in cells around the epithelial cells that line the resin duct. (D) IKI stained induced USB symptoms induced by limonene injection showing starch granules in cells surrounding resin duct epithelial cells. rd, resin duct; Sta, starch granules; USB, under-skin browning. Scale bars = A, B, C, D, 20 μ m.

USB symptoms were induced by treatment of ‘Honey Gold’ mango fruit skin with terpinolene and limonene as described in Chapter 6. Healthy control fruit showed no starch retention in cells

surrounding the epithelial cells that lined resin ducts (Figure 7.13A). In contrast, IKI staining revealed more extensive starch retention in cells surrounding the epithelial cells of tissues in which USB was induced (Figure 7.13C) as compared to natural USB (Figure 7.13B).

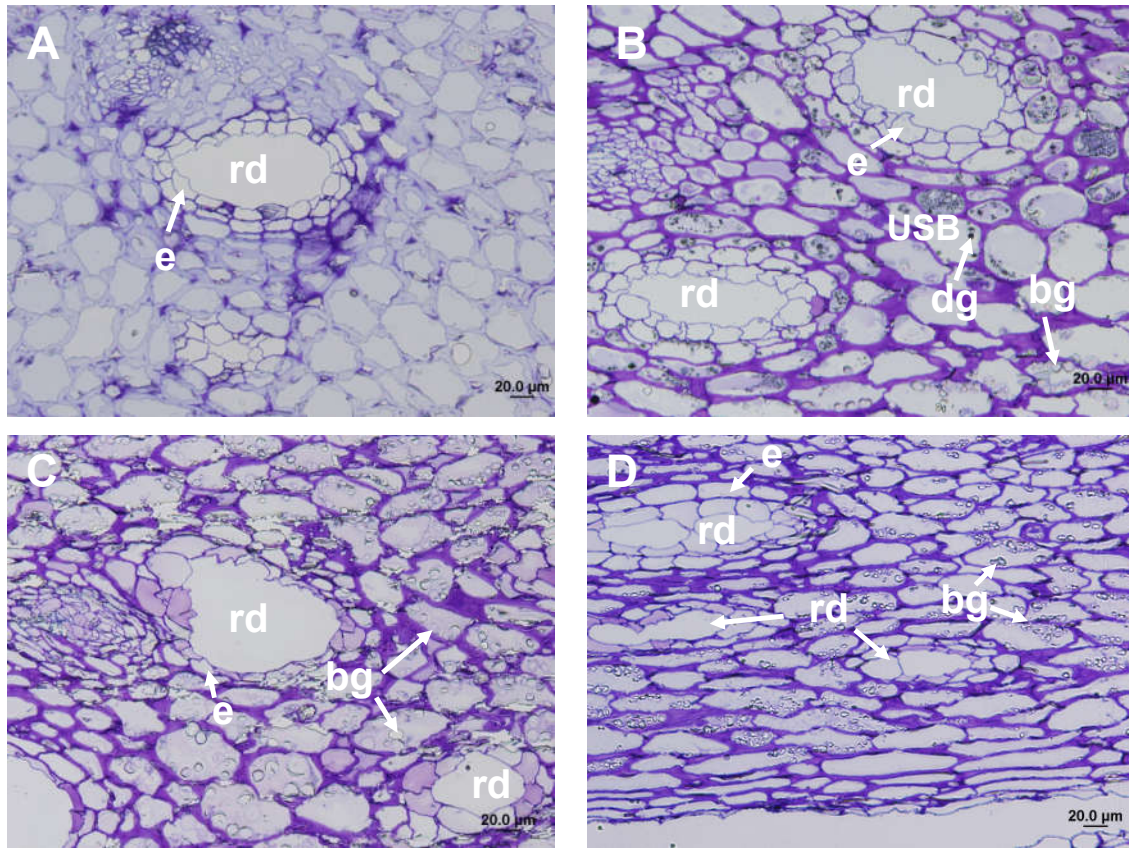


Figure 7.14 LM images of control (A), natural USB (B), induced USB symptoms by terpinolene (C) and limonene (D) in ‘Honey Gold’ mango fruit skin stained by TBO. (A) TBO stained control tissue showing no granules was present in the epithelial cells that line the resin duct. (B) TBO stained natural USB-affected tissues with bright white and dark granules present around the resin duct epithelial cells. (C) TBO stained induced USB symptoms induced by terpinolene injection showing that bright white granules were visible surrounding the epithelial cells that line the resin duct. (D) TBO stained induced USB symptoms induced by limonene injection showing bright white granules were present surrounding the epithelial cells. bg, bright granules; dg, dark granules; e, epithelial cells; rd, resin duct; USB, under-skin browning. Scale bars = A, B, C, D, 20 μm.

LM imaging of control asymptomatic tissue sections stained with TBO affirmed that no granules were present in epithelial cells that line the resin duct (Figure 7.14A). Cross sections of natural USB-affected regions showed two types of granules; bright white and dark black, in cells around the epithelial cells (Figure 7.14B). TBO stained sections of tissues in which USB had been induced

showed only bright white spherical granules in cells surrounding resin duct epithelial cells (Figure 7.14C-D). No dark granules were found in the induced USB symptoms in cells around the epithelial cells. In view of this difference, further anatomical comparison might ideally be conducted to provide better histological understanding of natural USB and induced USB symptoms.

7.3.2.1 Further anatomical comparison of natural USB and induced USB symptoms by TEM

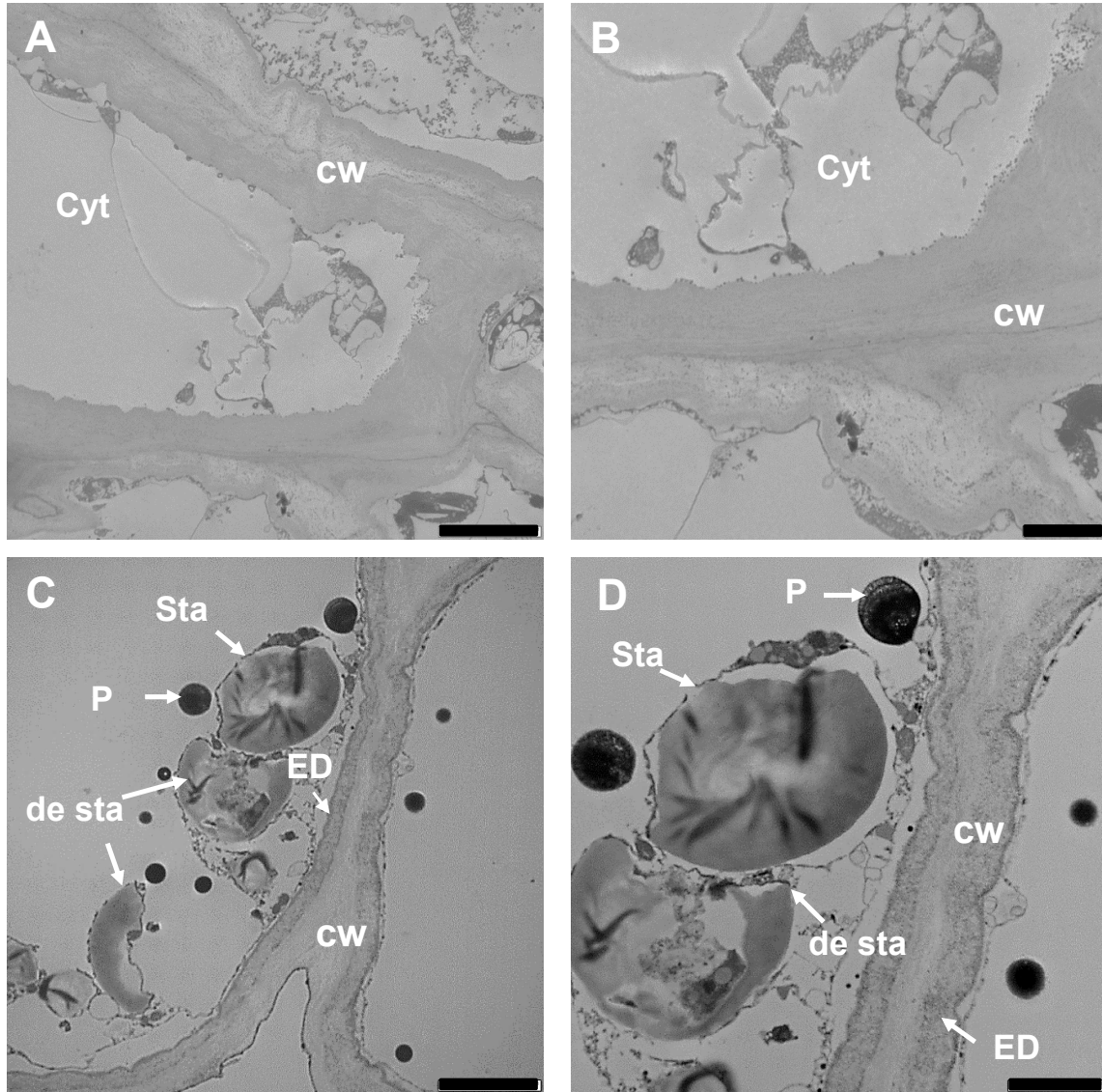


Figure 7.15 TEM of control (A, B), natural USB (C, D) and induced USB symptoms by terpinolene (E, F) and limonene (G, H) on ‘Honey Gold’ mango fruit. (A, B) A normal cell wherein no starch retention and phenolic deposition are present. (C, D) A natural USB-affected cell showing starch retention and phenolic deposition inside the cytoplasm. Starch granules were partly degraded. (E) An induced USB-affected cell by terpinolene showing only the starch retention in the cytoplasm. No phenolic deposition was found in the induced USB-affected cell. (F) Presence of electron-dense

material in the cell wall of induced USB-affected cell by terpinolene. (G) An induced USB-affected cell by limonene showing starch retention only in the cytoplasm. (H) Presence of electron-dense material in the cell wall of induced USB-affected cell by terpinolene. cw, cell wall; Cyt, cytoplasm; de sta, degraded starch; ED, electron-dense material; P, phenolics; rd, resin duct; Sta, starch granules. Scale bars = A, C, E, G, 5 μ m; B, D, F, H, 2 μ m.

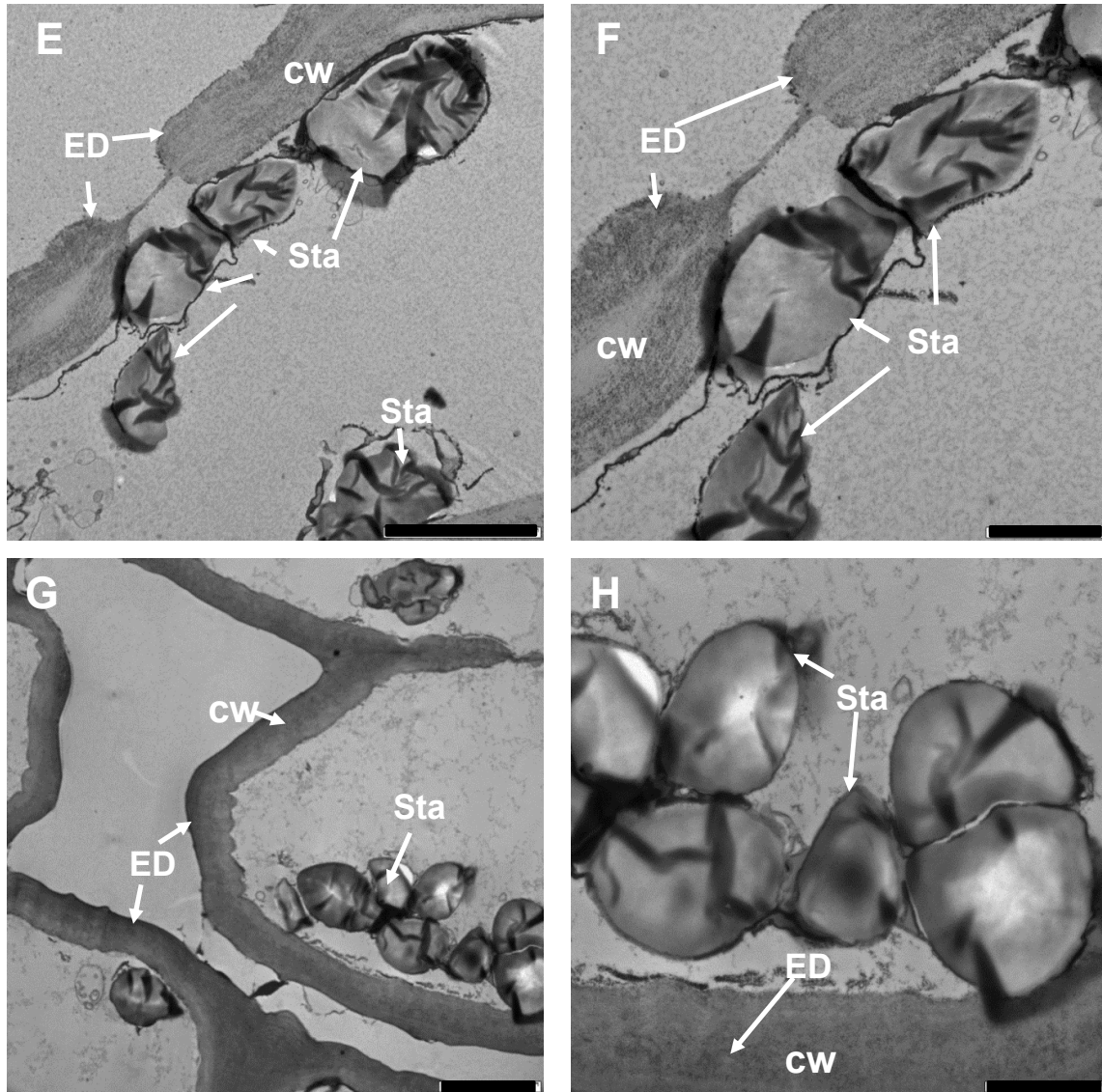


Figure 7.15 (Continued).

There was no starch retention and phenolic deposition in cells surrounding the epithelial cells from ripe control fruit (Figure 7.15A-B). Phenolics and starch granules were abundant in the natural USB-affected region (Figure 7.15C-D). However, some starch granules were partly degraded. There was also starch retention in sections through tissue in which USB had been induced by terpinolene and limonene. The starch granules were still evident after fruit had ripened (Figure 7.15E-H).

Control healthy sections showed no electron-dense material in the cell wall (Figure 7.16A). In contrast, the natural USB sections showed the presence of electron-dense material in the cell wall of cells surrounding the epithelial cells (Figure 7.16B). In tissues in which USB had been induced by terpinolene and limonene, similar electron-dense materials were observed in the cell wall of cells surrounding the epithelial cells (Figure 7.16C-D).

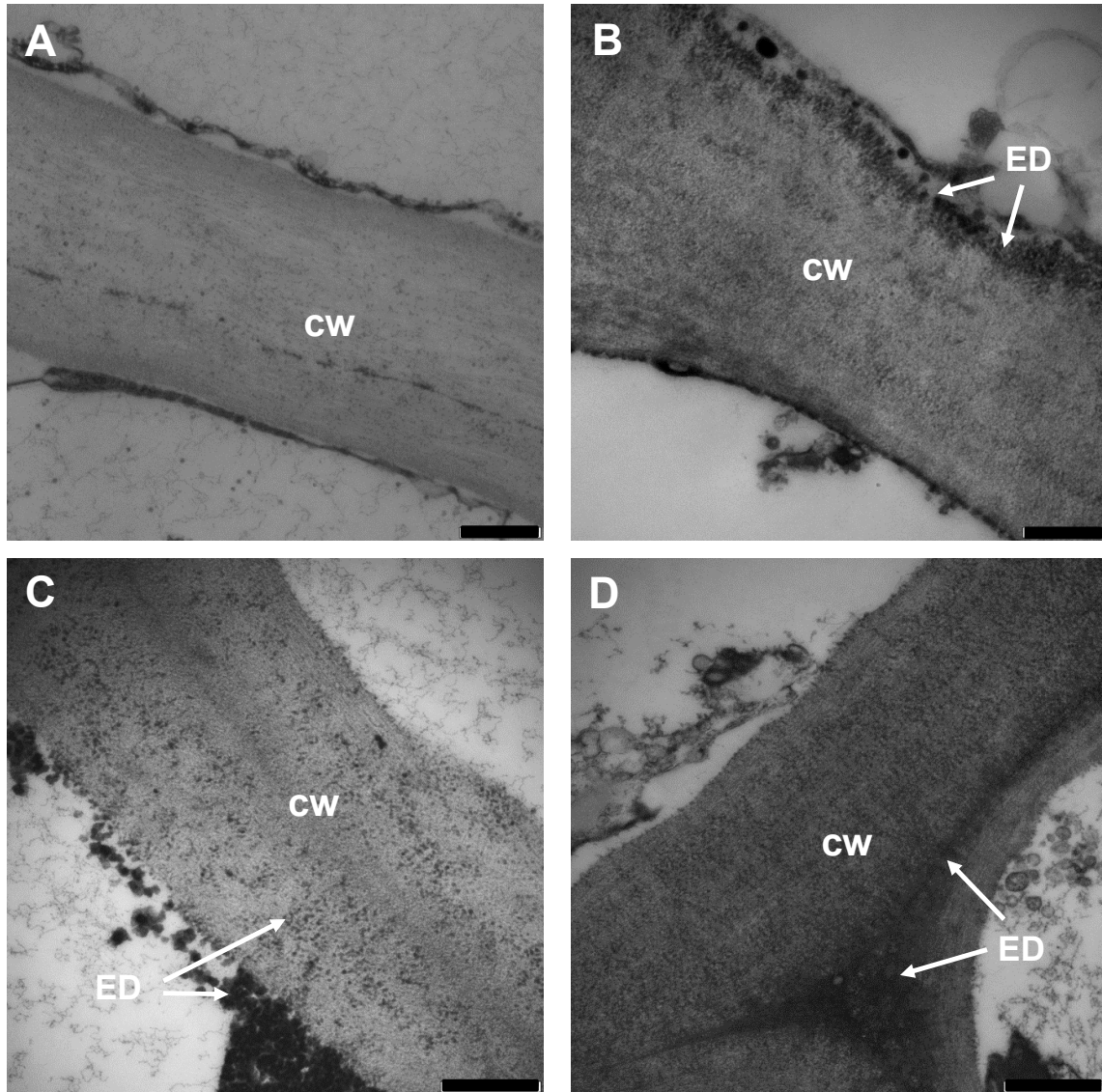


Figure 7.16 TEM of cell wall of control (A), natural USB (B), induced USB symptoms by terpinolene (C) and limonene (D) on ‘Honey Gold’ mango fruit. (A) A control cell showing no electron-dense material in the cell wall. (B) A natural USB-affected cell showing the indication of electron-dense material in the cell wall. (C) An induced USB-affected cell by terpinolene showing the presence of electron-dense material in the cell wall. (D) Similar electron-dense material was

found in the cell wall of the induced USB-affected cell by limonene. cw, cell wall; ED, electron-dense material. Scale bars = A, B, C, D, G, 500 nm.

7.3.3 Localisation of PPO and POD by tissue printing

7.3.3.1 USB

Tissue prints of healthy control samples stained bright pink in colour (Figure 7.17A) due to the presence of PPO. It was likely that the tissue prints from fruit expressing natural USB and induced USB symptoms showed a darker pink colour as compared to the healthy control tissue (Figure 7.17B-D). PPO apparently more strongly stained in the natural USB and induced USB tissue sections as compared to healthy fruit tissue section. PPO enzyme activity was generally distributed throughout the sections, but was not located inside resin ducts.

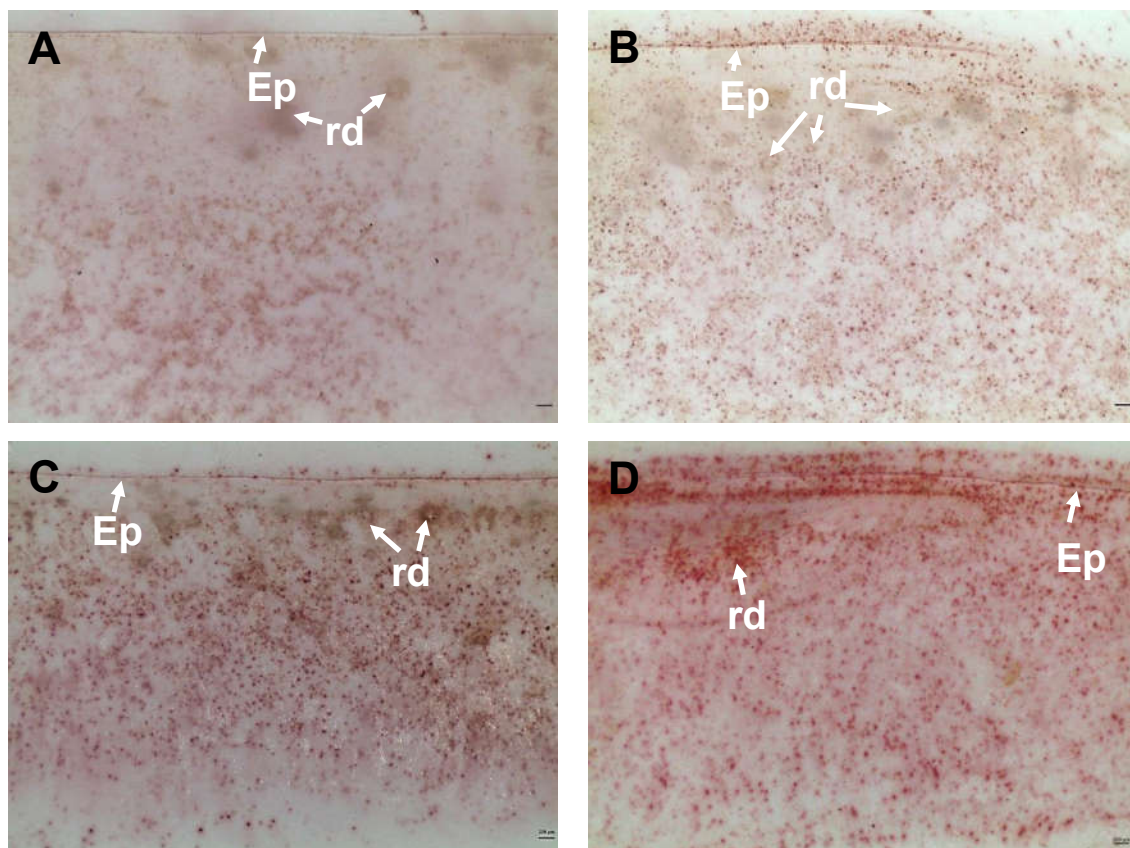


Figure 7.17 PPO localisation in control (A), natural USB (B), and induced USB-affected tissue sections by terpinolene (C) and limonene (D) on 'Honey Gold' mango fruit. (A) A control tissue section showing bright pink staining. (B) A natural USB tissue section stained pink colour. The bright pink colour is due to the presence of PPO. (C) An induced USB section by terpinolene

showing the PPO activity. (D) An induced USB tissue section by limonene showing the PPO activity. Ep, epidermis; rd, resin duct. Scale bars = A, B, C, D, 200 μ m.

There was no visible evidence of POD activity in the section of healthy control fruit tissue (Figure 7.18A). POD was located beneath the epidermis of the natural USB-affected region (Figure 7.18B, C). There was no visible evidence of POD expression in the regions showing USB symptoms induced by treatment with terpinolene and limonene (Figure 7.18D, E).

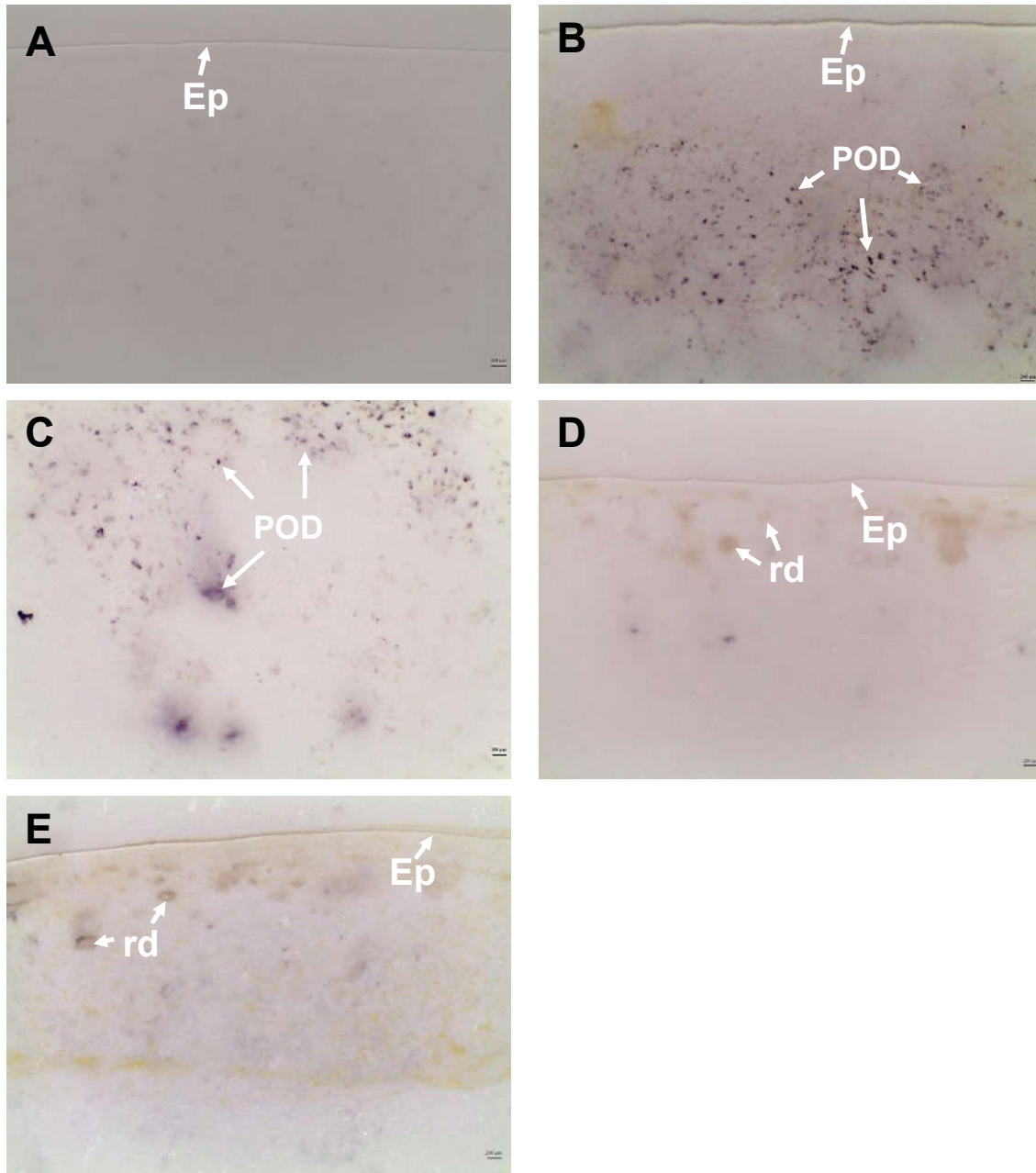


Figure 7.18 POD localisation in control (A), natural USB (B), induced USB-affected tissue sections by terpinolene (C) and limonene (D) on 'Honey Gold' mango fruit. (A) A control tissue section

showing no visible evidence of POD activity. (B, C) A natural USB-affected tissue section stained dark purple. The dark purple is due to the presence of POD. (D) An induced USB-affected section by terpinolene showing no POD activity was present in the affected region. (E) An induced USB-affected section by limonene showing no POD activity was present in the affected region. Ep, epidermis; rd, resin duct; POD, peroxidase. Scale bars = A, B, C, D, E, 200 μ m.

7.3.3.2 RCD

There was no visible evidence of PPO activity inside and surrounding the resin ducts of healthy tissue (Figure 7.19A). In contrast, PPO staining was evident in RCD-affected tissue (Figure 7.19B). The intensity staining of PPO which was strongly distributed around the resin ducts of RCD-affected region characterised by darker pink colour (Figure 7.19E). Tissue prints of RCD-affected tissue stained dark purple in cells surrounding the resin ducts, indicating the presence of POD (Figure 7.19D, F). Figure 7.19G-H shows the expression of both PPO and POD activities in cells surrounding resin ducts of RCD-affected tissues.

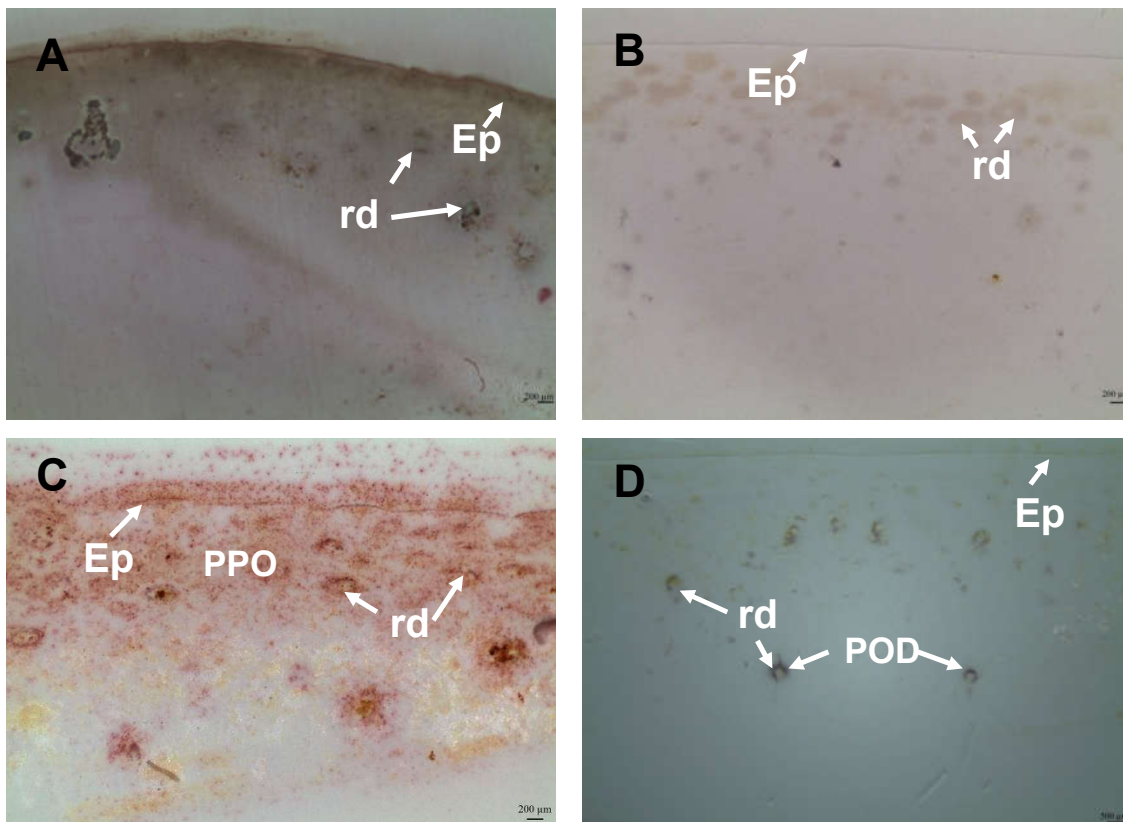


Figure 7.19 PPO and POD localisation in control (A, B) and RCD-affected tissue sections (C-H) on ‘Kensington Pride’ mango fruit skin. (A) A control tissue section showing no visible evidence of PPO activity. (B) A control tissue section showing no visible evidence of POD activity. (C, E) A

RCD-affected tissue section stained dark pink around the resin ducts and the affected region indicating the presence of PPO. (D, F) A RCD-affected tissue section stained dark purple surrounding the resin ducts. The dark purple indicated the presence of POD. (G, H) A RCD-affected tissue section wherein the localisation of both PPO and POD activity surrounding the resin ducts presents. Ep, epidermis, rd, resin duct; POD, peroxidase; PPO, Polyphenol oxidase. Scale bars = A, B, C, D, E, 200 μ m.

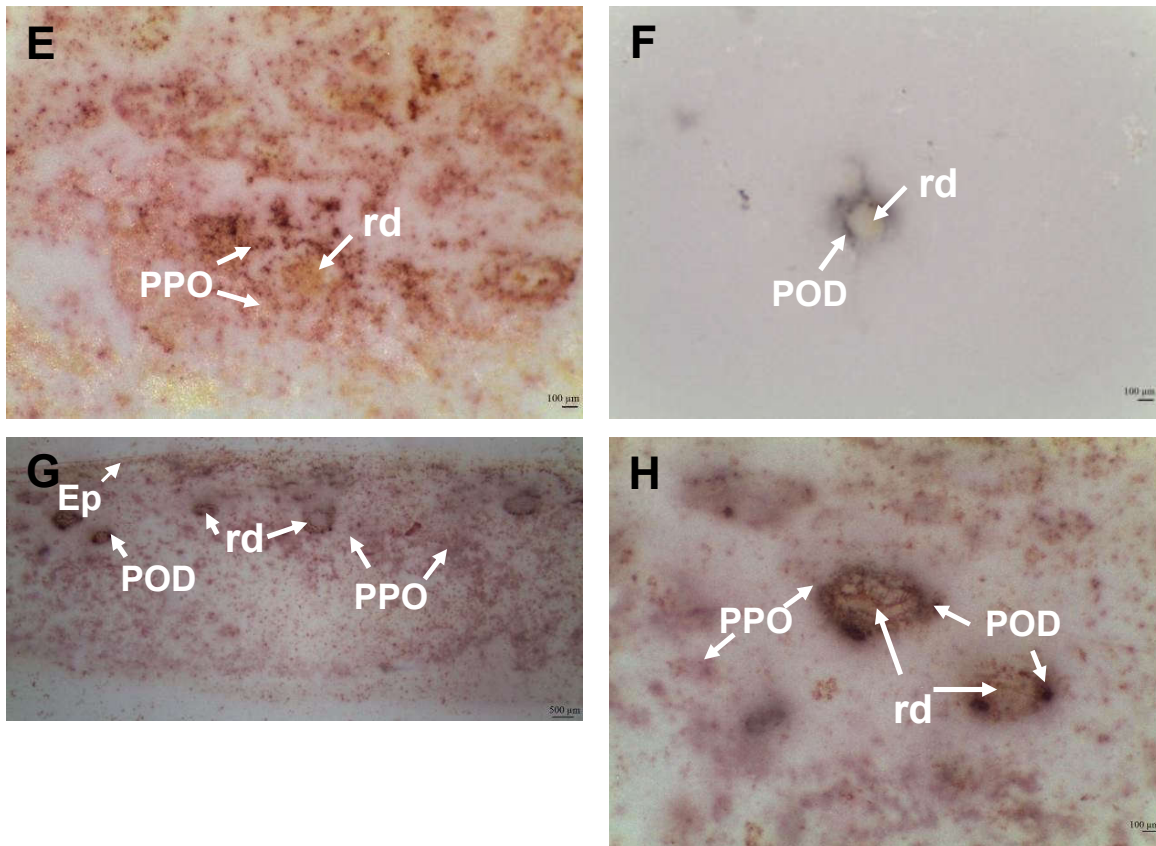


Figure 7.19 (Continued).

7.4 Discussion

7.4.1 Anatomy and histochemistry of USB

USB was characterised at the anatomical level by the presence of dark-brown cells that immediately surrounded the epithelial cells of resin ducts. Consistent with Marques et al. (2012), histochemistry imaging showed that starch retention in the parenchyma cells surrounding the epithelial cells of resin ducts was associated with USB (Figure 7.7). Nevertheless, LM histology did not reveal the fine structure of and within cells in USB-affected areas. Thus, it offers little information regarding both location within cells and granule structure.

TEM observations affirmed that the parenchyma cells of unripe fruit contained starch in the form of granules which otherwise would be converted into sugars during ripening (Figure 7.10B). Simão et al. (2008) reported the retention of starch in cells of unripe 'Keitt' mango fruit. Espinosa-Solis et al. (2009) showed that starch granules of 'Tommy Atkins' mango fruit had spherical dome shapes using SEM. Based on TEM imaging, the starch granules observed in 'Honey Gold' fruit in the current study were partially degraded (Figure 7.10D) in USB-affected tissues at the ripe stage. By contrast, starch granules were intact in the parenchyma cells of fruit tissue at green mature stage (Figure 7.10B). Somewhat similarly, Jacobi and Gowanlock (1995) reported that starch granules were located in the mesocarp of heat-treated ripe 'Kensington' mango fruit due to the prevention of enzyme activities related to carbohydrate metabolism. No starch granules were observed in the epithelial cells within both control and natural USB-affected tissues at the ripe stage such that there was no starch retention in the epithelial cells of mature-green fruit.

In addition, phenolics were present as granular bodies of various sizes ranging from 0.1 to 1 μm surrounding the epithelial cells of USB-affected tissue (Figure 7.11B). Electron-dense phenolic material was found in the boundaries of the junctions and cell wall of epithelial cells in the USB-affected areas (Figure 7.11D, F). No such electron-dense materials were observed in this region of control tissue sections (Figure 7.11C, E). This suggested that there was an apoplast in the cell wall. Jacobi and Gowanlock (1995) also noted that heat treatments of 'Kensington Pride' mango fruit caused phenolic as well as starch retention in mesocarp parenchyma cells. Similarly, traumatic resin ducts in conifers with parenchyma cells in the phloem contained starch granules and phenolics (Krokene et al., 2008). These TEM results supported the idea that the inhibition of starch degradation and phenolic deposition in USB were related to the self-defence to an external stress.

7.4.2 Anatomy and histochemistry of RCD

In contrast to USB, RCD was distinguished by localised browning of the lumen of resin ducts. Histological examination revealed phenolics (tannins) inside the lumen of resin ducts in RCD-affected epidermal tissue. TEM imaging confirmed the presence of polymerised phenolics inside resin duct lumen. Polyphenolic parenchyma cells containing phenolics and starch granules are associated with defence in many conifers (Krokene et al., 2008). Resin, produced in resin ducts, is a prominent defence constituent in many conifer families (Phillips and Croteau, 1999; Ruel et al., 1998). Resin or exudate gum, synthesised in secretory ducts in cashew trees, can inhibit the development of some bacteria, fungi and insects (Marques et al., 1992; Miranda, 2009) It is likely that a combination of production factors contribute to fruit susceptibility, along with a combination

of postharvest factors that generate symptom expression (Macnish et al., 2014). Like USB, natural defence mechanisms seem to be associated with RCD development.

7.4.3 Effects of non-aqueous fraction of sap on USB development

The histology of LM sections from fruit in which USB had been induced by two major components of non-aqueous fraction (terpinolene and limonene) revealed no apparent differences as compared to fruit with natural USB symptoms (Figure 7.13). Nonetheless, starch granules were present relatively more densely in cells surrounding the epithelial cells and also distributed further from the resin ducts in tissues in which USB had been induced as compared to in natural USB sections. Preservation of starch grains in USB-affected tissue in ripe fruit was apparently due to the inhibition of starch degradation (Figure 7.10D). Moreover, two different types of granules, bright white and dark, were present in cells surrounding the epithelial cells of natural USB (Figure 7.14B). In contrast, there were only spherical granules in cell layers surrounding the epithelial cells of induced USB symptoms (Figure 7.14C-D). Further anatomical comparison by TEM should be conducted to provide better histological understanding of natural versus induced USB symptoms.

TEM imaging revealed phenolics and starch granules in tissues showing natural USB (Figure 7.15C, D). However, only starch granules were retained in the sections of tissues in which USB had been induced USB. Starch grains in the induced USB tissue sections remained intact and were not degraded during fruit ripening (Figure 7.15E-H). Thus, high amounts of terpinolene and limonene (100 μ l) resulted in the inhibition of starch degradation. Terpenoid compounds including terpinolene, 3-carene and limonene are major components of the non-aqueous fraction of mango sap (Loveys et al., 1992). Terpinolene and limonene possibly interfered with and/or damaged cell membranes leading to cell decompartmentalisation. Regarding a putative role in defence, Martin et al. (2002) reported that terpenoids accumulated upon induction of traumatic resin ducts using methyl jasmonate treatment.

USB is seemingly a discolouration of the latex vessels, which spreads to surrounding tissues (Hofman et al., 2010b). It is possible that terpenoids diffuse from affected resin ducts into their surrounding epithelial cells and thence onto cell layers surrounding the epithelial cells. The reactions quite evidently retarded the starch degradation and promoted phenolic accumulation in parenchyma cells surrounding the epithelial cells that line resin ducts in USB-affected regions. (Krokene et al., 2008) considered the presence of starch granules and phenolics in parenchyma cells of traumatic resin ducts in conifers as defense responses against biotic or abiotic stress. Electron-dense regions were observed in the walls of cells surrounding epithelial cells of USB and induced

USB symptoms. Electron-dense materials were reported by Du Plooy et al. (2006) to indicate an apoplastic pathway for the accumulation of phenolics in lenticel discolouration.

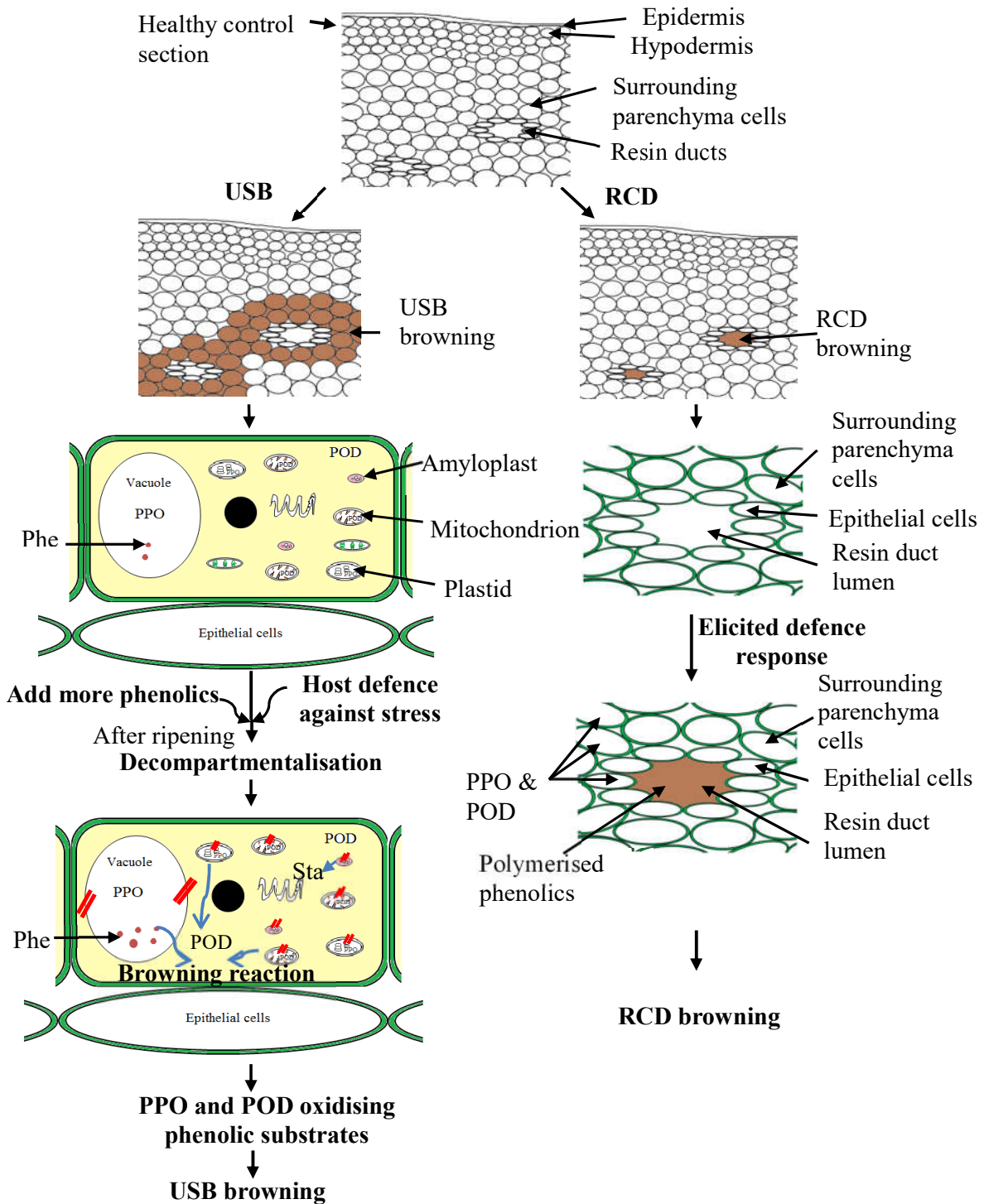


Figure 7.20 A comparative diagrammatic model of USB and RCD development mechanisms. USB symptoms are visible as browning in cell layers surrounding the epithelial cells. In contrast, RCD is visible as browning inside resin duct lumen. The USB model in ‘Honey Gold’ mango fruit is based

on current findings (this chapter) and published work by Gong et al., 2001; Nakano and Asada, 1981; Prasad et al., 1995; Toivonen and Brummell, 2008. Host tissue stress-induced decompartmentalisation likely occurs along with newly synthesised phenolics being added to those that are constitutively present. Damaged membranes of cell compartments release enzymes (e.g. PPOs in vacuoles and plastids, PODs in chloroplasts and mitochondria) and perhaps starch granules from amyloplasts into the cytoplasm. Browning enzymes are then free to oxidise phenolic substrates to yield brown polyphenols. The RCD model for ‘Kensington Pride’ mango fruit is based on current findings (this chapter). The browning is presumably polymerised phenolics inside the resin duct lumen (Figure 7.8). PPO and POD are evidently involved in RCD development (Figure 7.19). POD activity was mainly distributed in cells surrounding the lumen (Figure 7.19). Red slash, membrane disruption; POD, peroxidase; and, PPO, polyphenol oxidase.

7.4.4 Localisation of PPO and POD on USB and RCD browning

Tissue printing showed higher PPO levels in tissues from fruit with natural and induced USB symptoms as compared to the controls (Figure 7.17). Thus, involvement of PPO activity was attributed to development of USB symptoms. However, POD activity was only detected in fruit with natural USB. It appeared beneath the epidermis of the natural USB-affected regions. No POD activity was detected by tissue printing in tissues in which USB had been induced USB-affected tissue. A possible explanation is that host defence against stress-induced decompartmentalisation process increased phenolics in affected tissues (Figure 7.20). PPOs are mainly distributed in vacuoles and plastids (Murata et al., 1997). PODs are mostly found in chloroplasts (Nakano and Asada, 1981), mitochondria (Prasad et al., 1995; Toivonen and Brummell, 2008) and plant cell plasma membranes (Vianello and Macri, 1991). It was likely that the membranes of cell components such as vacuoles, plastids, chloroplasts and mitochondria were damaged through the physical and cold stress, so these PPOs and PODs were released in the cytoplasm. PPO and / or POD enzymes could then oxidise phenolic substrates to induce the browning.

PPO and POD activities were evidently associated with RCD browning in ‘Kensington Pride’ mangoes (Figure 7.19B). POD activity was mainly distributed in cell layers and epithelial cells surrounding the lumen of resin ducts (Figure 7.20). Somewhat similarly, John et al. (2002) found PPO and POD in the peel as the primary enzymes involved in browning that was associated with mango sap-burn injury. Thipyapong et al. (2004) reported increased PPO activity was correlated with the stress defence reaction in tomato. Accordingly, increased PPO and POD activities likely contributed to stress-related defence response browning of USB and RCD.

7.5 Conclusion

In short, USB and RCD evidently involve different cell biology processes. USB was associated with the starch retention and phenolic deposition in cells surrounding the epithelial cells of resin ducts through the apoplast in the cell wall. RCD involved polymerised phenolic deposition inside the lumen of resin ducts. Both PPO and POD activities contributed to oxidative browning in USB and RCD. This comparative study at the anatomical level contributes to better understanding of the browning mechanism for USB and RCD symptom expression. This improved knowledge should inform formulation of pre- and /or postharvest practices to lessen and ideally eliminate these disorders.

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CHAPTER 8. GENERAL DISCUSSION AND CONCLUSIONS

Mango is an economically important crop in Australia (Johnson and Parr, 2000). ‘B74’, ‘Honey Gold’, ‘Kensington Pride’ and ‘R2E2’ are the major cultivars produced in Australia (Dillon et al., 2013). However, the visual appeal and marketability of these cultivars can be limited by physiological skin disorders.

Lenticel discolouration (LD), under-skin browning (USB) and resin canal disorder (RCD) are three important skin browning disorders affecting the Australian mango industry (Hofman et al., 2010; Macnish et al., 2014; Marques et al., 2016). LD is evident as brown-black spots on the fruit surface. It reduces the appearance attribute of many mango cultivars in the world (Bezuidenhout and Robbertse, 2005; Self et al., 2006) including those cultivated in Australia (Hofman et al., 2010; Li et al., 2016; Marques et al., 2016). USB is an emerging important disorder that mainly occurs in ‘Honey Gold’ mango fruit. It develops under the epidermis and is visible as a diffuse brown region. Although no damage is caused to the flesh, the unattractive appearance of the fruit skin decreases consumer’s decision to purchase. RCD is visible as darkened outlines of the finely branched network of resin ducts under the skin and/or in the flesh. This disorder predominantly affects the ‘Kensington Pride’ cultivar. These three physiological disorders are of great concern to mango marketers because consumers often equate external blemishes to poor internal quality, including limited useable flesh and off flavours.

8.1 Method development for quantification of key aroma volatiles for mango fruit and sap

A high throughput and precise analytical method was developed to quantify characteristic aroma volatiles produced by fruits and sap of Australian mango cultivars (Chapter 3). This method utilises stable isotope dilution analysis (SIDA) in conjunction with headspace (HS) solid-phase microextraction (SPME) coupled with gas-chromatography mass spectrometry (GCMS).

More specifically, the combination of SIDA together with HS-SPME-GC-MS produced a highly sensitive and precise analytical tool for key volatiles released by Australian mango fruit and sap (Chapter 3, Table 3.2). The importance and impact of the technique is found in the ability to now eliminate variability related to sample preparation and matrix effects (i.e. improves recovery of the analytes), which has not previously been possible for quantitative analysis of key aroma volatiles in mango fruit and sap (Chapter 3). To highlight this further, in previous studies, the odour activity value of some compounds could not be resolved due to the lack of a suitable internal standard to

detect aroma volatiles at low levels. The accuracy and precision realised in the present work facilitates meaningful comparisons to be made using odour activity values, as the concentration data collected are sensitive and reliable (Chapter 3, Table 3.5).

Moreover, it has been applied to studies aimed at exploring and optimising the flavour of other mango varieties including 'B74', 'Kensington Pride', 'Honey Gold', 'R2E2' in other studies (Chapter 4) and those yet to be instigated. Accordingly, this method is of value to those in the community that measure key aroma volatiles produced by mango sap accurately and precisely in support of current research into the field of mango physiological disorder studies (in Chapter 6).

8.2 LD and other mango quality attributes

8.2.1 Effects of irradiation on LD and other mango quality attributes

γ -Irradiation is an effective post-harvest quarantine treatment. However, γ -irradiation at 0.5 (commercial dose) and 1.0 kGy (twice the commercial dose) reduced the appearance quality of 'B74', 'Kensington Pride' and 'R2E2' mango fruit by inhibiting the loss of green skin colour and stimulating development of severe LD during ripening (Chapter 4). Decreased rates of green skin colour loss presumably reflected a reduction in chlorophyll degradation. This finding was consistent with Hofman et al. (2010) who reported a significant increase in LD development on 'B74' mangoes irradiated at 0.543 kGy relative to non-irradiated fruit. In general, there was no difference in the severity of LD for fruit exposed to 0.5 or 1.0 kGy (Chapter 4). The γ -irradiation-induced LD was usually evident within 2-4 d of treatment and increased slowly towards the end of shelf-life or not at all as fruit ripened.

'Honey Gold' fruit were the least sensitive in response to irradiation in terms of LD and the loss of green skin colour as compared to other three tested cultivars (Figure 8.1). This variation in response to irradiation was likely largely due to the nature of their genotypic dissimilarity to the other cultivars. 'B74', 'Honey Gold' and 'R2E2' are hybrids of 'Kensington Pride' mangoes with different maternal parents (Dillon et al., 2013). In particular, the different cultivar-dependant susceptibility to LD may be due to compositional disparity of lenticel surface structure (Du Plooy et al., 2004). Marques et al. (2016) indicated that water contact and infiltration into lenticels during post-harvest handling can enhance LD development.

Exposure to 0.5 and 1.0 kGy γ -irradiation reduced initial rates of fruit softening in all 'B74', 'Honey Gold', 'Kensington Pride', 'R2E2' cultivars. Fruit softening in irradiated 'Tommy Atkins' fruit has

previously been reported to be due to the disintegration of the cells (Moreno et al., 2006). There was, however, no significant difference in firmness between irradiated and non-irradiated fruit by the end of ripening. It is possible that the effect of irradiation was lessened as fruit progressively ripened.

In general, γ -irradiation up to 1.0 kGy did not affect the TSS and carotenoid concentration in the skin of the four tested cultivars although this varied with cultivar. γ -Irradiation doses of up to 1.0 kGy showed different responses which are cultivar dependant on the other chemical properties and volatile production of mango fruit. Exposure to 0.5 and 1.0 kGy significantly ($P \leq 0.05$) reduced biosynthesis rates of the important aroma volatile 2-carene, 3-carene, α -terpinene, p-cymene, limonene, α -terpinolene, and ethyl octanoate in the ripened pulp of 'Kensington Pride' and 'R2E2' mango fruit. However, there was no significant ($P > 0.05$) effects of γ -irradiation at 0.5 and 1.0 kGy on volatile production of 'B74' and 'Honey Gold' mangoes. The irradiation-associated reduction in the rates of ripening-related decreases in TA and chlorophyll degradation during ripening of 'Kensington Pride' and 'R2E2' fruit, in combination with a decline in volatile production at higher doses, may translate into diminished flavour. In contrast, there was no major effect of irradiation treatment on the 'Honey Gold' cultivar (Chapter 4, Figure 8.1). Understanding the response of different Australian mango cultivars to irradiation in terms of appearance acceptability and physical-chemical properties is essential and meaningful for the mango industry to maximise the quality of treated fruit. Further post-harvest research should be undertaken to optimise the irradiation dose with a view to reducing the negative impacts of this phytosanitary treatment and delivering high-quality fruit to consumers.

8.2.2 Anatomy of LD

In Chapter 5, the anatomical features of LD in 'B74' mango fruit was illustrated using binocular microscope observation, light microscopy (LM) and serial block face scanning electron microscopy (SBF-SEM). LD is a physiological discolouration process that is visible as a dark brown to black region surrounding a white corona of the lenticel. LM imaging highlighted that LD was characterised by browning of sub-lenticellular cells around the lenticel cavity (Chapter 5). This browning was related to the phenolic deposition. It was likely that there was the phenolic deposition in cell walls (Chapter 5, section 5.3.3) and vacuoles (Du Plooy et al., 2009) of discoloured lenticels. However, this possible process was not very clearly demonstrated by LM, so SBF-SEM was employed to clarify.

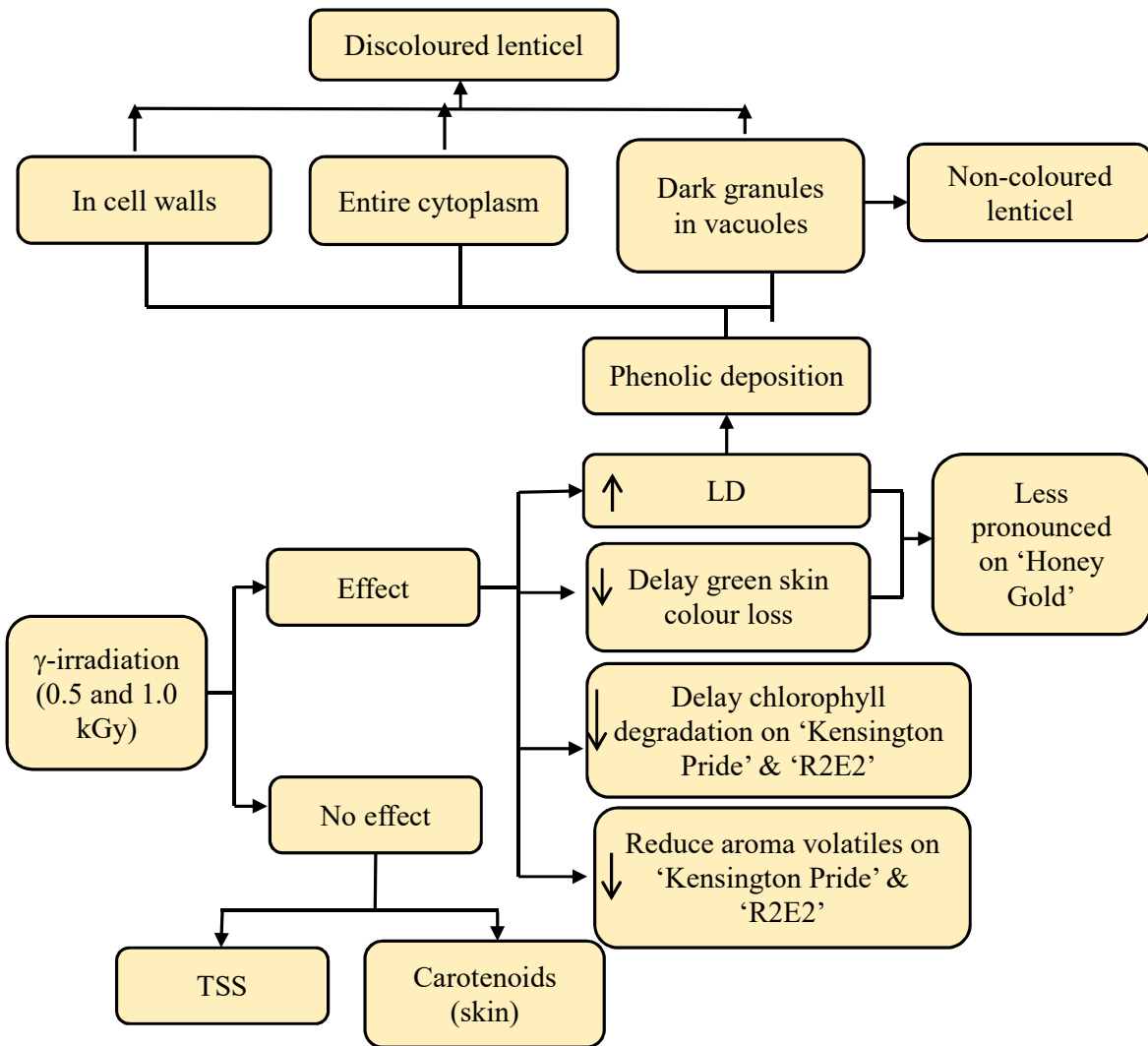


Figure 8.1 A model representing the effects of γ -irradiation on different Australian mango cultivars ('B74', 'Honey Gold', 'Kensington Pride' and 'R2E2') in terms of LD and other quality attributes. The upward arrows mean 'increase' and the downward arrows mean 'decline'.

SBF-SEM observation showed that the discolouration manifested beneath the cuticle to the sub-epidermal layers of pericarp. LD was associated with polymerised electron-dense phenolic depositions in the cell wall to the inside cell cytoplasm of cell layers surrounding the lenticel cavity (Chapter 5, Figure 8.1). There were two different kinds of phenolic deposition occurring inside the cell cytoplasm. Firstly, phenolics were observed to completely fill the cytoplasm of cells next to the cavity. Secondly, dark granules of differing electron density, which most likely represented varying concentrations of phenolics, were observed in the cytoplasm of sub-lenticellular cells surrounding the lenticel cavity. Nonetheless, only the second type as a discrete deposit of phenolics was observed in non-coloured lenticel. Furthermore, an illustration from an individual LM or TEM image of 50 nm-thick sections is not representative for a 500 μm lenticel. The visual illustration of

structural changes in detail and internal structural continuity of entire lenticel during discolouration using SBF-SEM provides a better understanding of the functional microstructure and potentially the mechanism of LD.

8.3 USB on ‘Honey Gold’ mango and RCD on ‘Kensington Pride’ mango

USB is often associated with physical injury damage and exposure to low temperature during extended transport and delivery (Marques et al., 2012a). Exposure of ‘Honey Gold’ mango fruit to physical damage by abrasion with sand paper and storage at low temperature (12 °C) can mimic these conditions and induce USB development (Hofman et al., 2010). Reducing fruit-to-fruit contact may reduce USB that otherwise expresses after long distance road transport (Li, 2015; Marques et al., 2012b). However, there is limited published information about the mechanism and control of USB development.

RCD is associated with discolouration of the finely branched network of sub-cuticle resin canals. Relative to USB, even less is known about the cause and prevention of RCD. This quality defect is likely attributable to a combination of production factors that contribute to increase fruit susceptibility and postharvest factors that contribute to fruit stress and symptom expression (Macnish et al., 2014).

8.3.1 Comparative anatomy of USB and RCD

At the anatomical level, USB was characterised by dark-brown cells predominantly in the cells surrounding the epithelial cells of resin ducts (Chapter 6). In contrast, RCD was distinguished by localised browning of the lumen of resin ducts. Accordingly, different cell biology processes hypothesised to be behind the development of USB and RCD at the cell and tissue levels. USB was associated with starch retention and phenolic deposition in the cell layers surrounding the resin ducts. TEM observation illustrated that the starch granules were only partly degraded in USB-affected regions of ripe ‘Honey Gold’ fruit tissue. In control fruit, starch was completely converted to sugars (Chapter 7, Figure 7.15). This finding implies that starch degradation in USB-affected regions was suppressed during fruit ripening. These results suggest that USB might be associated with self-defence to external stress (Chapter 7).

In contrast with USB, the browning of resin ducts in RCD-affected tissues was only related to polymerised phenolic deposition inside the lumen (Chapter 6, Figure 8.2). Polyphenolic parenchyma cells associated with phenolic and starch deposition were related to the defence response of many conifers (Krokene et al., 2008). Resin was major defence component in the resin

duct system of many conifer families (Phillips and Croteau, 1999; Ruel et al., 1998). Taken together, these findings suggest that the defensive mechanism may contribute to RCD development and expression.

8.3.2 Mechanism of USB development

8.3.2.1 Effect of diurnal harvest cycle on USB development

In Chapter 6, a postulated effect of harvest time over the diurnal cycle was evaluated in regard to the propensity of abraded fruit to develop USB at low temperature (e.g. < 10 - 12°C) exposure. USB incidence was markedly less in night- and early morning-harvested fruit than in post-midday-harvested 'Honey Gold' fruit (Chapter 6). USB was also observed on areas of the fruit that had not been abraded, most likely because of physical damage caused during road freight from the Northern Territory to south-east Queensland for evaluation. USB incidence on these non-abraded regions was also significantly less in fruit harvested at night and in the early morning compared with those harvested in the afternoon. Delayed cooling increased the incidence and severity of normal USB in the afternoon-harvested fruit. The higher USB incidence was related to a stress response to elevated physical damage (e.g. abrasion or transport) and/ or cold storage in combination with the afternoon harvest time (Figure 8.2). This result is consistent with the study by Marques et al. (2012) that physical injury from transport and delivery was a major factor inducing USB development (Marques et al., 2012b). Likewise, the severity of sap burn injury to the fruit skin was reported by Amin et al., 2008 to be significantly higher in 'Samar Bahisht Chaunsa' mango fruit harvested at noon and in the afternoon relative to those harvested at morning (7 AM).

The concentration of key aroma volatile compounds (2-carene, 3-carene, α -terpinene, *p*-cymene, limonene and α -terpinolene) in 'Honey Gold' fruit sap was significantly higher at 1400 h as compared to all other harvest times (0600 h, 1000 h, 1800 h, 2200 h, 2400 h and 0200 h). This diurnal variation in USB sensitivity was concomitant with changes in the concentration of fruit sap volatiles. The sap was more concentrated in the afternoon versus the morning as the water content of sap presumably decreases during the day (Chapter 6). This decrease in water content is because the plant pulls back water from organs into the tree via the resin canal system (Downton, 1981; Milburn et al., 1990).

8.2.2.2 Effect of sap components on USB incidence

Spurt sap or the non-aqueous phase (oil fraction) of the sap was responsible for inducing browning damage. These observations suggest that two factors played a role in increasing the sensitivity of

afternoon-harvested fruit to USB; more sensitive skin on the afternoon-harvested fruit, and higher volatile components of the afternoon sap had greater potential to cause skin browning damage (Chapter 6). These injurious components were associated with the spurt sap and were more abundant in the non-aqueous phase as compared to in the aqueous phase (Chapter 6, Figure 6.15). This is in agreement with another study of mango sap burn in which it was shown that skin damage was mainly due to the upper non-aqueous phase (Loveys et al., 1992).

It is apparent that the terpenoid components of spurt sap are mainly responsible for browning damage. Terpinolene and limonene, major components of the oil fraction, resulted in USB-like symptoms on mango skin (Chapter 6, Figure 8.2). The symptoms were similar but not identical to USB, but illustrated the potential for sap to cause significant damage. This was concomitant with the findings by Loveys et al. (1992) that the upper phase of sap and especially terpinolene caused tissue damage and enzymic browning in ‘Kensington’ mangoes.

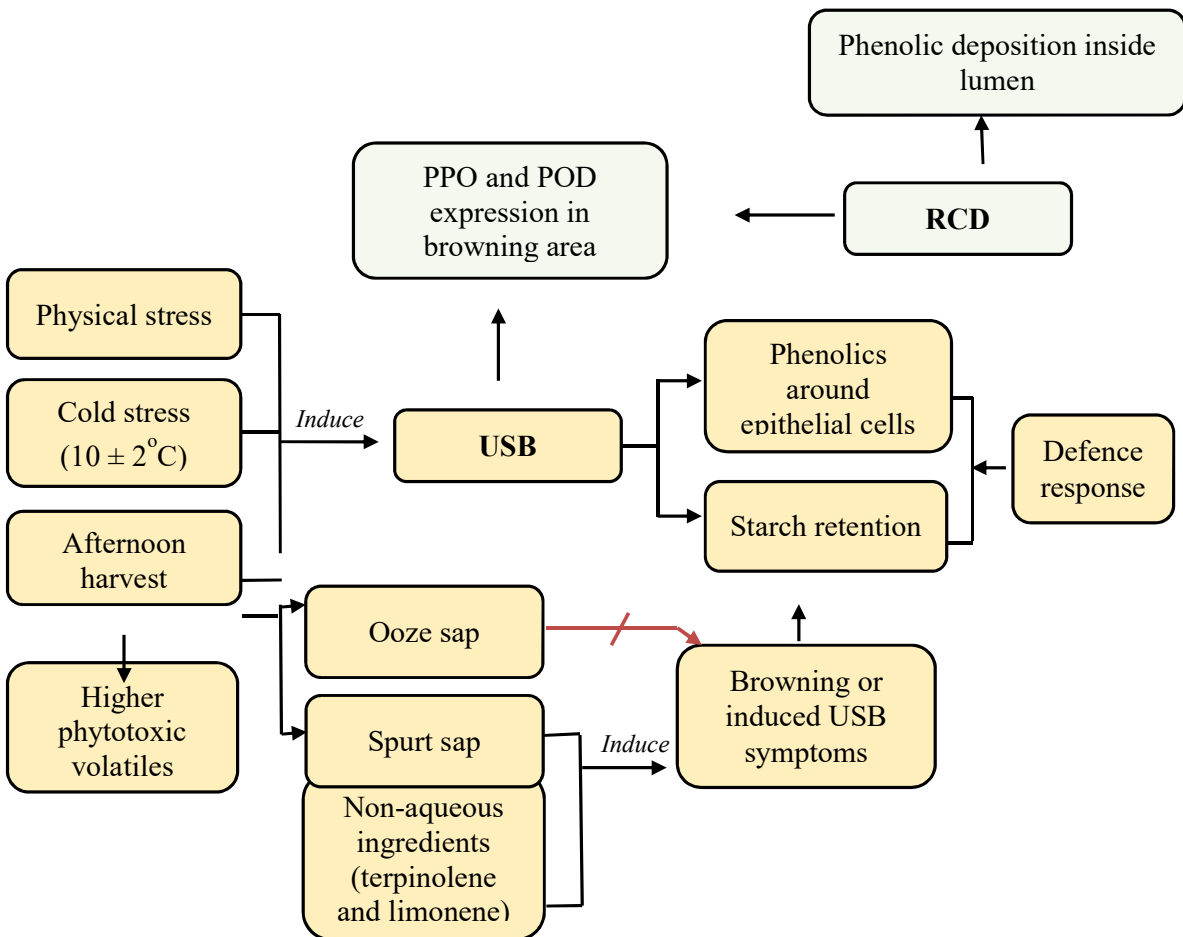


Figure 8.2 A model representing the comparative anatomy and enzymatic browning of USB on ‘Honey Gold’ fruit and RCD on ‘Kensington Pride’ fruit and factors affecting USB development.

The black arrows mean ‘related with’ and the red arrow means ‘did not affect’.

USB in 'Honey Gold' mango was also associated with phenolic deposition and the inhibition of starch degradation surrounding the epithelial cells of resin ducts through the apoplastic pathway (Chapter 6, Figure 8.2). Some starch granules were partly degraded in USB-affected tissues as fruit ripened. Parenchyma cells surrounding traumatic resin ducts were also reported to contain starch granules and phenolics (Krokene et al., 2008). This was due to the defensive response against biotic or abiotic stress in conifers. Likewise, it is possible that USB is associated to stress-related self-defence response.

Induced USB symptoms by treatment of fruit with terpinolene and limonene were also associated with starch retention (Figure 8.2). In these induced USB regions, starch granules were intact after the ripening process indicating the starch degradation process was completely suppressed by chemical-defence response to high concentration of terpenoids. It indicated that terpenoids like terpinolene and limonene were the browning inducers related to chemical defence response. It was possible that the terpenoids were dispersed from the resin duct into the epithelial cells and the cell layers surrounding the epithelial cells. Then they inhibited the starch degradation and accumulated phenolics surrounding the epithelial cells as the defence response.

8.3.3 Comparative enzymatic browning of USB and RCD

PPO and POD activities were responsible for the browning symptoms associated with USB and RCD (Chapter 7). PPO was evidently involved the browning of natural USB, induced USB symptoms and RCD. POD activity appeared to contribute to the enzymatic browning of natural USB and RCD. No such POD existence was illustrated in the induced USB symptoms. POD activity was localised to different tissues of natural USB and RCD-affected regions. Specifically, POD activity appeared all over the natural USB-affected regions, whilst it was mainly localised to the resin ducts of RCD-affected areas. Similarly, John et al. (2002) found that PPO and POD in the peel were the key enzymes involved in the skin browning reactions associated with mango sap-burn injury. Thipyapong et al. (2004) reported that PPO activity was correlated with a stress defence reaction in tomato. Collectively, the findings from the current study support the working hypothesis that increased PPO levels as well as the presence of POD contribute to tissue browning in USB and RCD disorders in Australian mango cultivars as a general stress-related defence response.

8.4 Conclusions and suggested future directions

Overall, a combination of SIDA and HS-SPME-GC-MS represents a marked improvement over current methods for detecting and measuring the concentration of key volatiles released by

Australian mango fruit and sap. This method has contributed to explore the flavour of Australian mango varieties affected by irradiation and the field of mango physiological disorder studies like under-skin browning. New techniques and methods in this area are highly sought after not only for the Australian market, but the many other countries that produce mango fruit for consumption. It could be beneficial in future mango flavour optimisation studies of other commercial cultivars, their parent lines and also wild types.

The present study highlighted genotypic variation of 'B74', 'Kensington Pride', 'Honey Gold', 'R2E2' cultivars in their tolerance to γ -irradiation in terms of LD, skin colour development and volatile production. 'Honey Gold' fruit were typically the most tolerant of irradiation while 'Kensington Pride' and 'R2E2' were the least tolerant. The genotypic dissimilarity and /or different composition of lenticel surface structure (Du Plooy et al., 2004) may contribute to this variable response to γ -irradiation. These findings underscore the importance of screening different cultivars for their response to commercial irradiation treatments with a view to devising/optimising handling and treatment protocols that minimise fruit quality loss. The results also highlight an opportunity to further study and quantify possible relationships between different cultivar sensitivity to irradiation-induced LD and lenticel anatomy and biochemistry. Moreover, sensory evaluation would be recommended to evaluate whether the irradiation-induced decrease in TA and aroma are discernible by consumers. It would be interesting to study the effect of irradiation on 'shared' pigment and aroma biochemical pathways.

The current research also contributed to a greater understanding of USB and RCD disorders by distinguishing their uniquely different cell biology processes at the cell and tissue levels. USB was associated with starch retention and phenolic deposition in the cell layers surrounding the resin ducts. USB development was likely due to a natural defence response to an external stress through the apoplast in the cell wall. RCD was characterised by polymerised phenolic deposition inside the resin duct lumen. An increase in PPO and POD activities contributed to the tissue-specific browning of these physiological disorders. A greater understanding of PPO and POD gene expression and the activation of the plant defence system should aid in the management of these physiological disorders. At present, there is limited information on the mechanism of RCD. It is unclear whether the existence of RCD occurs in the stem before the stem removal as a self-defence response to external stress factors. Another possibility is that bacteria could contribute to RCD symptoms. It is not known if the spurt resin contains bacteria or if the removal of the stem transmits infection. Determining the potential role of bacteria in RCD could be analysed by a molecular marker approach such as quantitative real-time PCR.

Stress response to elevated physical damage (e.g. abrasion or transport) and/ or cold storage in combination with afternoon-harvest were shown to be key contributing factors that induce USB development. One of the contributing factors towards higher USB sensitivity in afternoon-harvested fruit is a concomitant increase in 'aggressive' terpenoid volatile constituents in sap from these fruit. Overall, these results support practise change from conventional afternoon picking to night and early morning harvesting of 'Honey Gold' mango fruit to reduce the incidence and severity of USB. Night and early morning harvesting offers tremendous opportunities to prevent USB on fruit and reduces physiological stress on mango pickers. In order to improve the quality of 'Honey Gold' mango in coming seasons, growers of susceptible fruit are encouraged to adopt this practice.

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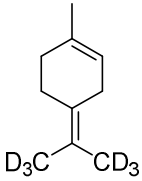
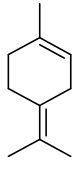
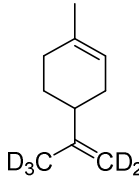
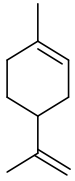
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APPENDICES

Appendix 1

Table A 1.1 ^{13}C NMR shifts of the labelled compounds and literature values of and unlabelled^{1,2} compounds.

Compounds				
1	134.2	134.0	133.8	133.7
2	120.8	120.5	120.7	120.7
3	29.5	29.5	30.6	30.6
4	127.6	127.6	41.0	41.1
5	26.6	26.6	27.9	27.9
6	31.5	31.5	30.8	30.8
7	23.4	23.4	23.4	23.5
8	121.3	121.6	150.0	150.3
9	-	19.7	-	108.4
10	-	19.8	-	20.8

¹Zou et al. (2008)

²Guo et al. (2016)

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Appendix 2. Scanning electron microscopy (SEM)

A 2.1 SEM protocol

Tissue samples ~ 2 mm width, ~ 2-3 mm length and ~ 1 mm depth were prepared from thin slices of mango pericarp using a scalpel blade (Swann-Morton®, England) and fixed in 2.5% glutaraldehyde (ProSciTech, Australia) in 0.1M sodium phosphate buffer pH 6.8. The fixed tissues were washed in ultra-high quality (UHQ) water (Pure lab UHQ, Elga, UK) 3 times and stored in UQH water. The samples were frozen by quickly plunging into liquid nitrogen and then transferred into a freeze-dryer (TLMCSP-80, Thermoline L+M, Australia). They were dried at -30 °C for 24 h and then allowed to warm to room temperature (ca. 22 °C) overnight under vacuum. The samples were then mounted on specimen stubs using conductive double-sided adhesive carbon tabs (ProSciTech) and conductive silver paint (Proscitech). The stubs were sputter coated with platinum using a BalTec MED 10 Coater (BalTec, Lichenstein). SEM samples were examined using an XL30 SEM (Philips, The Netherlands) at 5kV accelerating voltage.

A 2.2 SEM observations

SEM imaging of brown ‘Kensington Pride’ and ‘B74’ lenticels showed small irregular elliptical openings with a maximum diameter of 200 µm were evident on the surface of discoloured lenticels (Figure A 2.1). There was no difference in colour in surface appearance between the bordered dark brown centre and the lighter white corona that surrounded the pores because the discolouration of lenticel occurred internally.

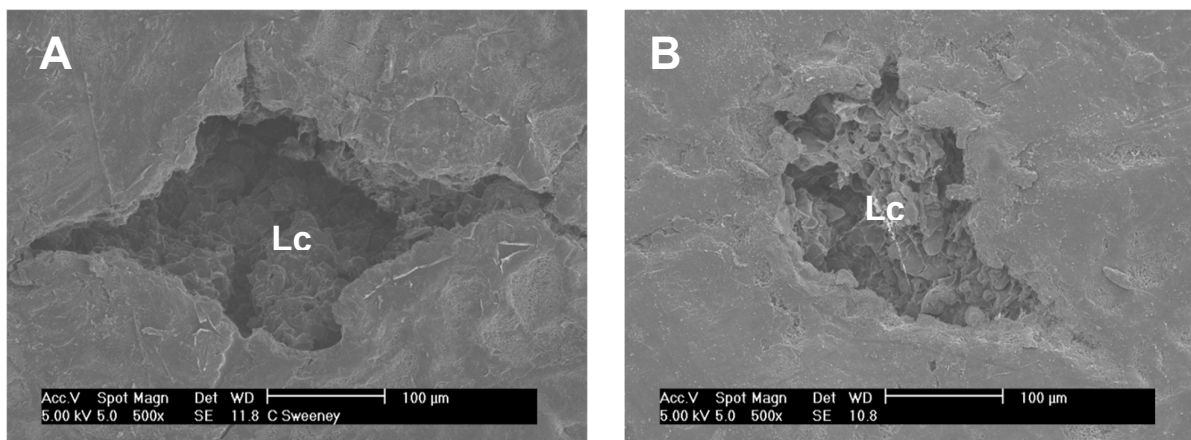


Figure A 2.1 Scanning electron micrographs showing the surface of discoloured lenticels of green ‘Kensington Pride’ (A) and ‘B74’ (B), mango fruit. Lc, lenticel cavity. Scale bars = A, B, 100 µm.

Appendix 3. Dry matter

The dry matter (%) was determined using the method of Hassan et al. (2007) with minor modification. Individual fruit was taken as the replicate. The flesh of one cheek of each fruit was removed using a sharp knife. A vertical section of flesh from the proximal stem end to the distal fruit tip was diced into small pieces. A sample of approximate 20 g mango flesh was added to a plastic cup and dried at 65 °C to constant weight.

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Analytical Methods

Stable isotope dilution assay (SIDA) and HS-SPME-GCMS quantification of key aroma volatiles for fruit and sap of Australian mango cultivars



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Volatiles

ABSTRACT

Reported herein is a high throughput method to quantify in a single analysis the key volatiles that contribute to the aroma of commercially significant mango cultivars grown in Australia. The method constitutes stable isotope dilution analysis (SIDA) in conjunction with headspace (HS) solid-phase microextraction (SPME) coupled with gas-chromatography mass spectrometry (GCMS). Deuterium labelled analogues of the target analytes were either purchased commercially or synthesised for use as internal standards. Seven volatiles, hexanal, 3-carene, α -terpinene, p-cymene, limonene, α -terpinolene and ethyl octanoate, were targeted. The resulting calibration functions had determination coefficients (R^2) ranging from 0.93775 to 0.99741. High recovery efficiencies for spiked mango samples were also achieved. The method was applied to identify the key aroma volatile compounds produced by 'Kensington Pride' and 'B74' mango fruit and by 'Honey Gold' mango sap. This method represents a marked improvement over current methods for detecting and measuring concentrations of mango fruit and sap volatiles.

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1. Introduction

Mango fruits are highly desired by consumers because of their distinctive and evocative flavour as a function of their aroma and taste. Australia produces ca. 50,000 ton of mango fruit for domestic and export markets annually (AMIA, 2016). 'Kensington Pride', 'B74', 'Honey Gold' and 'R2E2' are the main cultivars grown commercially (Dillon et al., 2013). These Australian varieties are not grown widely overseas. Nonetheless, they are in high demand both domestically and as premium products in export markets due to their distinctive fruit odour profiles. The intense flavour of the fruit of Australian mango cultivars has been reported to be a crucial attribute that drives repeat purchases by consumers (Florkowski et al., 2014). The mango industry has responded to this driver by focusing horticultural research into optimising agronomic and postharvest handling practices to maximise flavour production

by fruit; for example, a 'guaranteed sweet' program (Florkowski et al., 2014).

Several hundred volatile compounds have been identified in mango fruits (Andrade, Maia, & Zoghbi, 2000; Lalel & Singh, 2006; Lalel, Singh, & Tan, 2003; MacLeod & de Troconis, 1982; MacLeod, Macleod, & Snyder, 1988; MacLeod & Snyder, 1985; Pino, Mesa, Muñoz, Martí, & Marbot, 2005; Shivashankara, Isobe, Horita, Takenaka, & Shiina, 2006). The volatile profile varies considerably among different mango cultivars. The selection of the seven targeted compounds was based on their presence in the headspace analysis and their contribution to the flavour of mango fruit. For example, α -terpinolene is considered a key aroma volatile responsible for the characteristic flavour of 'Kensington Pride', the most commonly grown cultivar in Australia (Lalel et al., 2003). It was also shown to be present in relatively high abundance in 'Cheiro', 'Chana', 'Bacuri', 'Cametá', 'Gojoba', 'Carlota', 'Coquinho' and 'Comum' mango fruits (Andrade et al., 2000). Other volatile compounds, including hexanal, 3-carene and limonene, are also considered important as they have been found in mango fruits at concentrations above their sensory thresholds (Pino, 2012). 3-Carene has a sweet and pungent odour and quantitatively

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represented the second main volatile compound produced by 'Kensington Pride' mango fruit (2003). In 'Kensington Pride' mango, α -terpinene was present as the major volatile component at the climacteric stage, while the highest concentrations of limonene and *p*-cymene were found during the pre-climacteric stage. Ethyl octanoate replaces α -terpinolene in dominance after fruit reach the fully ripe stage. Ethyl octanoate has a low odour activity value. Nonetheless, it is important in the aromatic equilibrium phase as the most abundant constituent, and is a potential indicator of 'Kensington Pride' mango fruit senescence.

Mango sap has two phases including an oily upper non-aqueous phase and a milky lower aqueous phase. It is interesting to note that the non-aqueous phase, which is rich in terpenoids, plays an important role in the plants defence against bacteria and fungus (Negi, John, & Rao, 2002). In this study, limonene was reported as the dominant terpenoid compound. Moreover, a 'sapburn' browning injury was largely due to sap exudation on the mango fruit surface (Loveys, Robinson, Brophy, & Chacko, 1992). This blemish reduces the visual quality of fruit and leads to the loss of market confidence. Terpinolene was abundant in the non-aqueous phase of 'Kensington Pride' mango sap that is evidently related with this browning (Loveys et al., 1992).

The purpose of the present study was to develop a rapid, accurate and precise analytical method utilising a combination of Stable Isotope Dilution Analysis (SIDA) and Head-space Solid phase microextraction (HS-SPME) in combination with gas chromatography mass spectrometry (GC-MS) to quantify characteristic aroma volatiles produced by fruits and sap of Australian mango cultivars to assist flavour optimisation research. This analytical work was conducted towards better understanding the role of sap components in antimicrobial activities and sapburn injury. Seven key aroma volatile compounds, hexanal, 3-carene, α -terpinene, *p*-cymene, limonene, α -terpinolene, ethyl octanoate, were targeted for ripe 'Kensington Pride' and 'B74' mango fruit and for 'Honey Gold' mango sap.

HS-SPME coupled with GC-MS is a relatively simple, rapid, inexpensive, and solvent-free technique for quantifying volatiles (Butkhup et al., 2011; Sánchez-Palomo, Díaz-Maroto, & Pérez-Coello, 2005). However, matrix effects and other potential sources of variation (e.g. MS detector, ionic strength and other matrices) can considerably impact on the quantification of results, particularly their accuracy and precision (Yuan, Li, Xu, & Fu, 2013). SIDA provides an alternative approach to eliminate variability related to sample preparation and matrix effects and to improve the recovery of the analytes (Maraval et al., 2010). The combined analytical method of SIDA and HS-SPME-GC-MS is considered more accurate, precise, rapid and sensitive than other quantitative techniques, such as liquid-liquid extraction, especially for low volatile and polar odorants (Siebert et al., 2005).

Quantification methods have been developed previously for the analysis of volatiles in mango. The common examples are where analytical methods have been applied either to compare the most abundant aroma compounds between mango cultivars (Munafó, Didzbalis, Schnell, Schieberle, & Steinhaus, 2014; Nicola & Fontana, 2014; Pandit, Chidley et al., 2009; Pino et al., 2005), to measure differences in volatile aroma components between ripened and mature green mango fruits (Lalel et al., 2003; Pandit, Kulkarni et al., 2009; Shivashankara et al., 2006) or to quantify the major aroma-active compounds in tree-ripened mango fruit (Munafó, Didzbalis, Schnell, & Steinhaus, 2016). These methods only provide definite and accurate quantification results if recovery rates and response factors are measured for each compound against a suitable internal standard. Some studies (Loveys et al., 1992; Saby John, Jagan Mohan Rao, Bhat, & Prasada Rao, 1999) demonstrate only the percentage composition of sap and not the quantitative concentrations of aroma volatiles in sap. Musharraf,

Uddin, Siddiqui, and Akram (2016) quantified the volatile concentration of different Pakistan mango sap using gas chromatography triple quadrupole mass spectrometry. However, limited information is given regarding the effect of the sap matrix on the quantification. In most cases, such rigor in analytical method development has not been employed for the analysis of mango volatiles.

Ideally in analytical methods development, the response of analytes should be calibrated to an internal standard that behaves very similarly both chemically and physically to the analyte in a given matrix. This is particularly important where SPME is employed due to variation in the adsorption properties of different analytes to the fibre. Techniques that overcome these challenges use either deuterium or carbon-13 labelled analogues of target analytes as internal standards. Deuterium labelled standards are commonly applied as they are readily accessible (Florkowski, Shewfelt, Brueckner, & Prussia, 2014) and relatively inexpensive as compared to ^{13}C -labelled standards. This technique known as SIDA has been applied in combination with SPME for the analysis of volatiles in rice (Maraval et al., 2010), freeze-dried Cheonggukjang (Park, Choi, Kwon, & Kim, 2007), olive oil (Dierkes, Bongartz, Guth, & Hayen, 2012), juice and wine (Kotseridis et al., 2008), for which target analytes are successfully measured in concentrations ranging from $\mu\text{g/l}$ down to ng/l . To date, however, the combination of SIDA and HS-SPME-GC-MS has not been proven for quantitative analysis of key aroma volatiles in mango fruit and sap, which inspired our method development in this space.

2. Materials and methods

2.1. Materials

All solvents were HPLC grade and all reagents were purchased from Sigma-Aldrich, Australia. The standards used included hexanal (Sigma-Aldrich, Australia), 3-carene (Sigma-Aldrich, Australia), α -terpinene (Sigma-Aldrich, Australia), *p*-cymene (Sigma-Aldrich, Australia), limonene (Sigma-Aldrich, Australia), α -terpinolene (Fluka, Australia) and ethyl octanoate (Sigma-Aldrich, Australia). d_{15} -Ethyl octanoate and d_{12} -hexanal were purchased from CDN Isotopes (CDN, Canada). Deuterium labelled internal standards d_6 - α -terpinolene and d_5 -limonene were synthesised in-house, as they were not commercially available.

2.2. Synthesis of isotopes

The commercially available methyl 4-methyl-cyclohex-3-ene-1-carboxylate was reacted with an excess of d_3 -methyl magnesium iodide to provide d_6 -terpineol (Ketter & Herrmann, 1990). Phosphoryl chloride mediated elimination of water (procedure for unlabelled terpineol given below) produced a mixture of d_6 -terpinolene and d_5 -limonene (Korstanje, de Waard, Jastrzebski, & Klein Gebbink, 2012; Rudloff, 1961). Both compounds were partially separated by column chromatography on silver impregnated silica gel (Mander & Williams, 2016; Williams & Mander, 2001) to give 9:1 and 1:1 fractions of d_6 -terpinolene and d_5 -limonene. The fractions were characterised by high field ^1H and ^{13}C NMR as well as by Bruker AV400MHz mass spectrometry. For ^1H NMR, residual CHCl_3 in CDCl_3 was referenced at δ 7.26 ppm. For ^{13}C NMR, the central peak of the CDCl_3 triplet (δ 77.0 ppm) was used to reference chemical shifts. GC/MS was performed using a Shimadzu-17A GC equipped with J&W Scientific DB5 column (internal diameter 0.2 mm; 30 m) coupled to a Shimadzu QP5000 Mass Spectrometer (70 eV).

d_6 - α -Terpinolene (Zou, Wang, & Goeke, 2008)

^1H NMR (CDCl_3 , 400 MHz): δ 1.65 (s, 3H, H₃₋₇), 1.99 (m, 2H), 2.30 (t, J = 6.4 Hz, 2H), 2.71 (m, 2H), 5.35 (m, 1H, H-2).

^{13}C NMR (CDCl_3 , 125 MHz): δ 23.4, 26.6, 29.5, 31.5, 120.8, 121.3, 127.6, 134.2.

d_5 -Limonene (Guo et al., 2016):

^1H NMR (CDCl_3 , 400 MHz): δ 1.49 (m, 1H), 1.64 (br s, 3H, H_3 -7), 1.73–2.12 (m, 4H), 2.30 (m, 1H), 2.71 (sextet, $J = 2$ Hz, 1H, H-4), 5.35 (m, 1H, H-2).

^{13}C NMR (CDCl_3 , 125 MHz): δ 23.4, 27.9, 30.6, 30.8, 41.0, 120.7, 133.8, 150.0.

Elimination of water from terpineol: A solution of α -terpineol (1.0 g, 6.5 mmol) in anhydrous pyridine (10 ml) was added in phosphoryl chloride (1.2 ml, 13.0 mmol) under a N_2 atmosphere at 0 °C. The reaction mixture was gradually warmed up to room temperature and stirred for 20 h. The reaction mixture was then added to cold water and extracted with petroleum spirit (2 \times 10 ml). The organic phases were washed with a saturated solution of copper sulfate, dried over anhydrous magnesium sulfate and concentrated to give a yellow oil (0.75 g, 85%) consisting of terpinolene and limonene (1:1 by GC–MS) along with a small amount of chlorinated side product (8% by GC–MS).

2.3. Mango fruit

'B74' mango fruit were collected at commercial maturity (dry matter contents of 13.9, and 13.6%) from orchards near Dimbulah, Queensland, Australia (–17.149 °S, 145.111 °E) and Goodwood, Queensland, Australia (–25.10 °S, 152.37 °E), respectively. 'Kensington Pride' mango fruit were harvested at commercial maturity (dry matter contents of 13.1 and 13.6%) from orchards near Mareeba, Queensland, Australia (–16.992 °S, 145.422 °E) and Childers, Queensland, Australia (–25.17 °S, 152.17 °E), respectively. Thirty biological fruit replicates with three technical replicates per biological replicate were used for each 'B74' mangoes and 'Kensington Pride' mangoes. All fruit were de-stemmed in Mango Wash[®] (Septone, ITW AAMTech, Australia) for 2 min and graded for uniform quality as per commercial procedures. 'Nam Dok Mai' mango fruit for method validation were collected from the major commercial market at Rocklea near Brisbane, Queensland, Australia (–27.28 °S, 153.03 °E). The fruit were packed as a single layer into cardboard trays with plastic liners and transported within 24 h by air-plane and/or car to the Maroochy Research Facility postharvest laboratory near Nambour, Queensland, Australia. They were then treated with 10 $\mu\text{l/l}$ ethylene for 2 d at 20 °C to trigger ripening as per commercial practice. Thereafter, the fruit were maintained at 20 °C until ripe. The flesh of one cheek of each fruit was then removed using a sharp knife. A vertical section of flesh from the proximal stem end to the distal fruit tip was diced into small pieces and stored in glass bottles at –20 °C for 2–3 months pending analysis. Prior to instrumental analysis, samples were thawed and blended with an Ultra-Turrax[®] (IKA, Germany) stick blender ready for sampling directly into headspace vials.

2.4. Mango sap

'Honey Gold' mango fruit were harvested at commercial maturity from an orchard near Katherine, Northern Territory, Australia (14.28 °S, 132.16 °E). Fruit with a 2–5 cm-long pedicel attached were carefully transported to a nearby packing shed. The pedicel was broken at the abscission zone and the fruit inverted to allow the sap to drain over 4–5 min. The sap was collected into glass vials. The sap from 20 fruit was collected for each replication. The vial opening was covered with a piece of aluminium foil and closed with an aluminium screw-on cap. The collected sap was stored at –20 °C pending analysis. The aqueous phases of sap

(lower phase) was separated using a 5810 R centrifuge (Eppendorf, Germany) at 3000 rpm for 10 min and used in the validation.

Aliquots of 0.1 g of sap, either whole sap or aqueous sap lower phase, were dissolved in 10 ml of distilled water. The diluted samples were mixed well using a ball mill MM400 (Retsch GmbH, Germany) for 30 s and then diluted a further 50 times with distilled water. A volume of 2.5 ml diluted sap was then added to a 20 ml HS-SPME vial (Merck, Australia) containing 2.5 ml of saturated sodium chloride (NaCl) solution and a magnetic stir flea (5 \times 2 mm) for aroma analysis. Five biological replicates with two technical replicates per biological replicate were prepared for analysis.

2.5. Calibration and validation of method

Standard solutions were prepared using analytical reagent grade chemicals. A combined stock solution which contained each of the target analytes (viz., hexanal, 3-carene, α -terpinene, *p*-cymene, limonene, α -terpinolene and ethyl octanoate) in ethanol (Merck, Australia) was also prepared. Solutions were always made in duplicate from separately weighed reagents to ensure precision in their preparation. The stock concentration of target analytes were approximately 0.5 g/l (hexanal and *p*-cymene), 2 g/l (3-carene and α -terpinene), 10 g/l (limonene) and 20 g/l (α -terpinolene).

In duplicate, water, mango flesh and aqueous sap lower phase were spiked with increasing levels of the combined stock solution of analytes so that the resulting concentrations in water were approximately 0, 1.5, 3, 6, 15, 30, 60, 150, 300 and 600 $\mu\text{g/l}$ for hexanal and *p*-cymene, 0, 5, 10, 20, 50, 100, 200, 500, 1000 and 2000 $\mu\text{g/l}$ for 3-carene, α -terpinene and ethyl octanoate, 0, 25, 50, 100, 250, 500, 1000, 1500, 5000 and 10,000 $\mu\text{g/l}$ for limonene, and 0, 50, 100, 200, 500, 1000, 2000, 5000, 10,000 and 20,000 $\mu\text{g/l}$ for α -terpinolene. Mango flesh and aqueous sap lower phase contain minimal concentrations of analytes prior to spiking. A dilution of mango flesh of 1:400 was spiked with different amounts of α -terpinolene (0, 50, 100, 200, 500, 1000, 2000, 5000, 10,000 and 20,000 $\mu\text{g/l}$) in order to have a low α -terpinolene concentration for optimising the MS signal of α -terpinolene. The solutions were prepared in SPME vials for analysis ($n = 10 \times 2$). The concentrations of analytes in the samples were calculated from the peak area ratios for the unlabelled and labelled compounds versus the concentration ratio. Validation parameters including the calibration relative standard deviation (RSD) and recovery were calculated in spiked concentration levels of each standard compound. The limit of detection (LOD) and limit of quantification (LOQ) were calculated on the basis of $3.3\alpha/S$ and $10\alpha/S$, respectively (Musharraf et al., 2016). α is the standard deviation of the *y*-intercepts and *S* is the slope of calibration curve.

2.6. Headspace sampling of volatiles for analysis

A sample of either 2.5 g homogenized mango flesh or 2.5 ml aqueous solution of standards was added to a 20 ml SPME vial (Merck, Australia) containing 2.5 ml of saturated sodium chloride (NaCl) solution and a magnetic stirrer flea (5 \times 2 mm). Vials were immediately sealed with a screw cap fitted with a silicone/PTFE septum. Subsequently, 100 μl of combined internal standard solution was injected through the septum and the vial was shaken well. The deuterated internal standards were added at concentrations of 30 $\mu\text{g/l}$ for d_{12} -hexanal, 50 $\mu\text{g/l}$ for d_{15} -ethyl octanoate and 106 $\mu\text{g/l}$ for both d_6 - α -terpinolene and d_5 -limonene as equivalent to the amount of mango flesh in the vial. The vial and its contents were heated to 40 °C with stirring at 250 rpm for 2 min followed by extraction with a divinylbenzene/carboxen/polymethylsiloxane (DVB/CAR/PDMS, 'grey', 1 cm) 50/30 μm fibre (Supelco, USA)

which was exposed to the headspace for 30 min. The SPME fibre was injected into the Programmable Temperature Vaporizing (PTV) inlet (Gerstel, Germany) set at 200 °C in splitless mode and the fibre was desorbed for 8 min.

2.7. Instrumental analysis of volatiles

An Agilent 6890 gas chromatograph (Agilent Technologies, USA) equipped with a Gerstel MPS2XL multi-purpose sampler and coupled to a 5975N mass selective detector was used for the analysis of samples. The instrument was controlled by and the data analysed with MSD Chemstation E. 02. 02. 1431 software (Agilent). The gas chromatograph was fitted with a $\sim 30\text{ m} \times 0.25\text{ mm}$ fused silica capillary ZB-5 ms column (Phenomenex, Australia) with 0.25 μm film thickness. Helium carrier gas (BOC Gas) using a linear velocity of 44 cm/s with a constant flow rate of 1.5 ml/min was used. The pressure was 75.7 kPa and total flow was 70.5 ml/min. The oven had an initial temperature of 40 °C that was held for 2 min, increased to 80 °C at 2 °C/min and then to 220 °C at 40 °C/min and held for 5 min. The PTV inlet was fitted with a 0.75 mm borosilicate glass SPME inlet liner (Agilent). The mass spectrometer quadrupole temperature was set at 230 °C, the source was set at 250 °C, and the transfer line at 280 °C. Ion electron impact spectra at 70 eV were recorded in selective ion monitoring (SIM) or scan (35–350 m/z) mode.

3. Results and discussion

3.1. Isotope synthesis

The mass spectra of the internal standard compounds are characterised (data not shown). The NMR data for d_6 -terpinolene matched those previously reported (Zou et al., 2008), except for the following observations: The signals for CH_3 -9 and CH_3 -10 were absent in the ^1H spectrum of d_6 -terpinolene and C-9 and C-10 appeared as complex multiplets slightly upfield of the reported shifts (Zou et al., 2008) in undeuterated terpinolene, as would be expected for C^2H_3 moieties. The NMR spectral data obtained for d_5 -limonene was also in agreement with in the literature values (Bollen & Emond, 2014), except that the CH_2 -9 methylene and CH_3 -10 methyl were largely absent from the ^1H NMR and the signals for C-9 and C-10 were vastly diminished in the ^{13}C NMR spectrum. Analysis of the molecular ion region in the mass spectrum of d_6 -terpinolene indicated that it was 96% d_6 and 4% d_5 , and lower isotopomers were not observed. Similar analysis of d_5 -limonene indicated that it was 95% d_5 and 5% d_4 , and lower isotopomers were not observed.

3.2. Mango volatile analysis method and validation

An accurate and precise analytical method involving a combination of HS-SPME-GCMS and SIDA was developed using selected ion monitoring (SIM) to quantify important aroma volatiles present in Australian mangoes. The concentration ranges targeted for each analyte were selected by considering both the sensorially active range of each compound by referring to the odour sensory threshold concentrations and the indicative concentration range as previously reported for that compound in mango (Table 5). The labelled internal standard used to quantify each target analyte, respective retention time and the target and qualifier ions used for SIM and relative percentages are given in Table 1. Schmarr, Slabizki, Müntnich, Metzger, and Gracia-Moreno (2012) reported an inverse isotope effect whereby the heavier deuterated compounds elute prior to their non-labelled counterparts as was also observed in our study.

The precision of the method was thoroughly validated at various levels in mango fruit and sap matrices. Duplicate standard addition calibration equations were developed in water, mango flesh and sap matrices (Table 2) for each of the seven target volatile compounds. Separate standard addition calibration curves were created for mango fruit using 'Nam Dok Mai' fruit and mango sap using the non-aqueous sap phase of 'Honey Gold' mango. These were selected because of their naturally lower profile of these aroma volatiles.

The calibration equations developed for 'B74' and 'Kensington Pride' mango flesh, including their range of linearity and the coefficients of determination (R^2) achieved, are presented in Table 2. In general, the LOD and LOQ were relatively lower than its odour threshold. Therefore the developed method showed very good sensitivity of detection. The calibration curves showed good linearity over the reported concentration range for all compounds with good correlation (R^2) from 0.93775 to 0.99741. The largest calibration range developed was for α -terpinolene at 50–20,200 $\mu\text{g/l}$ due to its relatively higher odour sensory threshold at 200 $\mu\text{g/l}$ and the higher concentration as is expected in mango (Lalel, Singh, & Tan, 2004). The lowest calibration range developed was for p -cymene at 1.3–1060 $\mu\text{g/l}$ since it has a much lower odour threshold and was expected to be found in mango at much lower concentration ranges. At higher concentrations in the spiked standards, p -cymene co-eluted with d_5 -limonene which limited the range of calibration to $\sim 550\text{ }\mu\text{g/l}$. Nevertheless, this was still higher than the reported sensory threshold at 11.4 $\mu\text{g/l}$. The minimum LOD and LOQ were obtained for p -cymene at 0.824 and 2.498 $\mu\text{g/l}$, respectively. The validation was performed to confirm the calibration developed in the water matrix was suitable for application (Table 2). Generally, the method was accurate and precise for all 7 compounds within the mango matrix. After this verification, the analytes in mango flesh and sap samples were quantified using calibration curves obtained from a water matrix.

Similarly, the validation curves constructed using aqueous sap phase were linear throughout the calibration range, with good correlation (R^2) which varied from 0.89069 to 0.99142 (Table 2). As mango sap is a complex and variable matrix to quantify accurately all these compound versus their labelled internal standards, the correlation efficiency (R^2) was lower for 3-carene (0.89069).

The recoveries were also demonstrated by spiking a known amount of analyte into the 'Nam Dok Mai' mango matrix at five replicate additions for each analyte. Recoveries of the spiked concentrations are reported in Table 3. All the relative standard deviations (RSD) were <8.8% for all 7 spiked analyte compounds.

3.3. Analysis of key aroma volatiles in mango fruits

A range of mango fruits within the two varieties, were collected, ripened and prepared for analysis so that the analytical method could be validated for its applied usefulness. A summary of the concentration data for the 7 key aroma volatile compounds produced by ripe 'B74' and 'Kensington Pride' mango fruit is reported in Table 4. Overall, the variety 'Kensington Pride' produced the highest average concentration of α -terpinolene at 19,719 $\mu\text{g/l}$, which is in agreement with published data (Lalel et al., 2004). 'B74' had an average terpinolene content (11,272 $\mu\text{g/l}$) lower than 'Kensington Pride' mango fruit. 3-Carene and limonene were the second and third, respectively, most abundant volatiles produced by both 'B74' and 'Kensington Pride' fruits. 3-Carene and limonene have been reported to be responsible for a 'resinous, sweet, leafy, green, pungent' (MacLeod & Snyder, 1985; Pino, 2012; Shivashankara et al., 2006) and 'citrus-like' aroma (Pino, 2012). p -Cymene, with a characteristic 'herbal or minty' odour (MacLeod & Snyder, 1985), was present in 'B74' and 'Kensington Pride' fruits at average concentrations of 12 and 14 $\mu\text{g/l}$,

Table 1
Analysis parameters for determination using SIM of seven important aroma volatiles.

Internal standard	t_R^a (min)	Target ion m/z	Qualifier ions m/z (%) ^b	Target compounds	t_R^a (min)	Target ion m/z	Qualifier ions m/z (%) ^b
d_{12} -Hexanal	5.51	64	62 (46) 80 (46) 92 (6)	Hexanal	5.71	56	57 (70) 82 (22) 67 (11)
d_6 - α -Terpinolene	21.12	142	124 (98) 93 (71) 108 (18)	3-Carene	16.01	93	91 (41) 77 (30) 79 (27)
			124 (98) 93 (71) 108 (18)	α -Terpinene	16.64	93	121 (103) 136 (89) 91 (55)
			124 (98) 93 (71) 108 (18)	<i>p</i> -Cymene	17.22	119	134 (27) 103 (5) 117 (16)
d_5 -Limonene	17.29	141	126 (56) 112 (54) 100 (14)	Limonene	17.48	136	121 (121) 107 (109) 93 (345)
d_6 - α -Terpinolene	21.12	142	124 (98) 93 (71) 108 (18)	α -Terpinolene	21.37	136	93 (160) 121 (198) 91 (87)
d_{15} -Ethyl octanoate	24.20	91	105 (32) 142 (29) 121 (6)	Ethyl octanoate	24.32	88	101 (38) 127 (29) 115 (9)

^a Retention time.^b This % represents the associated ions relative % to the target ion shown in the previous column, not necessarily the base peak.**Table 2**
Summary of calibration and main validation parameters for seven mango volatiles in water, mango flesh and mango sap matrices.

Analyte	Calibration range ($\mu\text{g/l}$)	Calibration equation	R^2	LOD ($\mu\text{g/l}$)	LOQ ($\mu\text{g/l}$)
<i>In a water matrix</i>					
Hexanal	1.4–578	$y = 1.809x$	0.99741	1.058	3.205
3-Carene	5.2–1077	$y = 2.260 \times 10^{-1}x - 4.347 \times 10^{-2}$	0.95909	2.284	6.922
α -Terpinene	5.1–1211	$y = 8.441 \times 10^{-1}x - 3.403 \times 10^{-2}$	0.93775	3.038	9.205
<i>p</i> -Cymene	1.3–548	$y = 3.158x - 1.124 \times 10^{-1}$	0.97666	0.824	2.498
Limonene	25–5041	$y = 1.919 \times 10^{-1}x - 1.315 \times 10^{-1}$	0.94393	12.870	38.999
α -Terpinolene	50–20,214	$y = 3.432 \times 10^{-2}x - 6.503 \times 10^{-2}$	0.97716	32.842	99.520
Ethyl octanoate	5.4–2172	$y = 2.389 \times 10^{-1}x + 3.592 \times 10^{-1}$	0.98584	5.572	16.884
<i>In a mango flesh matrix</i>					
Hexanal	1.4–724	$y = 7.744 \times 10^{-1}x$	0.99249	1.939	5.875
3-Carene	5.2–2300	$y = 3.695 \times 10^{-1}x + 1.347$	0.94224	0.379	1.149
α -Terpinene	5.1–2220	$y = 1.601x + 9.669 \times 10^{-1}$	0.97366	5.721	17.336
<i>p</i> -Cymene	1.3–1060	$y = 4.526x + 1.650$	0.90798	3.641	11.032
Limonene	25–10,082	$y = 5.226 \times 10^{-1}x + 5.637 \times 10^{-1}$	0.93014	3.391	10.275
α -Terpinolene	50–20,214	$y = 4.001 \times 10^{-2}x - 9.522 \times 10^{-2}$	0.98950	28.373	85.979
Ethyl octanoate	5.4–2178	$y = 8.366 \times 10^{-1}x + 1.166 \times 10^{-2}$	0.98859	21.906	66.383
<i>In a mango sap matrix</i>					
Hexanal	1.4–724	$y = 3.769 \times 10^{-1}x$	0.99142	2.362	7.157
3-Carene	5.2–1150	$y = 6.605 \times 10^{-2}x + 1.819 \times 10^{-2}$	0.89069	1.110	3.363
α -Terpinene	5.1–2220	$y = 6.198 \times 10^{-1}x + 2.010 \times 10^{-1}$	0.90845	4.246	12.866
<i>p</i> -Cymene	1.3–530	$y = 2.346x - 2.098 \times 10^{-2}$	0.90143	7.025	21.288
Limonene	25–10,082	$y = 1.920 \times 10^{-1}x - 5.211 \times 10^{-2}$	0.90406	1.828	5.540
α -Terpinolene	50–20,214	$y = 6.979 \times 10^{-3}x + 4.775 \times 10^{-2}$	0.99336	17.505	53.045
Ethyl octanoate	5.4–1122	$y = 5.139 \times 10^{-1}x + 2.972 \times 10^{-2}$	0.99632	16.656	50.474

Table 3
Recoveries and relative standard deviation (RSD) of compounds spiked into a mango fruit.

Samples with spiked compounds	Matrix spike concentration ($\mu\text{g/l}$)	Recovery (%)	RSD (%)
Hexanal	290	107–121	8.8
3-Carene	909	95–104	3.9
α -Terpinene	854	97–119	7.9
<i>p</i> -Cymene	419	72–88	8.2
Limonene	4102	80–97	7.7
α -Terpinolene	4028	99–114	5.9
Ethyl octanoate	898	86–106	8.0

 $n = 5$ (replication).

respectively, and above its odour perception threshold. The lowest average concentration of ethyl octanoate was measured in 'Kensington Pride' at 0.5 $\mu\text{g/l}$, while the lowest average contents of hexanal and ethyl octanoate were found in 'B74' mango fruit at 6.0 and 0.6 $\mu\text{g/l}$, respectively.

In order to compare the odour contribution and relative importance of each compound to the aroma of 'B74' and 'Kensington Pride', the odour activity value (OAV) was calculated for each compound (Table 5). The OAV is the ratio between the concentration of an aroma compound and its odour threshold (Pino & Febles, 2013). Reported odour sensory thresholds and nature of the odour of each target analyte are also shown in Table 5. The compounds

α -terpinolene, 3-carene, *p*-cymene and limonene were found to be odour active in all mango fruits analysed (i.e., OAV > 1).

The application of OAV's to compare and contrast odour volatile contribution has been reported previously (Du, Plotto, Baldwin, & Rouseff, 2011). However, the method utilised in that study could not approach the OAV of some compounds due to the lack of a suitable internal standard to detect aroma volatiles present at low levels. The accuracy and precision realised in the present work allow for meaningful comparisons to be made using OAV's, because the concentration data collected is sensitive and reliable.

3.4. Analysis of key aroma volatiles in mango sap

The SIDA HS-SPME-GCMS method was applied to analyse 'Honey Gold' mango fruit sap with some modification in the preparation steps, namely a dilution of a small amount of sap to reduce the initial concentration of all volatiles. Following that modification, the ensuing steps for sap analysis was the same as were utilised for mango flesh. Thereby, this modified SIDA HS-SPME-GCMS method is easily employed to quantify these analytes in different matrices, such as 'Honey Gold' mango sap.

Quantitative data of the predominant volatile compounds in 'Honey Gold' mango sap are shown in Table 4. α -Terpinolene was the most abundant component in 'Honey Gold' mango sap. It is most likely responsible for mango sapburn, being present at levels notably higher than its perception threshold (Loveys et al., 1992). A

Table 4
Aroma compounds concentrations in ripe 'B74' and 'Kensington Pride' mango fruits and in 'Honey Gold' mango sap.

Compounds	Concentration ($\mu\text{g/l}$) of 'B74' fruit ¹					Concentration ($\mu\text{g/l}$) of 'Kensington Pride' fruit ¹					Concentration (mg/g) of 'Honey' mango sap ²				
	Average	Min	Max	S.D. ^a	CV ^b	Average	Min	Max	S.D. ^a	CV ^b	Average	Min	Max	S.D. ^a	CV ^b
Hexanal	6	3	13	2	41	54	14	188	47	87	n.d.				
3-Carene	929	34	4122	986	106	383	33	1777	418	109	4.2	3.0	5.7	1.2	28.8
α -Terpinene	23	1	72	19	84	43	2	202	47	109	1.5	1.0	2.1	0.5	30.7
<i>p</i> -Cymene	12	2	25	6	55	14	5	40	9	66	0.1	0.1	0.2	0.0	30.7
Limonene	216	89	769	155	72	264	93	881	198	75	1.1	0.5	1.6	0.5	45.9
α -Terpinolene	11,272	329	49,122	11,966	106	19,719	672	87,900	21,624	110	59.0	36.9	73.9	18.0	30.5
Ethyl octanoate	0.6	0.2	1.4	0.3	51	0.5	0.0	2.5	0.6	108	n.d.				

n.d.: not detected.

^a Standard deviation.

^b Coefficient of variation.

¹ $n = 30$ (replication).

² $n = 5$ (replication).

Table 5
Odour activity values in ripe 'B74' and 'Kensington Pride' mango fruits.

Compounds	Odour description	Odour threshold ^a ($\mu\text{g/l}$)	OAV ^b of 'B74' fruit					OAV ^b of 'Kensington Pride' fruit				
			Average	Min	Max	S.D.	CV	Average	Min	Max	S.D.	CV
Hexanal	Green, fatty ¹	4.5 ¹	1.31	1	3	1	41	12	3	42	10	87
3-Carene	Leafy ²	5 ¹	186	7	824	197	106	77	7	355	84	109
α -Terpinene	Citrus, lemon-like ³	85 ^{4,5}	0	0	1	0	84	1	0.02	2	1	109
<i>p</i> -Cymene	Herbal, minty ⁶	11.4 ⁷	1	0	2	1	55	1	0	4	1	66
Limonene	Citrus-like ¹	210 ¹	1	0	4	1	72	1	0	4	1	75
α -Terpinolene	Piney ¹	200 ¹	56	2	246	60	106	99	3	439	108	110
Ethyl octanoate	Slightly nutty coconut ⁶	194 ⁷	0.0	0.0	0.0	0.0	51	0.003	0.000	0.013	0.003	108

$n = 30$ (replication).

^a Each sensory (odour) threshold reported was determined in a water matrix.

^b Odour activity values were calculated by dividing the concentrations by the respective odour threshold.

¹ Pino (2012).

² Shivashankara et al. (2006).

³ Costa et al. (2008).

⁴ Du, Finn, and Qian (2010).

⁵ Vincente, Manganaris, Ortiz, Sozzi, and Crisosto (2014).

⁶ MacLeod and Snyder (1985).

⁷ Pino and Mesa (2006).

relatively higher concentration of terpinolene (70 mg/g) was found in 'Kensington' mango sap (Loveys et al., 1992), while an average amount of 59 mg/g was found in 'Honey Gold' mango sap. Terpinolene was significantly more abundant in 'Honey Gold' mango sap than in 'B74' and 'Kensington Pride' mango fruit (Table 4). The average concentrations of 3-carene and of limonene in 'Honey Gold' sap varied from 3.0 to 5.7 mg/g and from 0.5 to 1.6 mg/g, respectively. α -Terpinene was found at average concentration of 1.5 mg/g. The mean content of β -cymene was 0.1 mg/g. It is notable that hexanal and ethyl octanoate were not detected in the sap samples. Considerable differences in major volatile constituents in sap exist between mango cultivars. For example, there were significant variations in the volatile sap components of Australian and Indian mango cultivars. Terpinolene and 3-carene were found to be the major components in Australian 'Kensington' and 'Irwin' (Loveys et al., 1992) fruit sap, whereas neither of these compounds was identified in any of the Indian cultivars (Saby John et al., 1999). For this study, SIDA HS-SPME-GCMS served as a rapid, accurate and precise method for determination of key aroma compounds in sap.

4. Conclusion

An accurate and precise method for the analysis of key volatiles was developed in a model system and subsequently validated with both Australian mango fruit and sap. The method of combining SIDA with HS-SPME-GC-MS was found to be highly sensitive and

allowed high throughput of samples. It produced calibrations with high coefficients of determination and excellent linearity across a range of concentrations relevant to mango flavour. This method represents a pronounced improvement over current methods for detecting and measuring the concentration of mango fruit and sap volatiles. It could be of wider benefit in future studies aimed at exploring and optimising the flavour of commercial mango varieties, their parent lines and also wild types.

Conflict of interest statement

We certify that all authors have approved the final version of the manuscript being submitted. We confirm that this work is original and has not been previously published nor is under consideration for other peer-reviewed publication elsewhere.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2016.11.130>.

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Evaluation of various methods to break dormancy of potato (*Solanum tuberosum* L. 'Granola') minitubers

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Abstract

Different postharvest treatments were used to evaluate dormancy break in 'Granola' potato (*Solanum tuberosum* L.) minitubers. Treatments included ethephon (5,000 and 10,000 $\mu\text{L L}^{-1}$), modified atmosphere packaging (MAP) using 0.002 mm polyethylene bags (PEB), calcium carbide (CaC_2 , 3 and 5 g kg^{-1}) plus MAP, gibberellic acid (GA_3 , 1 and 5 $\mu\text{L L}^{-1}$) and 1-methylcyclopropene (1-MCP, 1 and 10 $\mu\text{L L}^{-1}$, 48 h). As early as 3 weeks or at opening of the PEB, minitubers treated with 3 g kg^{-1} CaC_2 and MAP had the highest sprouting percentage of 50%. This trend was consistent up to nine weeks of storage wherein 3 g kg^{-1} CaC_2 and MAP treatments resulted in highest sprouting percentage (71 and 72%, respectively). Treatment of 3 g kg^{-1} CaC_2 gave the highest weight loss (11%) at 12 weeks. Sprouting resulting from the different treatments ranged from 60 to 77% at 12 weeks, indicating long dormancy in 'Granola' minitubers. The ethephon and 5 $\mu\text{L L}^{-1}$ GA_3 treatments exhibited more than 50% sprouting at 9 weeks after treatment (WAT), but not the control, 1 $\mu\text{L L}^{-1}$ GA_3 and 1-MCP. Dormancy break with the use of CaC_2 (3 g kg^{-1}) resulted in most sprouts per minituber (2.9) and longest apical sprouts (3.5 mm) relative to the control (0.90 and 1.4 mm, respectively). However, CaC_2 and MAP treatments resulted in greater minituber decay at four weeks.

Keywords: calcium carbide, minitubers, modified atmosphere packaging, sprouting, potato, *Solanum tuberosum*, dormancy, ethephon, gibberellic acid, 1-MCP

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the important high value crops of the world. There has been an increasing demand for potato worldwide because of its high nutritive value. The 2013 production data show that it is the fourth most important staple crop after wheat, maize and rice (Food and Agriculture Organization Stat, 2015). It ranks third after wheat and rice in terms of human consumption (International Potato Center, 2015). In the Philippines, potato production increased from 63,524 MT in 2000 to 117,722 MT in 2013. In the same year, the land area devoted to potato production also increased from 5,172 to 7,890 ha (Philippine Statistics Authority, 2014).

There is a need to sustain year round potato production because of the growing demand. Certain problems like disease build-up in tubers and inherent tuber dormancy immediately after harvest limit potato production. Minitubers are an alternative source of clean planting material. Progeny tubers, which range from 0.1 to 10 g or more, are produced from in vitro derived plantlets and are considered of high health quality (Struik and Wiersema, 2007). In the Philippines, minitubers are also known as G-0 (for generation 0) planting materials. These are grown to produce clean seed tubers. Potato tuber dormancy starts at tuber detachment from the mother plant and continues until it breaks dormancy with visible sprout growth (Mani et al., 2014). Uniform sprout growth is highly desirable as it maximizes crop yield and value (Bethke, 2002).

Exogenous application of plant hormone, chemical stimulants and manipulation of

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immediate environmental conditions are often needed to break the dormancy of seed potatoes (Struik and Wiersema, 2007). Several studies have been conducted in other countries to break the dormancy of different potato planting materials. High percentage of sprouting and longer sprouts were observed in minitubers of long dormancy cultivars 'Nooksack' and 'RNK-S3' treated with ethephon and gibberellic acid (GA₃) (Külen et al., 2011). Similarly, GA₃ shortened the natural dormancy and accelerated sprout rate on 'Burren' and 'Agria' potatoes (Hassani et al., 2014). GA₃ application on 'Marfona', 'Draga' and 'Agria' potatoes resulted in starch hydrolysis, and a consequent increase in total soluble sugars, which eventually triggered tuber sprouting (Barani et al., 2013). Ethephon, 2-chloroethyl phosphonic acid, releases ethylene gas to promote fruit ripening, dormancy break and other plant processes (USA EPA RED, 1995). The dormancy breaking capabilities of ethylene depend on the duration of postharvest application, its concentration and the species and cultivar of potato (Rylski et al., 1974).

Another chemical stimulant is calcium carbide (CaC₂), which generates acetylene gas. The postharvest effectivity of acetylene as a ripening gas is 100 times less than that of ethylene (Bautista and Esguerra, 2007). Acetylene gas is released when CaC₂ reacts with water from the transpiring produce and mimics the ripening effects of ethylene gas (Bautista and Esguerra, 2007). Using 3 g CaC₂ kg⁻¹ of 'Granola' tubers for 21 days, Bayogan et al. (1988) reported that following storage in ambient, seed tubers had longer sprouts, better emergence, better overall growth performance in the field and higher yield ha⁻¹.

On the other hand, 1-methylcyclopropene (1-MCP), an ethylene antagonist, binds to ethylene receptors thereby blocking ethylene activity and consequently lengthening the life of fresh produce (Rees et al., 2012).

Other methods used in breaking dormancy include increasing CO₂ levels, humidity and temperature, which act as stressors to induce sprouting (Booth and Shaw, 1981). Accumulation of higher CO₂ can be achieved through passive modified atmosphere packaging (MAP) conditions. In MAP, there is an initial increase of CO₂ and reduction of O₂, but due to the permeability of the package, gas exchange continues until equilibrium is reached (Kader, 1986).

Potato dormancy may last up to three months depending on genotype, tuber size, preharvest and postharvest factors (Mani et al., 2014; Hassani et al., 2014). In vitro plantlets from nodal cuttings grown in greenhouses produce G-0 potatoes or minitubers that have a longer dormancy than conventional seed potato tubers. It is desirable to find methods for breaking tuber dormancy which lead to a shorter period to sprouting, coupled with good and uniform quality of sprouts. The quality of planting materials greatly influences the yield and quality of crops, which in turn benefits farmers. In this study, the sprouting of 'Granola' minitubers derived from stem cuttings was evaluated after they were subjected to different treatments for breaking dormancy.

MATERIALS AND METHODS

'Granola' G-0 potatoes or minitubers (5 to 12 g) were procured from the Northern Mindanao Agricultural Research Center (NOMIARC), Malaybalay, Bukidnon. Samples were cured for a week under dark conditions before being subjected to various postharvest treatments. Minitubers were treated with: ethephon, calcium carbide (CaC₂) in polyethylene bags (PEB), gibberellic acid (GA₃), 1-methylcyclopropene (1-MCP) and modified atmosphere packaging (MAP) using PEB. Samples were stored in diffused light conditions at 27.5±0.74°C and 77.7±6.6% relative humidity (RH) for 12 weeks after treatment (WAT).

The liquid chemical Ethrel™ (active ingredient: 48% 2-chloroethyl phosphonic acid) was used for the ethephon treatments. Minitubers were soaked in ethephon (5,000 and 10,000 µL L⁻¹) for 5 min and air-dried.

The weight of minitubers in each replication was obtained and the dose rate of calcium carbide was calculated to achieve a rate of 3 or 5 g CaC₂ kg⁻¹ of potato. CaC₂ was wrapped in two layers of paper and taped. Along with the minitubers, the wrapped chemical was placed in 0.002 mm PEB that was later sealed. These treatments were opened after three weeks using a method developed by Bayogan et al. (1988).

Two GA₃ concentrations (1 and 5 μL L⁻¹) were prepared from GA₃ powder (90% purity). Minitubers were soaked in the corresponding solution for 10 min and air-dried.

For the 1-MCP treatments, minitubers were placed in separate airtight chambers (constructed with a built-in fan) along with a beaker containing the required 1-MCP powder (1 or 10 μL L⁻¹). Distilled water was added to the powder in the beaker through a tube that extended slightly to the outside of the chamber. The tube was sealed after the delivery of water to the beaker. Minitubers were removed from the chamber after 48 h.

Samples in the passive modified atmosphere were sealed in 0.002 mm PEB for three weeks. The bags were then opened and minitubers held in ambient conditions for further observation.

The number and percentage of minitubers with sprouts, as well as the number of sprouts per minituber were observed at regular intervals. Data measured every four weeks were apical sprout length (mm), weight loss (g), sprout weight (g), shriveling using a scale (1 – no shriveling; 2 – slight, 1 to 15% of the surface area shriveled; 3 – moderate, 16 to 30% ; 4 – severe, 31 to 49%; 5 – extreme, 50% or more of the surface area shriveled) and decay using the rating scale: 1 – no decay; 2 – slight, 1 to 5% of the surface area decayed; 3 – moderate, 6 to 10%; 4 – moderately severe, 11 to 15%; 5 – severe, 16% or more of the surface area decayed. All minitubers with a rating of 5 were discarded after recording the data. Percentage decay was calculated using the formula:

$$\text{Decayed minitubers \%} = \frac{\text{Number of decayed minitubers}}{\text{Initial number of minitubers}} \times 100$$

The denominator changed depending on the remaining minitubers at evaluation time.

Random samples of 10 minitubers per replicate were labelled and weighed before treatment at the start of the experiment. These minitubers were used to measure the various data mentioned earlier. Samples with ≥1 mm sprout lengths were considered sprouted.

The experiment was laid in completely randomized design (CRD). Data were analyzed using analysis of variance (ANOVA). Forty minitubers were used for each replication with treatments replicated three times. Treatment means were compared using the least significant difference (LSD) test at p≤0.05.

RESULTS

Percentage sprouting

Minitubers treated with calcium carbide (3 g kg⁻¹) and MAP sprouted the fastest with the highest sprouting percentage (Figure 1A). As early as 3 weeks, at opening of the film bags, both treatments exhibited sprouting at 50% or more. Minitubers treated with 5 g kg⁻¹ calcium carbide attained 50% sprouting at 5 weeks. This was followed three weeks later by minitubers treated with 10,000 μL L⁻¹ ethephon. It took longer at 9 weeks after treatment (WAT) for those treated with 5,000 μL L⁻¹ ethephon and 5 μL L⁻¹ GA₃, while it took 11 WAT for the two 1-MCP treatments and control to register ≥50% sprouting. Minitubers with 1 μL L⁻¹ GA₃ registered ≥50% sprouting at 10 weeks. The minitubers registered 60 to 77% sprouting at 12 WAT.

Sprout characteristics

At 12 WAT, the number of sprouts per minituber ranged from 0.8 to 2.9. Calcium carbide at 3 g kg⁻¹ resulted in more sprouts per minituber (2.9). This was followed by calcium carbide at 5 g kg⁻¹ (2.1), which did not vary from the passive MAP treatment (2.0) (Figure 1B). The control, along with the minitubers treated with GA₃, 1-MCP and ethephon, had a similar number of sprouts ranging from 0.8 to 1.4.

Among treatments, calcium carbide (3 g kg⁻¹) also exhibited the longest apical sprout of 2.5 mm at 4 weeks and averaged two and half times the length of the control at 12 WAT (3.5 mm, the control being 1.4 mm; Figure 2). At 4 weeks, the second longest sprout (1.7 mm) was in the calcium carbide (5 g kg⁻¹) treatment. Sprout weight did not vary among the



treatments (data not shown).

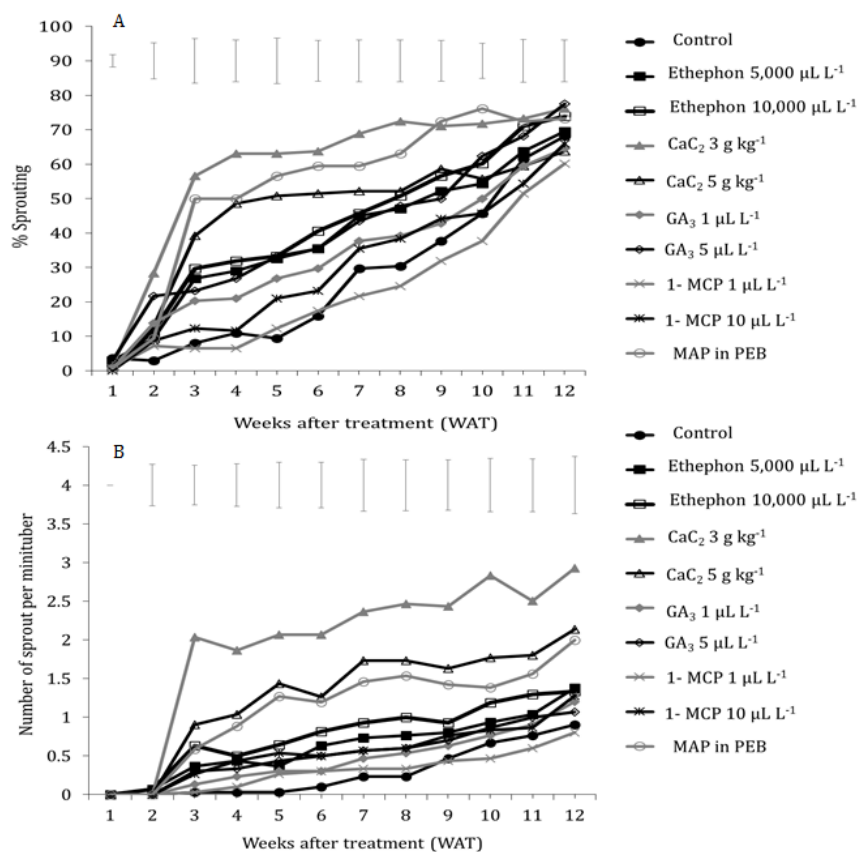


Figure 1. Percentage of sprouting (A) and sprout number minituber (B) as influenced by various treatments to break dormancy. Per evaluation period, vertical bars indicate LSD value at 5% level of significance.

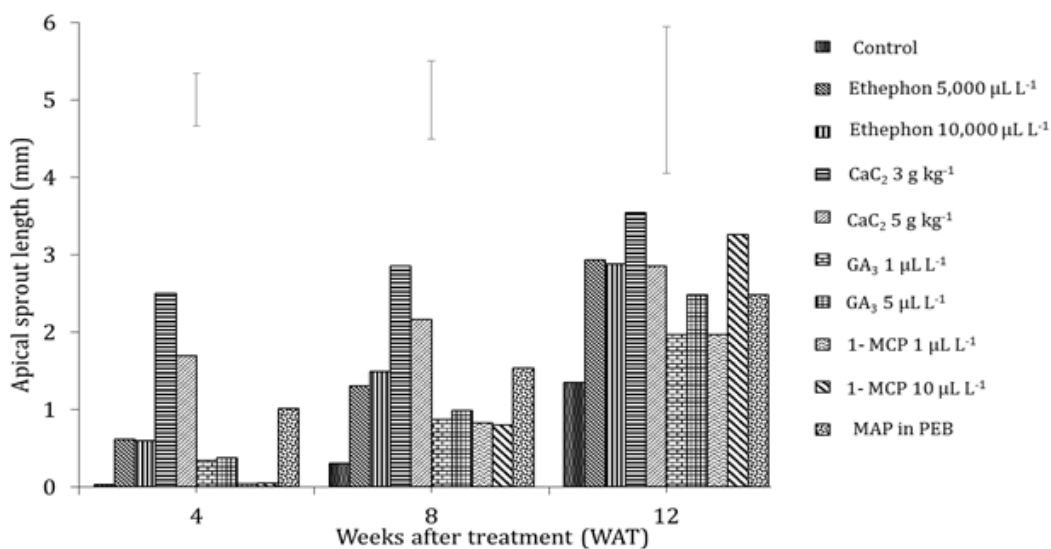


Figure 2. Apical sprout length in minitubers as influenced by various treatments to break dormancy. Per evaluation period, vertical bars indicate LSD value at 5% level of significance.

Weight loss, shriveling, and decay

Weight loss and shriveling of minitubers consistently increased from 4 to 12 WAT (Figures 3), with higher weight loss observed in calcium carbide (3 g kg^{-1}) at the end of the evaluation period. Minitubers treated with calcium carbide (3 g kg^{-1}) and MAP were more shriveled compared to other treatments (Figure 3B). Less shriveled samples were observed in the lower GA_3 and control lots.

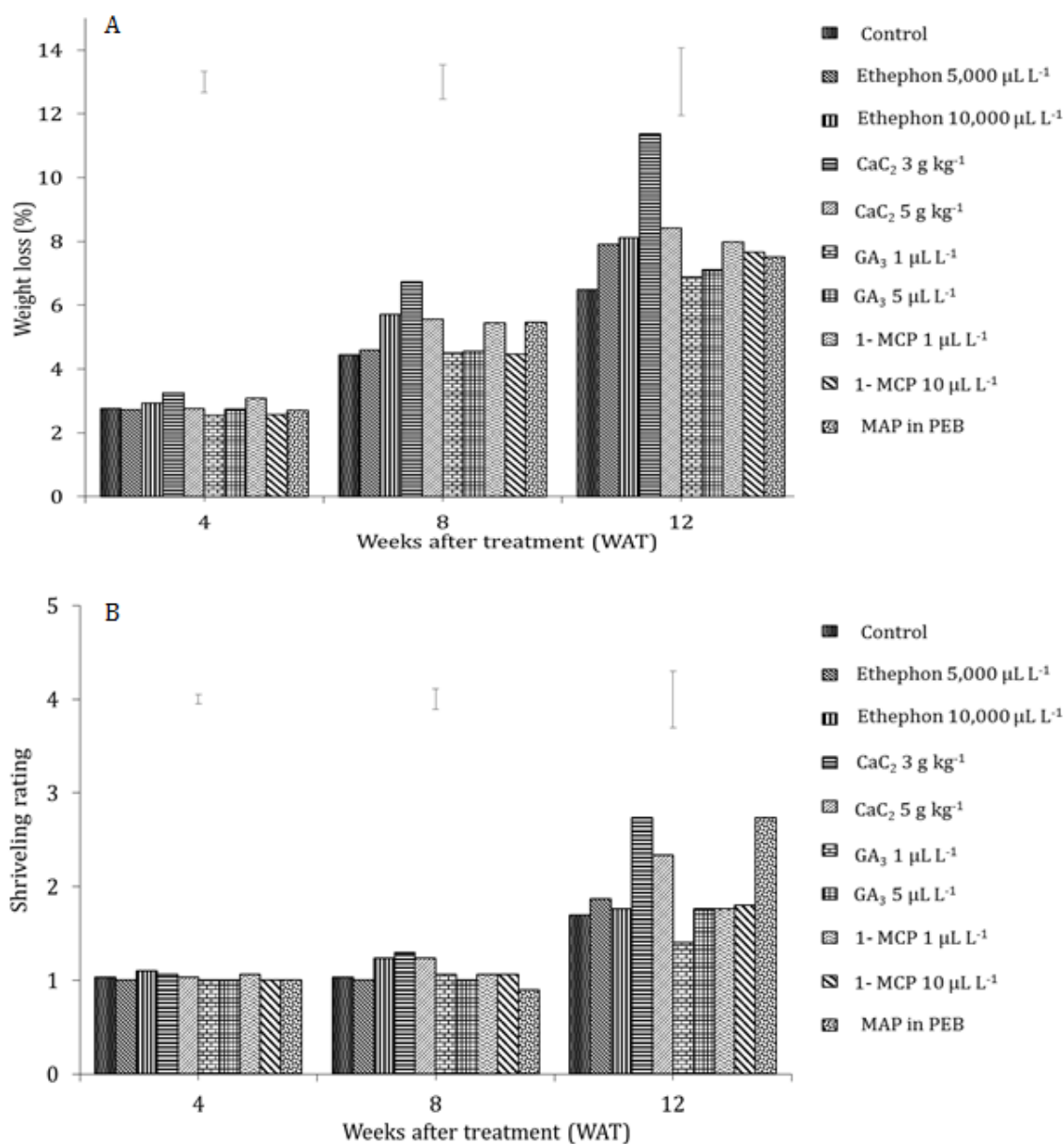


Figure 3. Weight loss (A) and shriveling (B) in minitubers as influenced by various treatments to break dormancy. Per evaluation period, vertical bars indicate LSD value at 5% level of significance.

A high incidence of decay was observed in treatments in the film bags as shown in Figure 4A (MAP at 10.1%, 3 g kg^{-1} calcium carbide at 8.7% and 5 g kg^{-1} calcium carbide at 9.4%). Figure 4B shows the same trend of degree of decay.

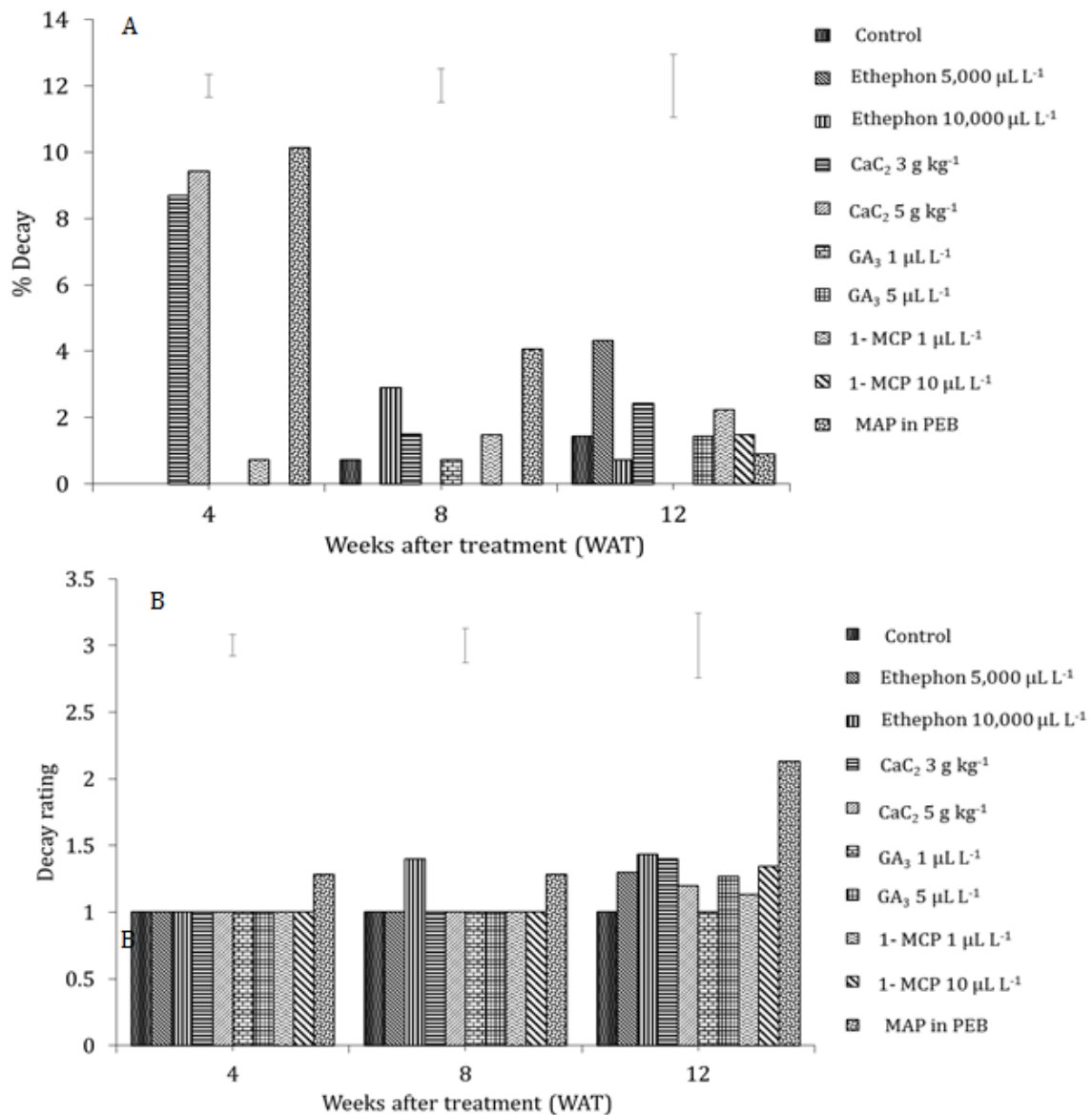


Figure 4. Percentage of decay (A) and degree of decay (B) in minitubers as influenced by various treatments to break dormancy. Per evaluation period, vertical bars indicate LSD value at 5% level of significance.

DISCUSSION

At three weeks, upon opening of the PEB, minitubers treated with 3 g kg^{-1} calcium carbide (CaC_2) already had 56.5% sprouting compared to 5 g kg^{-1} CaC_2 with 50.4% at 5 weeks. The time to reach 50% sprouting was delayed to 8 and 9 weeks for 10,000 $\mu\text{L L}^{-1}$ (50.7%) and 5,000 $\mu\text{L L}^{-1}$ (52.2%) ethephon treatments, respectively. The effect of acetylene gas produced in the reaction of calcium carbide and water was a higher percentage of sprouting of the minitubers. The MAP treatment followed very closely in terms of sprouting. In an earlier report in seed tubers, Bayogan et al. (1988) reported that calcium carbide (3 g kg^{-1}) treatment of 'Granola' potatoes resulted in a high sprouting percentage.

Acetylene is generated from calcium carbide. As a gas, it is less effective than ethylene because its postharvest effectivity is 100 times less than that of ethylene (Bautista and Esguerra, 2007). Ethephon allows the release of ethylene gas within the plant tissue because

cell sap has a pH of about 5 (Bautista and Esguerra, 2007). In aqueous solution, ethephon exists as an equilibrium between monoanionic and dianionic forms, the latter form undergoes decomposition releasing ethylene (Zhang and Wen, 2010). However, Zhang et al. (2010) indicated that the delayed decomposition reaction of ethephon and its uncontrollable conversion are matters that can affect its ability to mimic the effects of ethylene. The dual action of ethylene on dormancy break of potato was reported by Rylski et al. (1974). Ethylene significantly shortens dormancy duration but inhibits sprout elongation at extended treatment. In this study, the effect of ethephon on sprouting at three weeks was less than 30% with shorter apical growth length. This was not comparable with the effect of acetylene because calcium carbide hastened the sprouting in potato with longer apical growth length.

Heat produced during calcium carbide degradation could also have contributed in dormancy break. Heat treatments (18 to 25°C) could induce sprouting, especially when tubers are kept in dark conditions (Booth and Shaw, 1981). Moreover, Menza et al. (2008) observed high temperature can hasten sprouting in potato placed in pits. Slightly high temperature and high relative humidity coupled with slightly elevated CO₂ during the calcium carbide (in PEB) treatment could have contributed to the break of dormancy in the minitubers. Aksenova et al. (2013) reported that higher temperature up to 30°C and humidity up to 90%, as well as high CO₂, induce dormancy break and premature bud growth. High CO₂ causes acidification that loosens the cell wall, leading to an increase in the soluble sugars needed for sprout growth (Coleman, 1998).

Calcium carbide at 3 g kg⁻¹ enhanced sprout growth with more and longer sprouts per minituber than the 5 g kg⁻¹ CaC₂ treatment. The sprout weights at 12 WAT (data not shown) were however similar to those of other treatments because, although there were many sprouts, they were rather small ranging from 1.4 to 3.5 mm. In potato microtuber dormancy, ethylene action was limited only during the initial period of endodormancy development as exogenous ethylene inhibited precocious sprouting (Suttle, 1998). Ethylene, together with abscissic acid (ABA), is reported to induce dormancy break but bud dormancy was only maintained by ABA (Suttle, 2004). Ethylene concentrations from <0.005 to 10 µL L⁻¹ accelerated the breaking of dormancy and increased the number of sprouts per tuber, but reduced sprout length on 'Sebago' (Wills et al., 2004). In this study, the breaking of dormancy was more distinctly observed from the acetylene produced by the calcium carbide but not by ethephon. The uncontrollable conversion of ethylene and delayed ethylene release characteristics of ethephon as indicated by Zhang et al. (2010) may have contributed to its lesser efficacy in breaking the dormancy of the minitubers.

The high number of sprouts per tuber (3.5 sprouts for CaC₂ and 2 sprouts for MAP) and longer apical sprouts (3.5 mm for CaC₂ and 2.5 mm for MAP) have contributed to the high water loss and consequently higher weight losses. Minitubers treated with calcium carbide (3 and 5 g kg⁻¹) and MAP were more shriveled compared to other treatments for the same reason. Sprouts on a minituber compete for nutrients and are prone to water loss due to their permeability to water vapor, shriveling the tubers (Mani et al., 2014; Fernie and Willmitzer, 2001).

Minitubers in MAP showed similar responses as calcium carbide by minimizing the number of days to sprouting with a high sprouting percentage. Both treatments have microenvironments with higher relative humidity and CO₂ compared to other treatments. However, both treatments also showed higher decay ratings. Calcium carbide registered similar decay incidence, despite the presence of the paper used to wrap the chemical inside the PEB during treatment that could have absorbed some moisture from the produce. The bags containing the minitubers were held in ambient conditions, thus some fluctuation in temperature inside the PEB may have also contributed to the decay incidence (Kader, 1986). Moisture condensation on the minituber due to high RH is a favorable environment for pathogen growth.

Gibberellic acid has been reported to cause hydrolysis of starch, thus increasing soluble sugars and consequently hastening tuber sprouting (Barani et al., 2013; Hassani et al., 2014). However, in this study, dormancy break was not shortened by GA₃, as treated

minitubers reached 50% sprouting after 9 and 10 weeks of storage compared to 3 weeks in minitubers treated with 3 g kg⁻¹ calcium carbide. Lesser and shorter sprouts were also observed in GA₃ treatments leading to lower weight loss and shriveling. Dormancy break was not hastened by GA₃, probably because endogenous GA has no role in dormancy release, but only in sprout growth (Suttle, 2004). In this study, after 12 weeks, sprouting percentages were similar in GA₃ (77.54%) and 3 g kg⁻¹ calcium carbide (76.09%) treatments. Calcium carbide conferred the advantages of earlier sprouting and better sprout growth, as indicated by more sprouts with greater length.

The control samples slowly sprouted with over 50% of the minitubers sprouting before 11 weeks – the time in which minitubers may have reached the physiological age for dormancy break (Bethke, 2002). As an ethylene antagonist, 1-MCP can bind to ethylene receptors to inhibit sprouting. Plants can lessen their sensitivity to ethylene by modifying their ethylene receptor genes (Foukaraki et al., 2012). Minitubers treated with 1-MCP at 1 and 10 µL L⁻¹ reached ≥50% sprouting at 11 weeks. At this time, new ethylene receptors may have been formed thereby allowing sprouting to take place. Likewise, application of 1 µL L⁻¹ 1-MCP for 24 h did not affect the sprout number in potato tubers but it suppressed the action of ethylene in sugar accumulation (Foukaraki et al., 2012).

Overall, breaking of dormancy in minitubers was slow. Even at 12 weeks, the maximum percentage of sprouting was only 77.5% in 5 µL L⁻¹ GA₃, followed by 3 g kg⁻¹ calcium carbide at 76.1%. Some minitubers were unsprouted at 12 weeks. This was also compounded with the incidence of decay. Compared to farmer's seed tubers, minitubers are reported to exhibit a long dormancy period, which was seen in the 'Granola' minitubers used in this study. Hosseini et al. (2011) reported that it took more time, approximately 75 to 78 days, for untreated potato minitubers to obtain 80% sprouting compared to thiourea-treated minitubers. At 12 WAT, % sprouting among treatments ranging from 60 to 77% did not vary. After the rapid sprouting shown by calcium carbide- and MAP-treated minitubers, the treatments also sustained better sprout growth. It is possible that freshly harvested minitubers were more responsive than those treated one week after harvest (Hosseini et al., 2011). In this study, samples used were one week old minitubers which were cured for another week before treatment.

CONCLUSION

This study aimed to break dormancy of potato minitubers with early sprouting and uniform sprout growth. 'Granola' potato minitubers were subjected to various treatments to break dormancy, such as calcium carbide plus MAP, ethephon, gibberellic acid (GA₃), 1-methylcyclopropene (1-MCP) at different concentrations and modified atmosphere packaging (MAP) in polyethylene bags (PEB). 70% sprouting was attained in 'Granola' minitubers at 10-12 weeks after treatment. As early as three weeks or at opening of the PEB, minitubers treated with 3 g kg⁻¹ calcium carbide and MAP had the highest sprouting percentage. This trend was consistent up to nine weeks of storage. It also gave the highest weight loss at 12 weeks. At 12 weeks, sprouting resulting from the different treatments ranged from 60 to 77%, indicating long dormancy in 'Granola' minitubers. At nine weeks, ethephon treatments and 5 µL L⁻¹ GA₃ exhibited more than 50% sprouting, but not the control, 1 µL L⁻¹ GA₃ and 1-MCP. Dormancy break with the use of calcium carbide (3 g kg⁻¹) resulted in most sprouts per minituber (2.9) and the longest apical sprouts (3.5 mm) relative to the control (0.90 and 1.4 mm, respectively). Sprout weights were similar across all treatments. However, CaC₂ and MAP treatments resulted in less than 10% minituber decay at four weeks. The use of calcium carbide can hasten dormancy break in minitubers potato, but methods are needed to sustain better sprout growth and reduce decay.

ACKNOWLEDGEMENTS

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Effects of Organic Acids on Quality of Freshcut Cucumber

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Keywords: *Cucumis sativus*, ascorbic acid, lactic acid, antimicrobials, postharvest quality

Abstract

Enhancing quality and food safety of freshcut products is a great challenge in the fresh produce industry as consumers are increasingly patronizing ready-to-eat fruits and vegetables. This study was conducted to evaluate the antimicrobial efficacy of ascorbic and lactic acids and evaluate the physicochemical and sensory qualities of cucumber slices. Ascorbic and lactic acids were applied at 1.5-2.5% as 2-min dip, with 150 ppm chlorine and distilled water as control treatments. The product samples were packed in sterile resealable 50 µm-thick polyethylene bag and stored at 7-10°C for five days. Both organic acids were ineffective against bacterial load but markedly reduced yeast and mold population, with 1.5% level being sufficient. In most cases, the antimicrobial activities of organic acids were either comparable to or better than that of chlorine. These antimicrobial agents had also no remarkable effects on the physicochemical and sensory qualities after five days of cold storage.

INTRODUCTION

Cucumber is usually eaten raw or uncooked and is subject to contamination by spoilage and pathogenic microorganisms if no antimicrobial treatment is employed. Use of natural antioxidants such as organic acids has been singled out as emerging technologies where research should focus in order to develop safer and sustainable antimicrobial systems (Mastromatteo et al., 2009; Olliu et al., 2010; Vandekinderen et al., 2009). Limited information is available on the use of organic acids as antimicrobial treatment for freshcut products in the Philippines. Recently, the use of 1.5% ascorbic acid in freshcut jackfruit was found effective as antimicrobial agent but the efficacy was not sustained during subsequent cold storage (Acedo et al., 2011). This study evaluated the efficacy of ascorbic and lactic acids in maintaining quality of freshcut cucumber.

MATERIALS AND METHODS

Commercially mature defect-free cucumber fruits were sourced locally and under sanitary conditions, were washed, peeled, and cut into 7 mm thick slices. About 200 g fruit slices were applied with L-ascorbic acid and lactic acid (Hanna Chemicals) at 1.5-2.5% as 2-min dip, with 150 ppm chlorine and distilled water as control. Three replicates were used per treatment. After the immersion treatment, excess solution was removed using a commercial salad spinner. Samples were packed in sterile resealable 50 µm-thick polyethylene bag and stored at 7-10°C for five days. Microbial analysis was performed after 3 h from treatment and after five days of storage using plate count agar (total aerobic bacteria), violet red bile agar (coliform) and potato dextrose agar (yeast and mold) following US-FDA (2003) methods; microbial counts were expressed as log colony-forming units (CFU/ml). Produce quality was evaluated in terms of color using a Minolta CR-13 colorimeter, total soluble solids (TSS) using an Atago N1 Hand Refractometer, pH using an Orion pH meter model 210 A+, titratable acidity (% citric acid) by titration using standard 0.1 N NaOH and 1% phenolphthalein indicator, and sensory quality (color, aroma, taste, texture, and aftertaste) using 15 trained panelists and 1-5 rating scale describing the variation in each attribute and 9-point Hedonic scale for acceptability of each attribute; the general acceptability was also evaluated using a 9-point Hedonic scale.

Statistical analysis of results used the CROPSTAT version 7.2 program of the International Rice Research Institute.

RESULTS AND DISCUSSION

Ascorbic and lactic acids were ineffective against bacterial load but effectively reduced yeast and mold population with 1.5% level being sufficient (Figs. 1 and 2). Lactic acid, as produced by LAB or as additive to foods, is recognized as a bio-preservative with GRAS (generally regarded as safe) status, and numerous applications for decontamination using lactic acid have been described (Alakomi et al., 2000). They acidify the cells interior by being lipophilic and penetrating the plasma membrane (Akbas et al., 2007); reduce internal cellular pH, and disrupt membrane transport and/or permeability (Barry-Ryan et al., 2007).

In most cases, the antimicrobial activities of organic acids were either comparable to or better than that of chlorine. These antimicrobial agents had also no remarkable adverse effects on the physicochemical and sensory qualities of freshcut cucumber after five days of cold storage (Table 1).

CONCLUSION

Ascorbic and lactic acids can be considered as possible alternatives to chlorine or water as antimicrobial agents to ensuring food safety in sync that these treatments have no remarkable adverse effects on the physicochemical and sensory qualities of freshcut cucumber after five days of cold storage.

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Tables

Table 1. Colorimetric b* values, total soluble solids (TSS), titratable acidity (% citric acid), pH and overall sensory acceptability of organic acid-treated and untreated freshcut cucumber before and after five days storage at 7-10°C.

Treatments	Color (b*)	TSS (B*)	TA (% citric acid)	pH	Overall sensory acceptability
AA 1.5%	12.3	1.9a	0.31	5.55b	7.3a
AA 2.5%	10.0	1.8a	0.32	5.35b	7.0a
LA 1.5%	10.6	1.8a	0.26	5.23b	5.4c
LA 2.5%	7.4	1.5ab	0.39	4.30c	5.8bc
Cl	5.9	1.1b	0.25	6.11a	6.3b
Water	7.7	1.6a	0.23	6.18a	5.6c
Initial	9.0	1.7a	0.28	5.33b	-

b* indicates green (lower values) or yellow (higher values). Treatment means with a common letter are not significantly different at 0.05 level LSD.

Figures

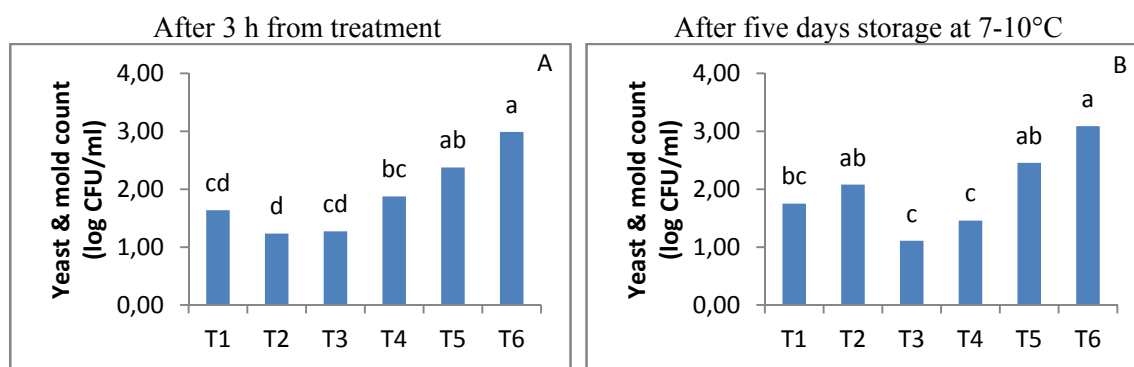


Fig. 1. Microbial counts on organic acid-treated and untreated freshcut cucumber. T1 – ascorbic acid 1.5%; T2 – ascorbic acid 2.5%; T3 – lactic acid 1.5%; T4 – lactic acid 2.5%; T5 – chlorine; T6 – water. Treatment means with same letter are not significantly different using LSD, 5%. % CV: A – 18.3; B – 32.6.

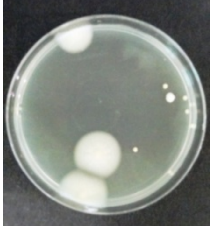
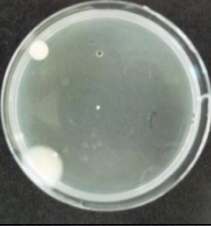
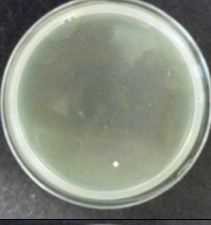



Ascorbic acid 1.5%	
Ascorbic acid 2.5%	
Lactic acid 1.5%	
Lactic acid 2.5%	
Chlorine	
Water	

Fig. 2. Yeast and mold colonies from cultured sample at 10^{-2} dilution, from organic acid-treated and untreated freshcut and cucumber after five days storage at 7-10°C.

Quality of Freshcut Tomato and Cucumber Treated with Probiotic Lactic Acid Bacteria

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Keywords: *Solanum lycopersicum*, *Cucumis sativus*, *Lactobacillus plantarum*, bioprotectant, postharvest quality

Abstract

Assuring food safety is vital to cater to consumer demand for quality and safe produce and improve the market competitiveness of fruit and vegetable industries. In an attempt to develop safe antimicrobial treatment, this study was conducted to optimize the application of probiotic *Lactobacillus plantarum* and evaluate the physicochemical and sensory qualities of tomato and cucumber slices. *Lb. plantarum* at 10^9 CFU/ml was applied at 1-3% (v/w) as protective culture. Control treatments included 150 ppm chlorine and distilled water as 2-min dip. The product samples were packed in sterile resealable 50 μ m-thick polyethylene bag and stored at 7-10°C for five days. *Lb. plantarum* inhibited coliform and aerobic bacteria and was consistently most effective at 3% on both tomato and cucumber. It also reduced yeast and mold population but only in tomato. In most cases, the antimicrobial activities of *Lb. plantarum* were either comparable to or better than that of chlorine. *Lb. plantarum* had no remarkable effects on the physicochemical and sensory qualities of the freshcut products after five days of cold storage.

INTRODUCTION

Lactic acid bacteria (LAB) are probiotics that could contain microbial contaminants of food products particularly in freshcut produce where microbial load and rates of deterioration are high (Makarova and Koonin, 2007). LAB carry out their reactions, i.e., conversion of carbohydrate to lactic acid, carbon dioxide, and other organic acids without the need for oxygen hence, they do not cause drastic changes in the composition of the food. *Lactobacillus acidophilus*, *Lb. bulgaricus*, and *Lb. plantarum* are examples of LAB routinely used as starter cultures in the manufacture of fruit, vegetable, meat, dairy, and bakery products (Abee et al., 1995). Probiotics are safe to humans, regulating the body's microenvironment to prevent dominance by harmful microorganisms. The use of *Lb. acidophilus* or *Lb. plantarum* (10^9 CFU/ml cell suspensions) as 1% (v/w) protective culture in freshcut jackfruit was explored (Acedo et al., 2011). This study optimized the efficacy of *Lb. plantarum* in maintaining quality of freshcut tomato and cucumber.

MATERIALS AND METHODS

Tomato fruits were washed and sliced circularly (7 mm thick) while cucumber fruits were washed and peeled before cutting into 7 mm thick slices. A small portion of pure culture of *Lb. plantarum* was aseptically transferred onto 50 ml of sterile Lactobacillus MRS broth to produce about 10^9 CFU/ml cell suspension. After 24 h of ordinary room incubation (26-33°C), product samples were applied with the cell suspension at 1, 2 or 3% (v/w) or 2, 4 or 6 ml per 200 g sample in sterile resealable 50 μ m-thick polyethylene bag and stored at 7-10°C for five days. Distilled water and 150 ppm chlorine served as control. Microbial analysis was performed after 3 h from treatment and after five days of storage using plate count agar (total aerobic bacteria), violet red bile agar (coliform) and potato dextrose agar (yeast and mold) following US-FDA (2003) methods; microbial counts were expressed as log colony-forming units

(CFU/ml). Produce quality was evaluated in terms of color using a Minolta CR-13 colorimeter, total soluble solids (TSS) using an Atago N1 Hand Refractometer, pH using an Orion pH meter model 210 A+, titratable acidity (% citric acid) by titration using standard 0.1 N NaOH and 1% phenolphthalein indicator, and sensory quality (color, aroma, taste, texture, and aftertaste) using 15 trained panelists and 1-5 rating scale describing the variation in each attribute and 9-point Hedonic scale for acceptability of each attribute; the general acceptability was also evaluated using a 9-point Hedonic scale. Statistical analysis of results was performed using the CROPSTAT version 7.2 program of the International Rice Research Institute.

RESULTS AND DISCUSSION

Effects of Probiotics LAB on Microbial Load

Lb. plantarum inhibited coliform and aerobic bacteria on both tomato and cucumber and was consistently most effective at 3%. It also reduced yeast and mold counts on tomato but not on cucumber (Figs. 1 and 2). It has been reported that *Lb. plantarum* is a Gram-positive aero tolerant bacterium that produces both isomers of lactic acid (D and L) and has the capacity to lower the pH in the surrounding media, thereby creating a hostile environment for the undesirable microflora. This species and related lactobacilli are unusual because they can respire with oxygen but have no respiratory chain or cytochromes. The consumed oxygen ultimately ends up as hydrogen peroxide which acts as a weapon to exclude competing bacteria on the food source. Furthermore, *Lb. plantarum* is commonly found in many fermented food products and when directly added to food, shelf life could be enhanced by competing with other microflora (both bacterial and fungal) for food and by producing antimicrobial metabolites such as lactic acid, hydrogen peroxide, and bacteriocins. The high levels of LAB in food make it an ideal candidate for the development of probiotics for human health promotion.

Physicochemical and Sensory Quality

In most cases, the antimicrobial activities of *Lb. plantarum* were either comparable to or better than that of chlorine. These antimicrobial agents had also no remarkable adverse effects on the physicochemical and sensory qualities of freshcut tomato and cucumber after five days of cold storage (Tables 1 and 2).

CONCLUSIONS

Lb. plantarum at 3% minimized microbial population on both freshcut tomato and cucumber. Probiotic treatments had no dramatic effects on the physicochemical and sensory qualities of freshcut tomato and cucumber. Hence, these treatments can be considered as possible alternatives to chlorine or water as antimicrobial agents to ensuring food safety in sync that these treatments have no remarkable adverse effects on the physicochemical and sensory qualities of freshcut tomato and cucumber after five days of cold storage.

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Tables

Table 1. Colorimetric b* values, total soluble solids (TSS), titratable acidity (% citric acid), pH and overall sensory acceptability of probiotic-treated and untreated freshcut tomato before and after five days storage at 7-10°C.

Treatments	Color (b*)	TSS (B*)	TA (% citric acid)	pH	Overall sensory acceptability
<i>Lb.</i> 1%	4.5	1.3	0.23	4.82	6.2
<i>Lb.</i> 2%	1.2	1.5	0.20	4.75	6.1
<i>Lb.</i> 3%	7.7	1.4	0.19	4.89	6.1
Cl	5.2	1.1	0.19	4.83	5.8
Water	4.2	1.3	0.22	4.79	5.7
Initial	0.1	1.3	0.22	4.79	-

Table 2. Colorimetric b* values, total soluble solids (TSS), titratable acidity (% citric acid), pH and overall sensory acceptability of probiotic-treated and untreated freshcut cucumber before and after five days storage at 7-10°C.

Treatments	Color (b*)	TSS (B*)	TA (% citric acid)	pH	Overall sensory acceptability
<i>Lb.</i> 1%	12.0ab	2.4	0.08	6.32bc	6.6a
<i>Lb.</i> 2%	11.0bc	2.1	0.07	6.55a	6.5a
<i>Lb.</i> 3%	13.4a	1.9	0.09	6.48ab	6.5a
Cl	10.7bc	1.8	0.05	6.31bc	5.6b
Water	9.4c	1.8	0.09	6.18c	4.9c
Initial	12.8ab	1.5	0.09	6.14c	-

b* indicates green (lower values) or yellow (higher values). Treatment means with a common letter are not significantly different at 0.05 level LSD.

Figures

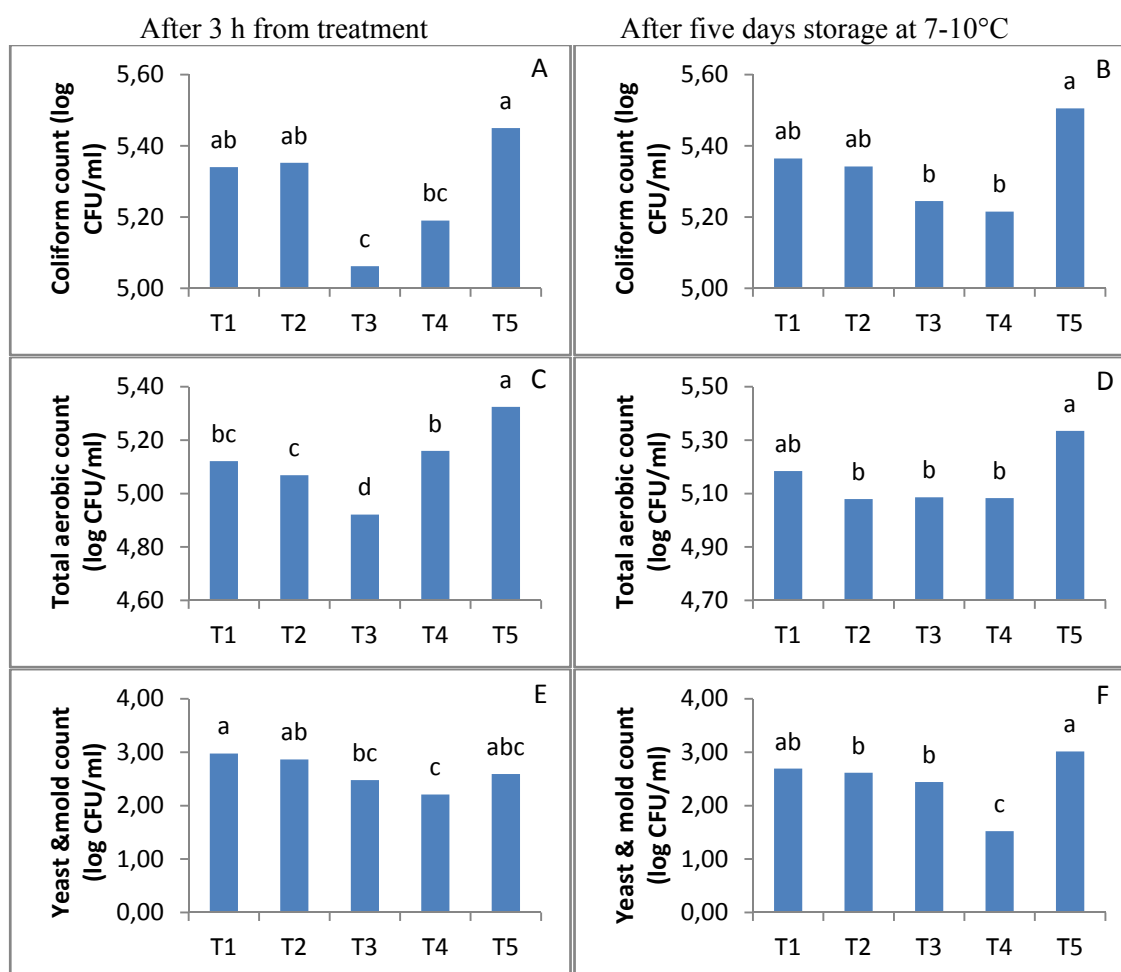


Fig. 1. Microbial counts on probiotic-treated and untreated freshcut tomato. T1 – *Lb. plantarum* 1%; T2 – *Lb. plantarum* 2%; T3 – *Lb. plantarum* 3%; T4 – chlorine; T5 – water. Treatment means with same letter are not significantly different using LSD, 5%. % CV: A – 2.5; B – 1.8; C – 0.8; D – 1.8; E – 9.3; F – 8.6.

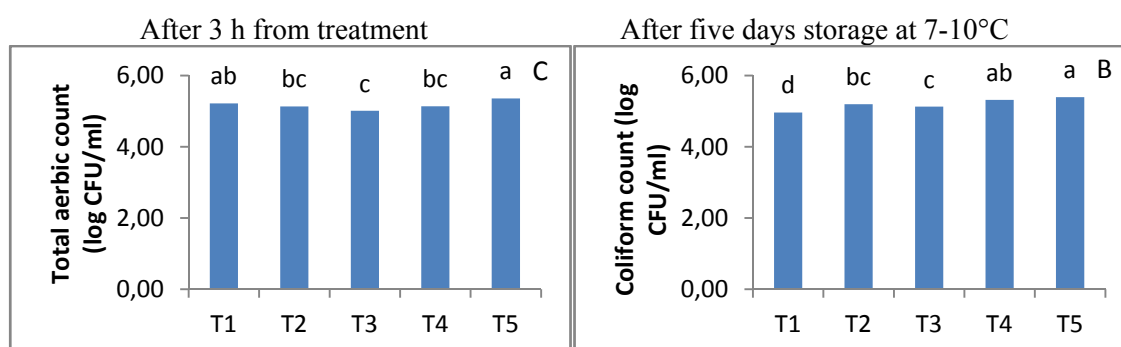


Fig. 2. Microbial counts on probioticsalt-treated and untreated freshcut cucumber. T1 – *Lb. plantarum* 1%; T2 – *Lb. plantarum* 2%; T3 – *Lb. plantarum* 3%; T4 – chlorine; T5 – water. Treatment means with same letter are not significantly different using LSD, 5%. % CV: B – 1.7; C – 1.7.

Bitter gourd (*Momordica charantia*) quality grading at two storage temperatures

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Abstract

This study characterized the fruit quality attributes of commercially mature bitter gourd (*Momordica charantia* L. 'Galaxy F1') from harvest to end of shelf life during storage at 7-10 and 21-25°C to develop quality grading standards for research, education and industry use. Quality at harvest was mainly determined by shape, size, uniformity of green surface color, and absence of defects that included yellowing (overmaturity) and preharvest damage due to insect pests, diseases and mechanical injury. During postharvest storage, loss in quality was due to yellowing, water loss that resulted in turgidity loss measured as weight loss and degree of shrivelling and softening, and/or soft rot. These quality changes occurred more rapidly at 21-25°C resulting in much shorter shelf life than at 7-10°C. Overall, the quality attributes were characterized using a scale for visual quality which was evaluated by a consumer panel. The results provide valuable information for establishing a system of grade standardization and classification along the production-marketing continuum.

Keywords: quality standards, grade classification, temperature effects

INTRODUCTION

Bitter gourd (*Momordica charantia* L.) is a member of the *Cucurbitaceae* family and is also called, depending on the country where it is cultivated, bitter melon, African cucumber, karela, carille, art pumpkin, balsam pear, maiden apple or koe (Mohammed, 2010). Perhaps better known for the use of the leaf, root and fruit for medicinal uses, the commodity has great potential as a food source in both developing and industrialized countries and is rich in iron, phosphorus and ascorbic acid. The fruit is intensely bitter, green and warty in appearance, and is highly fragile and perishable (Mohammed, 2010). The perishable nature of bitter gourd to a great extent leads to mixed variability in quality which doesn't coincide with consumer requirement. This study evaluated the fruit quality attributes of commercially mature bitter gourd (*Momordica charantia* L. 'Galaxy F1') from harvest to end of shelf life during storage at 7-10 and 21-25°C to develop grading scale for research, education and industry use.

MATERIAL AND METHODS

Bitter gourd (*Momordica charantia* L. 'Galaxy F1') at commercial maturity stage was harvested at a commercial farm at Brgy. Cabintan, Ormoc City. Right after harvest, the fruits were sorted for shape, size, uniformity of green surface color, and absence of defects that included yellowing (over maturity) and preharvest damage due to insect pests, diseases and mechanical injury. Each fruit was individually wrapped with soft paper and packed in cartons before shipment to Postharvest Technology Laboratory, Department of Horticulture, VSU, and were taken out from the box and allowed to equilibrate at ambient for about 2 h before the application of the storage treatments. Ten fruits were used as samples per replicate or a total of 30 fruits per treatment. The samples were then placed in plastic trays (40×30 cm) each representing a replicate. All samples were stored at 7-10°C using a refrigerator compartment to simulate prolonged refrigerated holding, and ambient room temperature at 21-25°C. Photomicrographs were taken daily for firmness/softening,



shriveling, and visual quality rating grade standardization. Sample fruits were also evaluated in terms of weight using a digital weighing scale. Statistical analysis of results was performed using the STAR, version 2.0.1 (2014) program of the International Rice Research Institute.

RESULTS AND DISCUSSIONS

Quality at harvest was mainly determined by shape, size, uniformity of green surface color, and absence of defects that included yellowing (overmaturity) and preharvest damage due to insect pests, diseases and mechanical injury factoring in producer, wholesaler, retailer and consumer quality requirements (Table 1). During postharvest storage, loss in quality was due to yellowing, water loss that resulted in turgidity loss measured as weight loss and degree of shriveling and softening, and/or soft rot. These quality changes occurred more rapidly at 21-25°C resulting in much shorter shelf life than at 7-10°C (Figure 1). Zong et al. (1995) stated that fruit quality was best maintained if bitter melon/gourd were stored at 10 and 12.5°C. Fruits stored above 10 and 12.5°C continued to develop, showing undesirable changes including seed development, loss of green color, and fruit splitting. Similar results were obtained from the study (Figure 1). Overall, the quality attributes were characterized using a developed scale or rating standard for visual quality which was evaluated by a consumer panel (Figures 2-3).

Table 1. Bitter gourd grades. Source: Mohammed (2010).

	Grade 1 ¹	Grade 2 ¹	Rejects ¹
a. Similar varietal characteristic, well-colored, keeping quality and presentation in the package	Excellent	Good	Fair to poor
Whole, fresh, firm, clean, well-trimmed, well-formed	95-100%	80-94%	Below 80%
b. Damage ² , over-mature, badly misshapen	Absent to slight	Slight to moderate	Moderate to extensive
c. Decay (bacterial soft rot), sunburn	(0-1%)	1-20%	>21%
Size classification: size was determined by length of ampalaya (cm):			
a. Extra-small: 3-9 cm			
b. Small: 10-17 cm			
c. Medium: 18-25 cm			
d. Large: 26-32 cm			
e. Extra-large: >32 cm			
Quality specification or definition:			
a. Similar varietal characteristic: the fruits in any container are similar in shape and color of skin and flesh.			
b. Firm: not soft, flabby or shriveled.			
c. Well formed: fairly typical shape of the cultivar, fairly symmetrical, not excessively tapered, and slightly curved. An ampalaya is slightly curved when the maximum distance obtainable between the concave side and the flat surface of the ampalaya is more than 1/6 in length.			
d. Clean: practically free from staining, dirt, or other foreign material.			
e. Damage: any defect which materially detracts from the appearance of the edible or shipping of the fruit.			
1. Sunburn: when causing flattening of the fruit, or drying out of the skin or flesh, or when affecting more than 10% of the surface of the fruit.			
2. Insect damage: scars, wormholes and other insect infestations which affect more than 5% of the surface area.			
3. Mechanical damage: bruises or scars, cuts, and other damages caused by improper handling.			
f. Length: the distance in a straight line from the stem to the blossom-end excluding the portion of the ampalaya that is less than 5 mm in diameter.			
g. Badly misshapen: is twisted or curved.			
h. Well-colored: for green colored ampalaya, half of the ampalaya surface is medium green or darker in color. For white ampalaya, the color is fairly typical of the cultivar.			
i. Over mature: characterized with traces of yellow color, soft fleshy wall, the seed cavity turns red and not suitable for human consumption.			
j. Decay: bacterial soft rot is the most serious decay encountered in cabbage characterized by a foul odor in advance stage.			

¹Percentage in the lot sampled.

²Damage caused by disease, insect and mechanical or other means: absent, 0%; slight, 1-20%; moderate, 21-40%; extensive, >40% area affected.

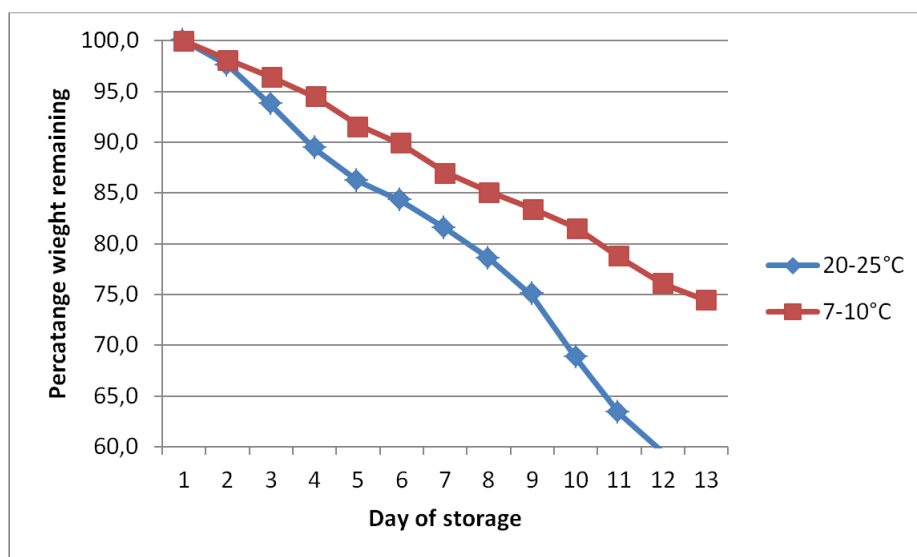


Figure 1. The average percentage weight remaining on a daily basis of 'Galaxy F1' cultivar placed in refrigerated storage (7-10°C) and ambient storage (20-25°C). Means are significantly different using t-test, 5%.

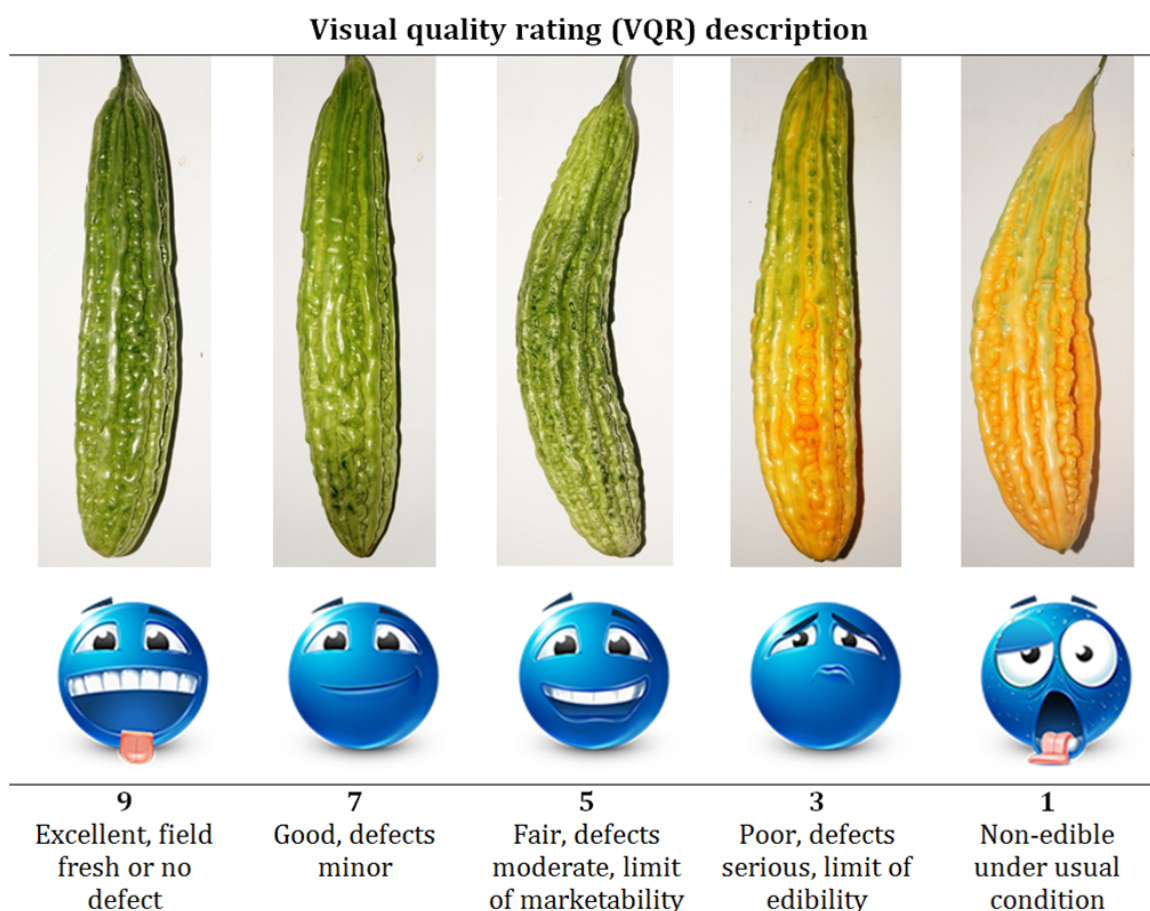


Figure 2. A rating standard developed for the visual quality rating (VQR) of 'Galaxy F1' cultivar.

Yellowing index (YI) and firmness index (FI)



Figure 3. A rating standard developed for the yellowing index (YI) and firmness index (FI) of 'Galaxy F1' cultivar.

CONCLUSION

Cold storage under 7-10°C reduced quality changes in bitter melon as compared to samples stored under ambient condition. The results provide valuable information for establishing a system of grade standardization and classification along the production-marketing continuum.

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Cabbage (*Brassica oleraceae* var. *capitata*) quality grading at two storage temperatures

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Abstract

Common cabbage (*Brassica oleraceae* var. *capitata*) is the leading leafy vegetable in the Philippines. Quality grading is vital to establish a system of grade standardization and classification that can be used as research, education and industry standards. This study evaluated the quality attributes of head cabbage at commercial mature stage and their changes during storage at 7-10 and 21-25°C. Quality at harvest was mainly determined by head solidity, size, weight, and absence of defects that included yellowing of outer leaves, insect damage particularly by the Diamond back moth, bacterial soft rot, immaturity (puffy heads) and mechanical injury. During postharvest storage, loss in quality was due to yellowing and wilting of outer leaves and bacterial soft rot that usually started at the cut butt end, which necessitated trimming of affected parts. Trimming loss and weight loss increased with storage at a faster rate at 21-25°C resulting in much shorter shelf life than at 7-10°C. Yellowing and wilting of outer leaves limited the shelf at both storage temperatures; additionally at 21-25°C, bacterial soft rot contributed to quality deterioration.

Keywords: quality standards, grade classification, temperature effects

INTRODUCTION

Cabbage's 1st quarter's production was 23.42 MT, 5.0% higher than the 2013 level of 22.29 MT (<http://www.bas.gov.ph/?ids=vegetablenessituation>). It's the fifth leading vegetable and the number one leafy with production of 125,309.48 metric t from an area of 8,549.63 ha (BAS, 2013). Cabbage is highly seasonal with an oversupply during production peaks and undersupply during lean season resulting in highly fluctuating prices aside from varying wholesaler, retailer and consumer requirements. Consumer preference which is a determining factor on cabbage quality must be integrated in standards or grades. These quality requirements are market information that should be effectively and efficiently transmitted from downstream to upstream industries. In short, requirements of ultimate consumers of vegetables should be known to farmers and all the players in the marketing system so that production can accurately respond to market signals (Digal and Hualda, 2003). Recently, the formulation of the PNS/BAFPS 17:2005, Philippine National Standard for Vegetables – Cabbage (*Brassica oleracea* var. *capitata* L.) – Grading and Classification, was initially drafted by the Bureau of Agriculture and Fisheries Product Standards (BAFPS). This study evaluated the quality attributes of head cabbage at commercial mature stage and their changes during storage at 7-10 and 21-25°C and developed a local grade standardization and classification that could serve as a common ground for determining and harmonizing market player and consumer preferences.

MATERIAL AND METHODS

Common cabbage (*Brassica oleraceae* var. *capitata*) at commercial maturity stage was harvested at a commercial farm at Brgy. Cabintan, Ormoc City, Leyte, Philippines. Right after harvest, the fruits were sorted for solidity, size, weight and absence of defects. Each produce was individually wrapped with soft paper and packed in cartons before shipment to Postharvest Technology Laboratory, Department of Horticulture, VSU, and were taken out



from the box and allowed to equilibrate at ambient for about 2 h before the application of the storage treatments. 10 cabbages were used as samples per replicate or a total of 30 fruits per treatment. The samples were then placed in plastic trays (40×30 cm) each representing a replicate. All samples were stored at 7-10°C using a refrigerator compartment to simulate prolonged refrigerated holding, and ambient room temperature at 21-25°C. Photomicrographs were taken daily for the development of firmness/softening, shriveling, and visual quality rating grade standardization. Sample fruits were evaluated in terms of weight using a digital weighing scale. Statistical analysis of results was performed using the STAR program, version 2.0.1 of the International Rice Research Institute (STAR, 2014).

RESULTS AND DISCUSSION

Quality at harvest was mainly determined by head solidity, size, weight, and absence of defects that included yellowing of outer leaves, insect damage particularly by the Diamond back moth, bacterial soft rot, immaturity (puffy heads) and mechanical injury (Table 1) (Gunter and Campbell, 2008). Cabbages were also immediately moved into a temporary shelter. Field heat should be removed as quickly as possible because an increase in temperature triggers an increase in physiological activity within the commodity (Bautista and Esguerra, 2007). Storing cabbage correctly is important to preserving its quality. Proper storage methods will help to slow down the respiration of the cabbage. This is important because the faster the cabbage respire, the quicker the cells metabolize and the cells' metabolic processes begin to break down, and the sooner the vegetable begins to spoil. Samples stored under 7-10°C showed a slower change in quality attributes (weight loss, yellowing and wilting of leaves) compared to samples stored under ambient room temperature at 21-25°C (Figure 1). Yellowing and wilting contributed to the decrease in quality of cabbage. Leaf yellowing was estimated from the total yellow area on each leaf surface using a scoring index of 1 (full green) to 5 (extensive yellowing) (Figure 2). When leaves reached a score >3 in the visual quality rating scale, they were considered unmarketable (Figure 3). Bacterial soft was also observed to develop faster at 21-25°C storage temperature which also significantly reduced the overall quality of the cabbage samples (Figure 4) (Cantwell and Suslow, 2013).

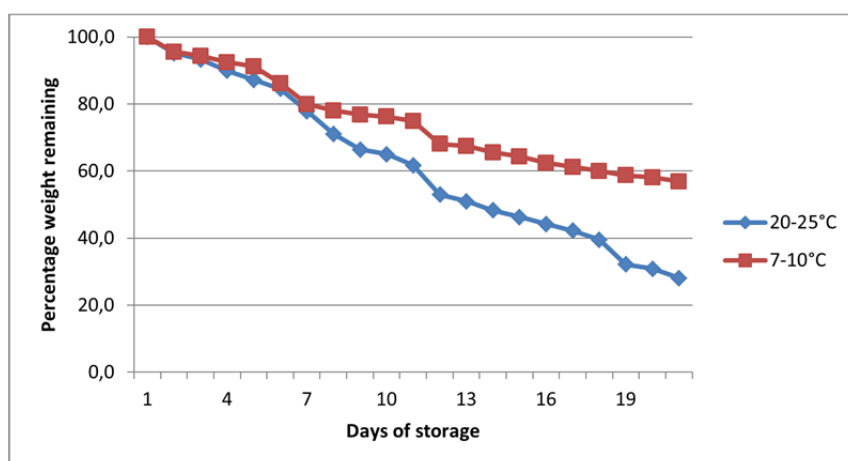


Figure 1. The average percentage weight remaining on a daily basis of cabbage placed in refrigerated storage (7-10°C) and ambient storage (20-25°C). Means are significantly different using t-test, 5%.

Table 1. Cabbage grades. Source: Philippine National Standards (2005) and Gunter and Campbell (2008).

	Grade 1 ¹	Grade 2 ¹	Rejects ¹
a. Appearance	Excellent	Good	Fair to poor
Fresh, firm, clean, well-trimmed	95-100%	80-94%	Below 80%
b. Damage ²	Absent to slight (0-1%)	Slight to moderate (1-20%)	Moderate to extensive (>21%)
c. Bacterial soft rot	Below 5%	5-10%	Above 10%
Size classification			
a. Small: below 0.5 kg			
b. Medium: 0.5-1 kg			
c. Large: over 1-1.8 kg			
d. Extra-large: over 1.8 kg			
Quality specifications/definitions			
a. Fresh and new harvested			
b. Firm: the head is closely formed and does not yield readily to pressure			
c. Clean: reasonably free from stain, dirt or other foreign material			
d. Well-trimmed: the head should have four wrapper leaves and the stem does not extend more than 1.5 cm beyond the point of attachment of the outermost leaf.			
e. Good head formation: the various section of the head must show about the same degree of firmness and the outer leaves must reach at least 2.5 to the top of the head			
f. It must be free from the following defects:			
1. Decay: bacterial soft rot is the most serious decay encountered in cabbage characterized by a foul odor in advance stage.			
2. Puffy head: the head is very light in proportion to size, due to presence of fairly larger spaces in the inner portion of the head causing it to yield readily to moderate pressure			
3. Excessively trimmed: the outer head leaves have been removed exposing the tender inner leaves			
4. Worm injury: slight worm injury maybe ignored. Any worm hole penetrating into the head to a depth more than 1.5 cm should be scored against the grade			
5. Mechanical damage: torn leaves, bruises and other physical damage caused by careless handling or poor harvesting			
6. Burst head: those which have cracked open, usually at the crown and sometimes at the base			
7. Tip burn: the leaf margin of the outer and inner head leaves has turned yellow or brown			
8. Insect injury: caused by thrips or aphids.			
9. Black leafspot: small sharply sunken brown or black specks affecting leaves			

¹Percentage in the lot sampled.

²Damage caused by worm, insect, black leafspot, tip burn: absent, 0%; slight, 1-20%; moderate, 21-40%; extensive, >40% area affected.

Leaf yellowing index (YI) and leaf wilting index (WI)

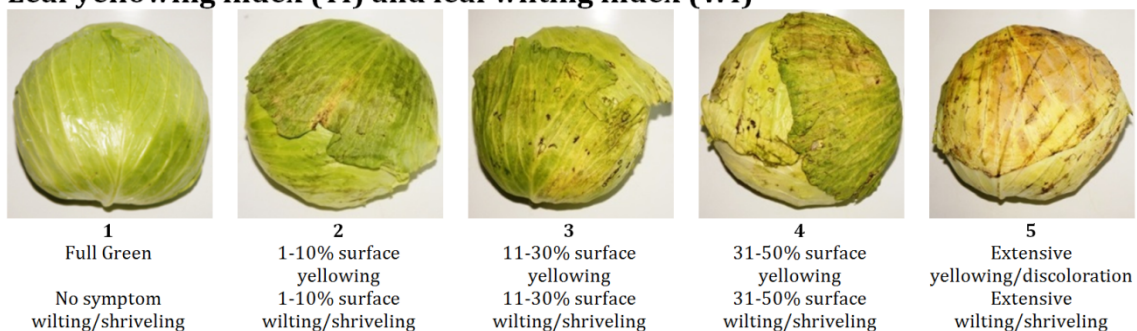


Figure 2. A rating scale developed for the leaf yellowing index (YI) and Leaf Wilting Index (WI) of cabbage.

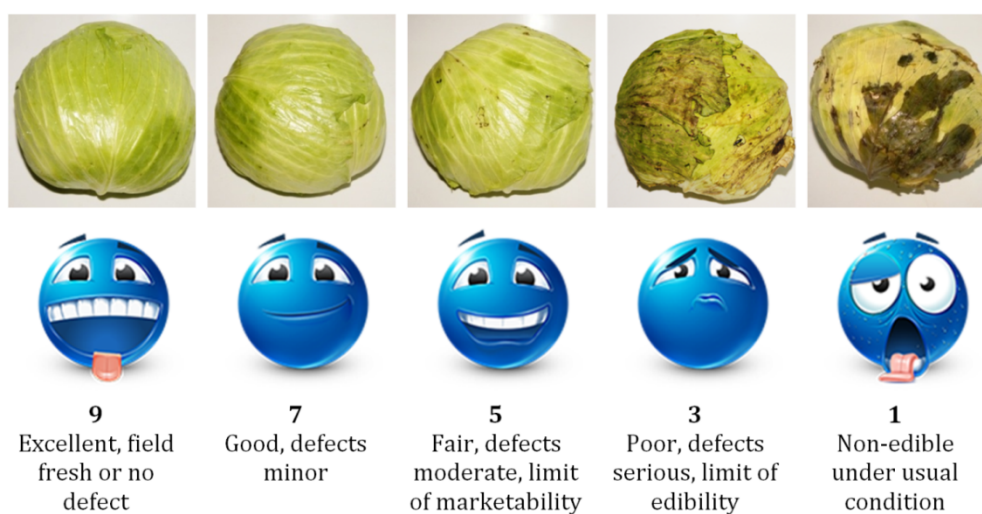


Figure 3. A rating scale developed for the visual quality rating (VQR) of cabbage.

Bacterial soft rot index (BSRI)

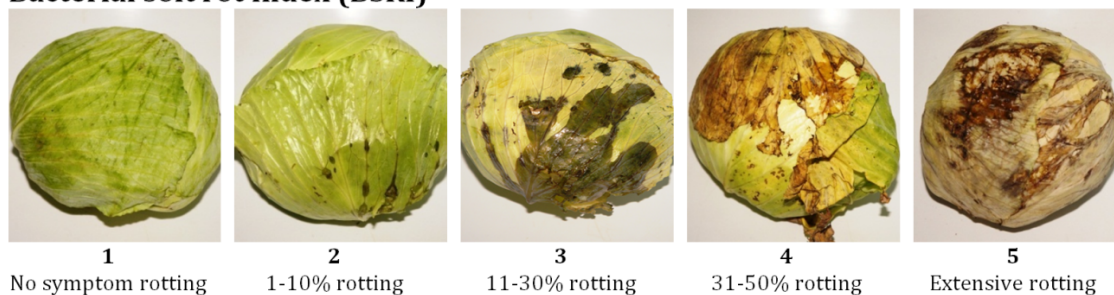


Figure 4. A rating scale developed for the bacterial soft rot index (BSRI) of cabbage.

CONCLUSION

Cold storage under 7-10°C reduced quality changes in cabbage as compared to samples stored under ambient condition. The results provide valuable information for establishing a system of grade standardization and classification along the value chain.

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Tomato (*Solanum lycopersicum*) quality grading at two storage temperatures

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Abstract

The study was conducted to evaluate the quality and shelflife of tomato (*Solanum lycopersicon* 'Diamante Max') at 7-10°C and 21-25°C and develop grading scale for research, education and industry use. Fruit quality at harvest was determined by maturity and absence of defects including deformation, blossom-end rot, and insect damage. During storage, quality changes were a result of the ripening process, with water loss and rotting as secondary factors. Storage at 7-10°C expectedly and more effectively delayed ripening, reduced weight loss, and prolonged shelf life of tomato fruits than at 21-25°C. Overall, the quality attributes were characterized using a scale for visual quality which was evaluated by a consumer panel. The results provide valuable information for establishing a system of grade standardization and classification along the production-marketing continuum.

Keywords: quality standards, grade classification, temperature effects

INTRODUCTION

The cultivated tomato (*Solanum lycopersicum* L.) is the second most commonly consumed vegetable crop after potato (*Solanum tuberosum* L.) in the world (FAOSTAT, 2008). Depending on the market, cultivar and production area, tomatoes are harvested at stages of maturity ranging from physiological maturity (mature-green stage) through full-ripe. Immature tomatoes are available for certain regional dishes. This denotes a certain degree of varying fruit quality which may not coincide with consumer quality requirements at a specific period without proper standardization. Recently, the formulation of this Philippine National Standard for Tomatoes, PNS/BAFPS 26:2006 was initially drafted by the Bureau of Agriculture and Fisheries Product Standards (BAFPS). The study was conducted to evaluate the quality and shelf life of tomato (*Solanum lycopersicon* 'Diamante Max') at 7-10°C and 21-25°C and develop a local grading scale to bridge the gap between producers and end-user to facilitate research, education and mainly industry use.

MATERIAL AND METHODS

Tomato (*Solanum lycopersicon* 'Diamante Max') at commercial maturity stage was harvested at a commercial farm at Brgy. Cabintan, Ormoc City. Right after harvest, the fruits were sorted by maturity (size and shape) and absence of defects including deformation, blossom-end rot, and insect damage. Each fruit was individually wrapped with soft paper and packed in cartons before shipment to Postharvest Technology Laboratory, Department of Horticulture, VSU, and were taken out from the box and allowed to equilibrate at ambient for about 2 h before the application of the storage treatments. Ten fruits were used as samples per replicate or a total of 30 fruits per treatment. The samples were then placed plastic trays (40×30 cm) each representing a replicate. All samples were stored at 7-10°C using a refrigerator compartment to simulate prolonged refrigerated holding, and ambient room temperature at 21-25°C. Photomicrographs were taken daily for color change, firmness/softening, shrivelling, and visual quality rating grade standardization (Table 1; Figures 1-3). Sample fruits were evaluated in terms of weight using a digital weighing scale. Statistical analysis of results was performed using the STAR, version 2.0.1 (2014) program of



the International Rice Research Institute.

Table 1. Tomato grades. Source: Philippine National Standard (2006) and UNECE STANDARD FFV (2012).

Grade 1	Grade 2	Grade 3
Similar varietal characteristics	Similar varietal characteristics	Similar varietal characteristics
Mature, not overripe or soft, clean, well developed, fairly well-formed and fairly smooth. It is free from decay, freezing injury and sunscald, and is not damaged by any other cause	Mature, not overripe or soft, clean, well-developed, reasonably well-formed and not more than slightly rough. It is free from decay, freezing injury, sunscald and is not seriously damaged by any other cause	Mature, not overripe or soft, clean, well-developed and may be misshapen. It is free from decay, freezing injury, not seriously damaged by sunscald, and is not very seriously damaged by any other cause
Tolerance: 5% by weight not satisfying the requirements of the grade	10% by weight not satisfying the requirements of the grade	
Classification: a. Large: diameter of 50 mm and above b. Medium: diameter of 40-50 mm c. Small: diameter of 30-40 mm		
Quality specification or definition: a. Similar varietal characteristic: the tomatoes are similar in firmness of flesh and shade of color in a lot. b. Mature: tomato has reached the stage of development, which will ensure a proper completion of the ripening process. c. Soft: tomato yields readily to slight pressure. d. Clean: practically free from dirt or other foreign material. e. Well: developed-shows normal growth. f. Fairly well-formed: tomato is not more than moderately deformed. g. Fairly smooth: tomato is not conspicuously rough. h. Damage: any specific defects or combination of defects, which materially detracts from the appearance, or the edible or marketing quality of the tomato. i. Reasonably well formed: is not decidedly deformed. j. Slightly rough: tomato is not decidedly grooved. k. Serious damage: any specific defects or combination of defects, which seriously detracts from the appearance, or the edible or marketing quality of the tomato. l. Misshapen: tomato is decidedly deformed. m. Diameter: the greatest dimension at right angles to the longitudinal axis. n. Very serious damage: any specific defects or combination of defects, which very seriously detracts from the appearance, or the edible or marketing quality of the tomato. 1. Anthracnose: a disease caused by fungus which appears as small, slightly sunken, water-soaked, circular spots on the surface of the fruit which becomes dark in the center because of the formation of a small, black fruiting bodies of the fungi. 2. Bacterial soft rot: the disease starts as a slightly sunken, water-soaked lesion on the fruit. It later enlarges rapidly and soon the whole fruit becomes very soft and watery. The skin is easily broken in the advanced stages and a foul odor usually accompanies the disease. 3. Bacterial spot: on the fruit, small, black, raised, circular spots with distinct narrow water-soaked borders appear which later turn brown and become slightly sunken and scabby. 4. Fusarium rot: the disease is characterized by water-soaked and slightly sunken lesions. Under favorable condition, white or pink fungal growth appears on the surface of the infected portion. 5. Gray mold: a grayish or yellowish green spots which take on a water-soaked appearance. 6. Nail head spot: spots on the fruit are small, circular, slightly sunken, superficial and tan to brown. As the spots enlarge, the centers become grayish-brown and definitely sunken and borders are more distinct which turns dark brown to black in color. Spots are confined only on the outer surface and seldom penetrate the wall of the fruit. 7. Sour rot or watery rot: infected fruits appear pickled with sour and fermented odor and often-times, the skin of the affected tissue ruptures revealing a white, cheesy fungal growth. 8. Blossom-end rot: a small water-soaked lesion appears near or around the blossom end of green fruits. This lesion soon enlarges and the affected tissues would dry out, darken and become leathery. Secondary rots occur in the tissue.		

Visual Quality Rating (VQR) Description

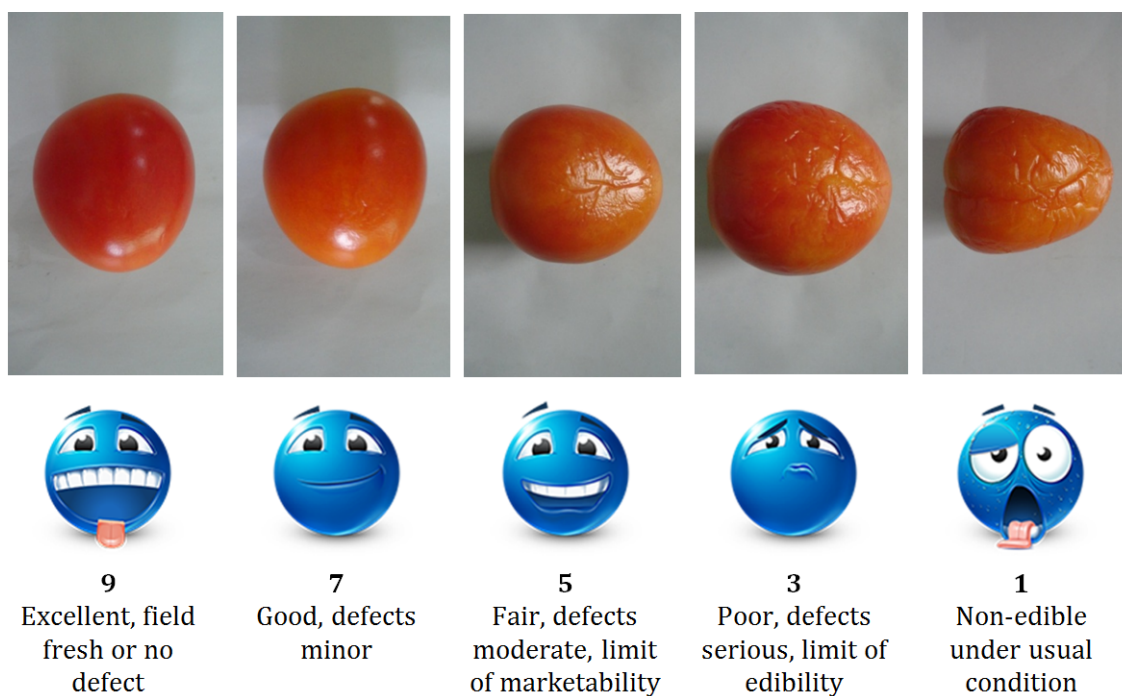


Figure 1. A rating scale developed for the visual quality rating (VQR) of 'Diamante Max' cultivar.

Shrivelling Index (SI) and Firmness Index (FI)

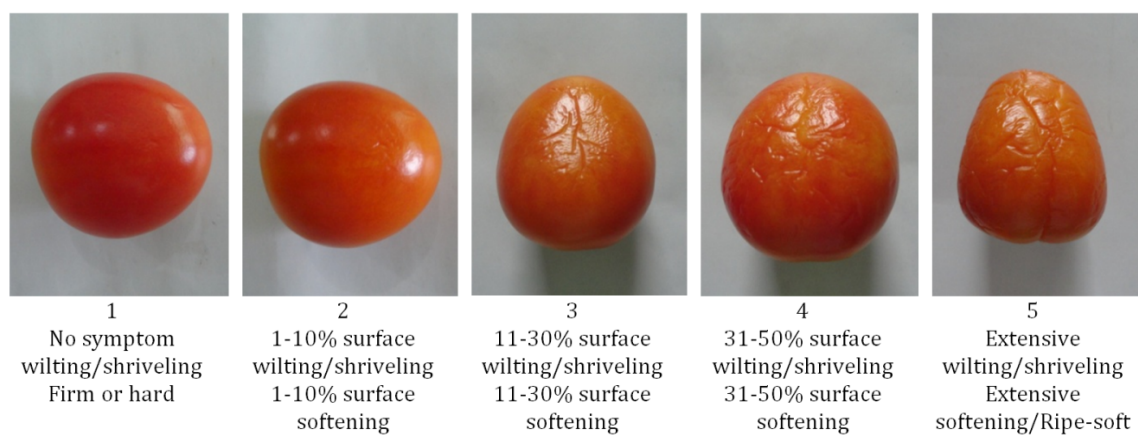


Figure 2. A rating scale developed for the firmness index (FI) and shrivelling index (SI) of 'Diamante Max' cultivar.

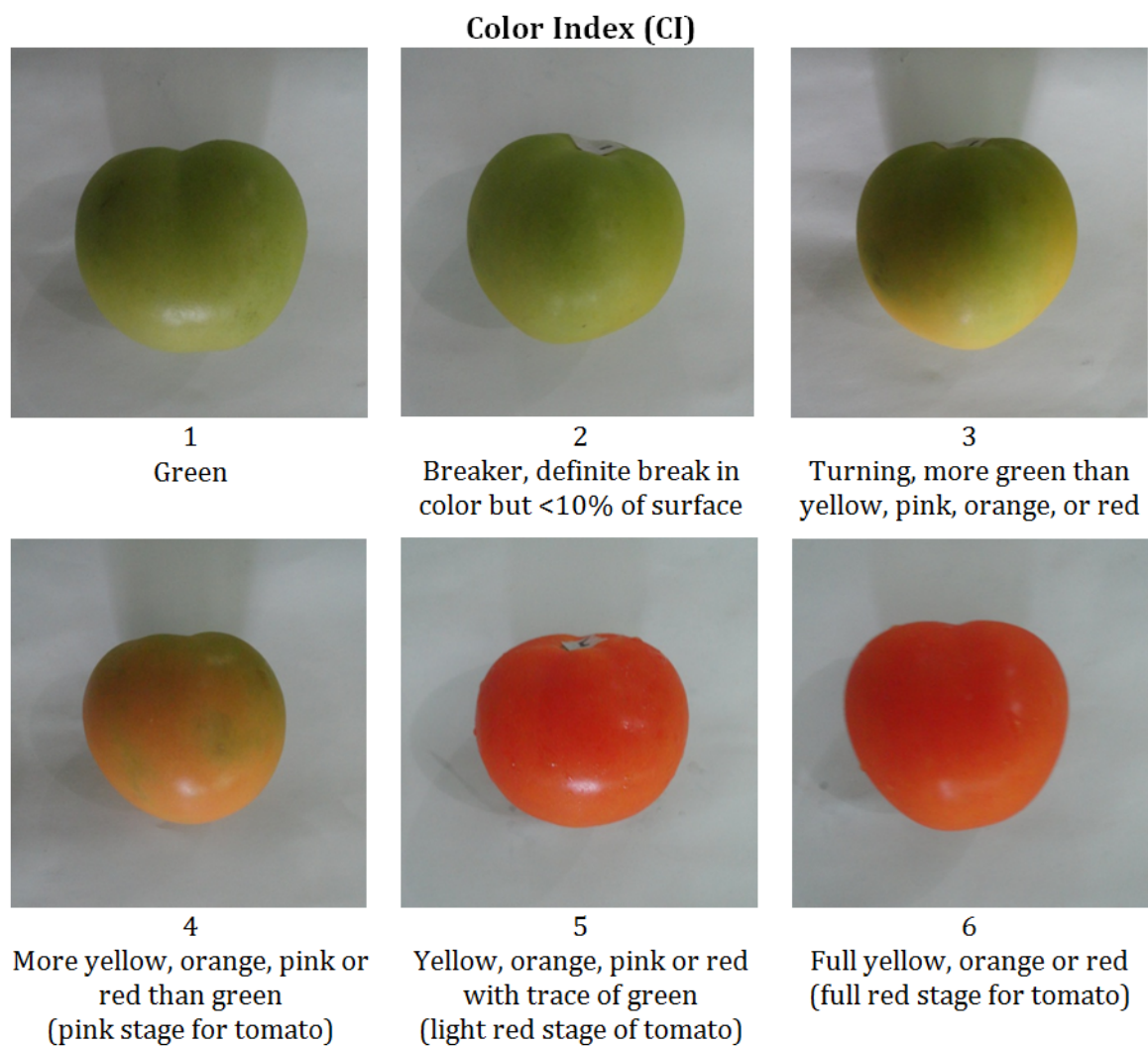


Figure 3. A rating scale developed for the color index (CI) of 'Diamante Max' cultivar.

RESULTS AND DISCUSSION

Fruit quality at harvest was determined by maturity and absence of defects including deformation, blossom-end rot, and insect damage (Table 1) (Cantwell and Suslow, 2013). Storage at 7-10°C effectively delayed ripening, reduced weight loss, and prolonged shelf life of tomato fruits than at 21-25°C (Figure 4). During storage, quality changes were a result of the ripening process, with water loss and rotting as secondary factors (Figures 1-2). The higher the temperature above the optimum temperature, the faster the respiration and shorter the time a commodity will last. Generally, for every 10°C increase in temperature there is an approximate doubling of respiration. Thus, this shortens the postharvest life by half (Bautista and Esguerra, 2007). Overall, the quality attributes were characterized using a scale for visual quality which was evaluated by a consumer panel (Figure 1).

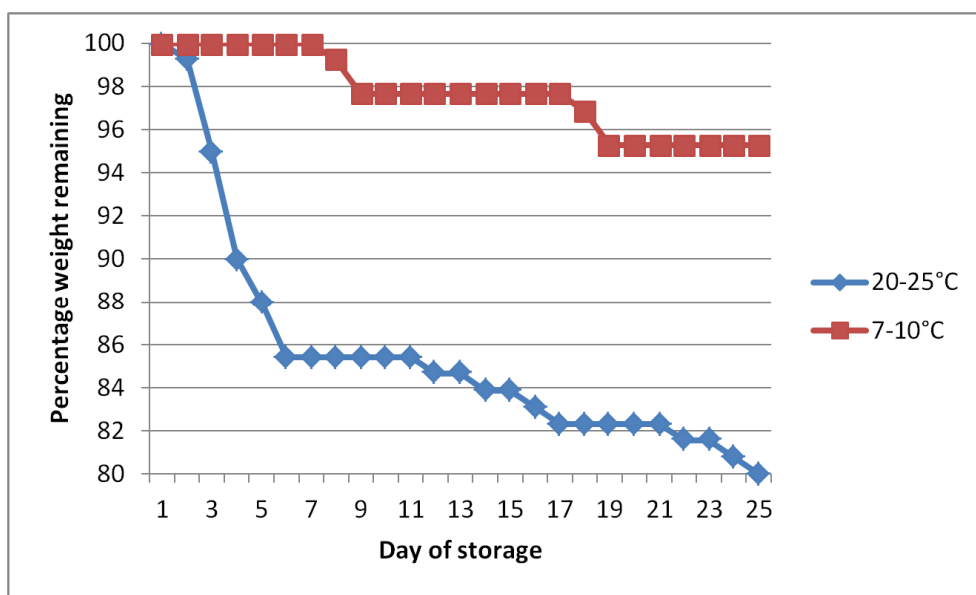


Figure 4. The average percentage weight remaining on a daily basis of 'Diamante Max' cultivar placed in refrigerated storage (7-10°C) and ambient storage (20-25°C). Means are significantly different using t-test, 5%.

CONCLUSION

Cold storage under 7-10°C reduced quality changes in tomato as compared to samples stored under ambient condition. The results provide valuable information for establishing a system of grade standardization and classification along the production-marketing continuum.

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