Managing chilli (*Capsicum* spp.) quality attributes: the importance of pre-harvest and postharvest factors

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Abstract

Demand for chillies and peppers continues to increase in many parts of the world as chillies (*Capsicum spp*.) are a good source of beneficial compounds. Optimising postharvest storage of chilli fruit is not enough to gain highest quality products in the market place if there is a significant variation in the quality such as size, colour and phytochemical compounds at the time of harvest, which may be a result of pre-harvest factors. The objectives of this research were to understand effects of pre-harvest and postharvest factors on chilli quality in order to produce consistent quality chilli fruit.

Storing of Habanero and Jalapeño at 8 °C can maintain low respiration rates and delay loss of firmness without the development of chilling injury symptoms for 4 - 5 weeks, while Paprika requires warmer storage temperatures as loss of firmness was found during storage at 8 °C, although overall appearance was still marketable. Chillies were very susceptible to shrivel when stored above 8 °C. In Jalapeño, water loss occurred approximately equally through fruit skin and through the calyx and pedicel area until cracking appeared on Jalapeño fruit which stimulated a significant increase in skin water loss. A model was developed to predict the shelf life (using 5 % water loss as time to shrivel development) of Jalapeño during storage by conducting a sensitive analysis on the potential factors (such as fruit weight, water vapour permeance (P'_{H_2O}), temperature and RH); RH was the most important factor on the impact on rate of water loss and time to shrivel. Application of wax on fruit skin or the whole fruit is recommended as waxing on calyx and pedicel of Jalapeño increased shelf-life by 10 % compared to control fruit.

Pre-harvest factors such as time of planting, position on plant, maturity at harvest and crop load significantly influence Jalapeño quality (i.e. fruit size, colour and phytochemical composition). Fruit weight, colour and ascorbic acid varied with time of planting and time of fruit set during the season demonstrating that growing conditions affected plant and fruit growth. Fruit from plants planted late in the season (October) were small and contained low ascorbic acid concentration. Position on plant also affected fruit size and ascorbic concentration despite fruit being of the same maturity stage. Different fruit size may be explained by the competition between plant

and fruit growth and also the distance from nutrients and water supply rather than fruit to fruit competition as there was no influence of crop load on fruit size. However, ascorbic acid accumulation in fruit was stimulated by competition between fruit on the plant as fruit from high crop load plants showed higher ascorbic acid concentration than fruit from low crop load plants. In addition, it may be influenced by plant age or time of fruit set during season, as late season or upper node fruit produce low ascorbic acid concentration. Maturity had a major effect on colour at harvest, but colour change was influenced by position on plant and growing conditions. Colour development of fruit at lower nodes which were set at cooler temperatures was slower than fruit at higher nodes which were set at warmer temperature. Capsaicinoid concentration seemed to be consistent along the plant. However, the observed results showed that measurement of total capsaicinoid concentration can be affected by the sub-sampling error from the proportion of each individual tissue (i.e. pericarp, placenta and seed) contained in the sample due to large differences in capsaicinoid concentration among tissues. Similar to capsaicinoids, antioxidant activity (AOX) and total phenolic concentration (TPC) seemed to be consistent along the plant. A weak correlation was found between AOX and TPC or AOX and ascorbic acid indicating that ascorbic acid or TPC was not a major contributor of the AOX in Jalapeño. Further work in this area is required, but needs to start with harmonisation of extraction solvents.

In conclusion, this research generates an overall understanding on the effects of pre-harvest and postharvest factors on chilli quality which will assist chilli growers in controlling sources of variation and help to produce more uniform chillies. Based on these results, to produce larger Jalapeño fruit with high concentrations of health beneficial compounds such as ascorbic acid, Jalapeño plants should be pruned not to higher than 12 nodes. Thinning leaders during production is essential for decreasing the risk from plant collapse due to weight but does not influence fruit size. As this research was focused on plants with two leaders and a single first flush fruit per node at high crop load, investigating the role of more leaders, a higher number of fruit per node and the second flush of fruit production should be investigated in future work.

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Abbreviations

A	pre-exponential factor
AOX	antioxidant activity
a*	CIE Lab ‘a’ value measured by colorimeter
a_w	water activity
b*	CIE Lab ‘b’ value measured by colorimeter
C.A.N.	calcium ammonium nitrate
C_a	chlorophyll a
$CaCO_3$	calcium carbonate
C_b	chlorophyll b
CO_2	carbon dioxide
C_{x+c}	carotenoids
d	day
ρ	density of fruit
dpi	dots per inch
DW	dry weight
E_a	activation energy
Eq.	equation
FAA	formalin alcohol acetic acid
$FeSO_4.7H_2O$	ferric sulfate
FRAP	ferric reducing antioxidant power
FW	fresh weight
H	high crop load
HPLC	high performance liquid chromatography
J	joule
K	potassium
KOAc	potassium acetate
kPa	Kilopascal
L*	CIE Lab ‘L’ value measured by colorimeter
L	low crop load
LDPE	low density polyethylene
LSD	least significant difference

M	fruit mass during time of storage
M_0	fruit mass at the beginning of the experiment
N	nitrogen
Na_2CO_3	sodium carbonate
$P_{CO_2}^{initial}$	CO_2 concentration immediately after closing container (%)
$P_{CO_2}^{final}$	CO_2 concentration after certain period (%)
P^{total}	total air pressure
P'_{H_2O}	water vapour permeance
Δp_{H_2O}	the difference in partial pressure of water vapour between the environment and fruit
$p_{H_2O}^{f}$	the partial pressure of water vapour in fruit
$p_{H_2O}^{e}$	the partial pressure of water vapour in the environment
$p_{H_2O}^{sat}(T)$	saturated partial pressure of water vapour at temperature
Pa	Pascal
PGU	Plant Growth Unit
γ	psychrometric constant
R	ideal gas constant
R_c	predicted respiration rate
RH	Relative humidity
r'_{H_2O}	rate of water loss
r_{CO_2}	respiration rate
SA	surface area
SE	standard error
t	storage time
T	temperature
T_e	temperature of environment
T_f	temperature of fruit skin
T_w	wet bulb temperature
TPC	total phenolic concentration
TPTZ	2,4,6 tripyridyl-s-triazine
UV	ultra violet radiation
Vf	fruit volume
Vjar	volume of jar

W	width
WAF	weeks after flowering
WAFS	weeks after fruit set

CHAPTER 1

Introduction

Chillies are used as food additives or spices in many national cuisines due to their sensory attributes of colour, heat, pungency flavour, and aroma. Chillies are a good source of vitamin A, C, and E, but the concentration depends on the cultivar (Bosland & Votava, 2000). Chillies are used fresh, canned, brined/pickled, frozen, fermented, dehydrated, or processed to chilli powder. Demand for chilli or pepper continues to increase in many parts of the world. The global production of chillies and peppers has increased since 1990 (FAOSTAT, 2012). The New Zealand contribution to production is low, but represents a high yield per planted area (Table 1.1), because of the use of greenhouse production systems. North America and Western Europe are the main importers of chillies and peppers while Pakistan, Mexico, India, China, and Chile are the main exporters (Bosland & Votava, 2000).

Table 1.1 Yields/ha, and total production of chillies and pepper around the world (FAOSTAT, 2012).

	Yields ($\times 10^3$ kg.ha^{-1})			Production ($\times 10^3$ kt)		
	1990	2000	2010	1990	2000	2010
Africa	7.6	5.7	7.5	1.6	2.1	2.7
America	10.9	14.1	17.5	1.4	3.1	3.9
Asia	9.2	13.9	15.6	5.2	12.7	18.1
Thailand	12.6	14.0	13.9	0.010	0.014	0.017
Europe	16.1	18.3	23.4	2.5	2.7	2.9
Australasia	16.2	18.5	21.7	0.023	0.049	0.056
New Zealand	26.5	31.2	35.6	0.003	0.005	0.006
World (total)	10.1*	12.5*	14.8*	10.9	20.8	27.6

*This data are from calculation and estimation by FAO.

In Thailand, chillies are a major exported fresh vegetable (Department of Agricultural Extension, 2007). The two main species grown are *Capsicum annuum*

(e.g. Cayenne) and *Capsicum frutescens* (e.g. Bird's eye chilli). Water loss, mechanical damage and microbial deterioration are the major problems affecting quality of fresh produce in Thailand because of a shortage of cool storage and refrigerated transportation resulting in inappropriate postharvest handling (Utto, 2000). Therefore, research related to improved postharvest handling and storage is needed for chilli growers and industries in Thailand.

After harvest, chillies and peppers fruit remain biologically active and change in respiration rate, colour, firmness and water loss. A good quality chilli or pepper should be firm with fresh calyx and pedicel and free from bruises, abrasions, and disease. Shrivel and wilting can have an important effect on visual quality of chillies (Bosland & Votava, 2000). Postharvest treatments e.g. low temperature storage, packaging etc. can delay these physiological changes, maintain quality and prolong storage life of chilli and pepper fruit. Previous research has found that the optimum temperature for pepper storage ranges between 7 - 13 °C to avoid chilling injury (Thompson, 1979; Gonzalez-Aguilar, 2004).

The colour, heat (pungency), aroma, and nutritional value of chilli are important factors that make chilli desirable as a food additive in many parts of the world (Pino et al., 2006; Pino et al., 2007). According to the level of maturity or ripeness, their colour, pungency, volatile compounds and phytochemical compounds are different. However, wide variation is found even in the same variety or cultivar. To study sources of variation and the difference of these characteristics during maturity would be useful to determine optimum harvest times and control the uniformity of chilli attributes to develop the maximum beneficial compounds.

Chillies belong to the genus *Capsicum*. At least 25 wild species and five domesticated species exist (Table 1.2).

Table 1.2 Five domesticated *Capsicum* species and popular varieties in each *Capsicum* species (Bosland & Votava, 2000).

Species	Popular varieties
C. annuum	Bell pepper, Paprika, Cayenne, Jalapeño, and Chiltepin
C. baccatum	Brown pepper and Aji
C. chinense	Habanero and Scotch bonnet
C. frutescens	Tabasco, Bird's eye
C. pubescens	Raccoto

C. annuum is the most widespread species which includes many common commercial varieties. *C. chinense*, is known as the hot species due to its content of heat and aroma compounds. Common *C. chinense* varieties include Habanero and Scotch bonnet (Bosland & Votava, 2000). However, among species there is a tremendous variety of chillies with the heat varying greatly from mild to hot. Chillies and pepper contain high amount of vitamin C and provitamin A as well as vitamin B_1, B_2, and B_3 (Table 1.3). The recommended daily intake (RDI) of vitamin C is 90 (in male) or 75 (in female) mg per day so even a single fruit of bell pepper (ca. 90 - 120 g of fruit; 128 mg of vitamin C per 100g of bell pepper) is enough to be a good source of vitamin C for a day (Anon, 2011).

Table 1.3 Nutrition values (per 100 g) of green bell pepper and red New Mexican (Bosland & Votava, 2000).

	Water	Carbohydrate	Fibre	Vitamins			
	(%)	(g)	(g)	A (IU)	C (mg)	B_1 (mg)	B_2 (mg)
Green bell pepper	93	5.3	1.2	530	128	0.09	0.05
Red New Mexican	88	9.5	1.8	770	242	0.09	0.09

Chillies are consumed fresh, processed as sauce or seasoning or used for decoration in a wide range of food products. The pungency of chillies is caused by the alkaloid capsaicin that varies among selections from mild to hot (Bosland & Votava, 2000). Chillies have pharmaceutical properties with capsaicinoid compounds used to alleviate pain or purported to cure or prevent some diseases (Bosland & Votava,

2000). To clarify terminology, the term 'variety' is used for characteristics, shaped or coloured types of chilli within a species; variety names are used without quotation marks. The term 'cultivar' is used for particular accessions of a variety and cultivars are always named in single quotation marks.

Levels of heat or pungency can be determined by several methods. The Scoville Heat Units (SHU) test is the traditional method where trained panellists taste chilli samples, record their assessment of heat levels, after which the samples are diluted until the heat is no longer be tasted. Pure capsaicin, heat compound, rates between 15,000,000-16,000,000 SHU while bell pepper has 0 SHU which means no heat is tasted from bell pepper (Table 1.4).

Table 1.4 Scoville heat units in type of peppers and chillies (Anon., 2008).

Scoville heat unit	Type of peppers and chillies
15,000,000-16,000,000	Pure capsaicin
8,600,000-9,100,000	Various capsaicinoids (e.g. homocapsaicin, homodihydrocapsaicin, nordihydrocapsaicin)
855,000-1,050,000	'Naga Jolokia'
350,000-580,000	'Red Savina' Habanero
100,000-350,000	Habanero, Scotch Bonnet, Datil pepper, Rocoto, Jamaican hot pepper, African Birdseye
50,000-100,000	Thai chilli, 'Pequin'
30,000-50,000	Cayenne, Aji pepper, Tabasco,
10,000-23,000	Serrano
2,500-8,000	Jalapeño, Guajillo, Hungarian wax pepper, Anaheim, 'Poblano', Rocotillo
500-2,500	Pimento, 'Pepperoncini'
100-500	Bell pepper
0	

The SHU test is limited by human subjectivity as different individuals have different heat tolerance. High performance liquid chromatography (HPLC) is a more reliable

and repeatable method capable of detecting capsaicinoid compounds in parts per million (ppm).

Quality criteria of chillies include uniformity in shape, size and colour that are typical of a specific variety. Fruit should be free of physical damage (including splits, cracks and bruises), physiological damage (such as pitting or shrivelling) and pathological damage (decay caused by fungi or bacteria). At room temperature, fresh chillies lose water quickly and begin to shrivel within a few days.

Quality of chillies changes during maturation and after harvest but little is known about the physiological and biochemical changes occurring during these periods for the diverse cultivars. If the physiological changes of chillies are understood then optimum maturity stage at harvest and excellent handling can be used to obtain high quality and uniform chillies. In addition, postharvest treatments could be applied to maintain quality of fresh chillies. Those are useful for chilli growers and industry to control quality and increase values in both domestic and international market.

1.1 Summary of varieties used in this research

1.1.1 Paprika

Figure 1.1 Paprika (*C. annuum*)

Paprika is a red powder spice or dry pods derived from *C. annuum* (Smith et al., 1987). Paprika is an important food flavouring spice used in a wide variety of dishes. Paprika has a Scoville Heat Units score of 1000-2000 and so is regarded as mild. Paprika fruit are grown outdoors in summer or in greenhouses in winter.

1.1.2 Jalapeño

Figure 1.2 Jalapeño *(C. annuum*)

Jalapeño is also in *C. annuum* species. It is 3.75 - 5 cm wide and 5 - 7.5 cm long with a rounded oblong shape. The colour changes from dark green at immature stage to red at mature stage with or without corky lines (a netting pattern) on the fruit skin. Jalapeño is used fresh, canned, pickled, and in sauces (Smith et al., 1987; Bosland & Votava, 2000). The range of heat rating of Jalapeño is 3500 - 4500 and so is classified as mild to medium. 'Conchos' is a Jalapeño cultivar with moderate heat widely grown in the U.S. and Mexico. Fruit are cylindrical and change from dark green to red when fruit are mature.

1.1.3 Habanero

Figure 1.3 Habanero (*C. chinense*)

Habanero *(C. chinense)* originates from Mexico; it is lantern-shaped with green colour at immature stage and changes to orange or red colour at maturity. Fruit are 2.5 cm wide at the shoulder areas and 6 cm long. Habanero is an intensely hot and aromatic fruit. The SHU scores range from 300,000 - 400,000 while 'Red Savina' cultivar shows 577,000 SHUs. Habanero is used fresh and as an ingredient in salsas or sauces (Bosland & Votava, 2000).

1.2 Morphological and physiological behaviours of chillies and peppers during fruit development and after harvest

1.2.1 Fruit size and shape

Fruit weight of chillies and peppers has been shown to rapidly increase at an initial stage of fruit development from 10 - 30 days after flowering (DAF) and then fruit weight remains stable until fruit reach fully developed stage (Biles et al., 1993; Tadesse et al., 2002; Barrera et al., 2005; Barrera et al., 2008), showing that chillies and peppers have a single sigmoid growth curve (Miller et al., 1979; Biles et al., 1993; Barrera et al., 2005). For example, New Mexican peppers increased size rapidly from 20 to 33 DAF until 40 DAF when fruit were fully developed (Biles et al., 1993). Later, fruit left on the plant showed a decline of fruit weight due to water loss resulting from senescence.

Chilli pods vary from cylindrical (e.g. Jalapeño and Paprika) to lantern shaped (Habanero) (Bosland & Votava, 2000). Fruit with cylinder shapes show an increase of length faster than diameter, particularly at the early stage of fruit development (Tadesse et al., 2002; Barrera et al., 2005; Barrera et al., 2008). Sweet pepper attains almost 75 % of the final length in 3 weeks after anthesis and gradually elongated until 10 weeks after anthesis while fruit diameter increased slower than fruit length at the early growth stage (Tadesse et al., 2002).

To increase fruit size, thinning or pruning fruit and/or leaves to reduce the number of fruit on the plant has been effective in some fruit such as apple (Stopar et al., 2002), cherimoya (*Annona cherimola*) (González & Cuevas, 2008) and kiwifruit (Atkins, 1990; Stopar et al., 2002; González & Cuevas, 2008). In addition, position of fruit on the plant also has effects on fruit size of pear (Wang et al., 2010), kiwifruit (Lawes et al., 1990; Tombesi et al., 1993; McPherson et al., 2001; Remorini et al., 2007), custard apple (*Annona cherimola* × *Annona squamosa*) (George & Nissen, 1988), cherimoya (González & Cuevas, 2008), and strawberry (Sachs & Izsak, 1972). Fruit weight of kiwifruit at the top of the canopy was higher than fruit from the bottom (Remorini et al., 2007) while cherimoya and strawberry fruit from basal positions developed larger fruit than fruit from apical positions (Sachs & Izsak, 1972;

González & Cuevas, 2008). This research indicates that fruit weight and shape do not develop similarly along the plant, therefore size grading needs to be done before marketing. Currently, effects of plant and crop load manipulation on the resulting fruit from the *Solanaceae* plant family are limited. These effects are confounded as *Solanaceae* plants grow rapidly simultaneous with fruit growth. In general, thinning chilli and pepper leaders is important for avoiding plant collapse due to weight and allowing the strong leaders to grow and produce quality fruit.

1.2.2 Water loss

Like most fruit and vegetables, approximately 80 - 90 % of chillies are water. Water loss of only 5 % can cause wilting or shrivelling in some commodities (Wills et al., 2007) and limit marketable life of fresh produce. Water loss results from transpiration that is driven by the difference of partial pressure between the inside of the fruit and the external environment. This difference influences a water vapour pressure gradient where moisture flows from high to low water vapour pressure (i.e. from inside to outside the fruit).

Chilli and pepper of different varieties have significantly different rates of water loss during storage (Lownds et al., 1993; Banaras et al., 1994; Lownds et al., 1994; Guerra et al., 2011) which can be influenced by storage condition and fruit characteristics. Temperature, RH and storage period greatly affect water loss in chilli and pepper fruit. A high water loss rate was found in bell pepper and Jalapeño during storage at high temperature (20 °C), while storage at low temperature can reduce the rate of water loss and prolong the shelf - life of peppers (Lownds & Bosland, 1988; Lownds et al., 1994). The water loss of pepper fruit increased with storage time during storage at 8 - 20 °C due to high transpiration or water evaporation at high temperature (Lownds et al., 1993; Banaras et al., 1994; Lownds et al., 1994) while storage at high RH can reduce water loss and delay senescence of harvested bell pepper fruit (Lurie et al., 1986; Lownds et al., 1994).

In addition, physical properties e.g. surface area (SA), initial water content, surface area to volume ratio (SA/V), surface area to fruit weight ratio (SA/FW), and surface morphology, which differ depending on chilli types or varieties, can also affect the

rate of water loss of chillies and peppers (Ben-Yehoshua, 1987; Wills et al., 2007). For example, Lownds et al. (1993) found a positive relationship between water loss of different pepper cultivars (*C. annuum*) and SA/V but a negative relationship between water loss and the amount of epicuticular wax. In case of bell pepper fruit which are hollow, Diaz-Perez et al. (2007) found a higher water loss rate (%.day^{-1} kPa^{-1}) in fruit with high SA/FW.

Water loss in chillies and peppers can occur via the calyx, pedicel, picking scar and skin surface (Lownds et al., 1993; Diaz-Perez et al., 2007). As stomata are absent on the fruit skin in chillies and peppers, water loss occurs solely through the cuticle (Blanke & Holthe, 1997). The fruit skin cuticle is mainly composed of lipid, wax and cutin (Maalekuu et al., 2005) resulting in water resistant properties that play an important role in inhibiting water loss by transpiration and maintaining high water content in fruit (Wills et al., 2007). The chemical composition of cuticle in pepper has a major effect on water loss with a negative correlation existing between water loss rate and the proportion of alkanes and aliphatic compounds (Parsons et al., 2012). These results indicate that simple straight chain aliphatic compounds create a packed and more impermeable cuticle. Meanwhile a positive correlation was found to exist between water loss rate and total triterpenoid and sterol content. The components create a complex structure which cannot be packed closely and caused a porous and permeable cuticle (Parsons et al., 2012). In similar, Lurie & Ben-Yehoshua (1986) also found high water loss in mature fruit which related to an increase of sterol content on fruit skin. Mechanical damage to the cuticle, caused by bruising, cracking, or cutting can also accelerate rate of water loss. This disagreement exists on the main route of water loss in the *Solanaceae* family. Lownds et al. (1993), Vogg et al. (2004) and Maalekuu et al. (2005) found that water loss mainly occurred through fruit skin in tomato and pepper respectively while Diaz-Perez, (1998) and Diaz-Perez et al. (2007) showed that calyx and stem was the major path of water loss in eggplant and bell pepper respectively.

To determine rate of water loss from fruit, water vapour permeance (P'_{H_2O}; mol.s^{-1}m^{-2}Pa^{-1}), which characterises the barrier properties of the fruit surface to water vapour and influences the rate of water vapour diffusion from the fruit, is

measured (Maguire et al., 1999a). Changes of P'_{H_2O} depend on many factors such as cuticular structure, fruit maturity, waxing, RH and temperature etc. For example, P'_{H_2O} of 'Cayene' chilli increased with temperature (Utto, 2001). Mature bell pepper showed lower fruit P'_{H_2O} than immature fruit while the P'_{H_2O} of calyx has been found to be 14 times higher than skin P'_{H_2O} (Diaz-Perez et al., 2007). In addition, a disorder on fruit skin (e.g. micro-cracking of cuticle in apple) also affects P'_{H_2O} and results in higher water loss (Maguire et al., 1999b). High variation of P'_{H_2O} was found in apple as influenced by grower line, cultivar, harvest date and orchard with no correlation to maturity (indicated as firmness, starch, colour and soluble solids content) (Maguire, 1998; Maguire et al., 1999a; Maguire et al., 2000). Although apple skin characteristics are different from chilli, the effects of cracking on P'_{H_2O} due to growing conditions may be useful to understand the resulting effect on water loss.

1.2.3 Colour changes

Colour change is the important component of visual quality. In addition, colour can be an important factor for commercial value: for example, prices of red bell pepper are higher than green bell pepper due to consumer preference. Colour uniformity of fruit is also considered for maturity assessment. Colour in chillies and peppers varies depending on species, cultivar, maturity, and growing conditions (Nagle et al., 1979; Gómez et al., 1998; Barrera et al., 2008).

Often colour of chillies and peppers is measured during fruit ripening (de Guevara et al., 1996; Gómez et al., 1998; Perez-Lopez et al., 2007). Hue angle was used to define colour changes on the skin of chillies during maturation and ripening in some accessions of *C. annuum* that change from green to orange or yellow at the full ripe stage. In this case, hue values changed from green (104°) to red (40°), orange (57°) or yellow (75°) in different accessions (Tadesse et al., 2002; Barrera et al., 2005; Barrera et al., 2008). Barrera et al. (2008) mentioned that hue angle of some hot peppers differed at each maturity stage and therefore could be used as a maturity index. Lightness or L* value was used to predict carotenoid concentration in Paprika grown in open air and sweet pepper (Gómez et al., 1998; Perez-Lopez et al., 2007).

However, in chillies or peppers which change from green to red colour during ripening, a* value would be appropriate to describe colour changes in these varieties.

Colour changes in chilli and pepper during ripening occur from a decrease or disappearance of chlorophyll content with an increase or unmask of carotenoids (Davies et al., 1970; Rahman et al., 1978; Biles et al., 1993; Minguez-Mosquera & Hornero-Mendez, 1993, 1994b; de Guevara et al., 1996; Deli et al., 1996; Gómez et al., 1998; Markus et al., 1999; Hornero-Mendez et al., 2000; Hornero-Mendez & Minguez-Mosquera, 2000; Hornero-Mendez et al., 2002; Hornero-Mendez & Minguez-Mosquera, 2002; Marín et al., 2004; Navarro et al., 2006; Perez-Lopez et al., 2007; Menichini et al., 2009). Carotenoid concentration of yellow bell peppers increased 12 - 70 fold during maturation from mature to fully ripe fruit (Rahman et al., 1978). The major carotenoid components in ripe red pepper are capsanthin, capsorubin, and cryptoxanthin, while lutein and violaxanthin are the major group in green fruit (Davies et al., 1970; Gómez et al., 1998; Hornero-Mendez et al., 2000). Generally, colour changes of chillies and peppers initiate in the range of 3 - 8 weeks after full bloom (Biles et al., 1993; Tadesse et al., 2002; Barrera et al., 2005; Barrera et al., 2008). Therefore, maturity at harvest can result in uniform colour change after harvest.

Growing conditions such as temperature also affect pigment compounds in pepper. Markus et al. (1999) found that red peppers grown in a cool and rainy season had more carotenoid than fruit grown in a hot summer season while Paprika fruit grown with less exposure to sunlight showed darker red colour (Gómez et al., 1998). As fruit on the plant differ in maturity, application of ethephon onto chilli plants enhanced ripening and induced fruit to reach marketable colour at the same time including increased the percent of red marketable fruit for both Paprika and cayenne chillies (Krajayklang et al., 1999). However, when ethephon was applied to fruit which were harvested at different maturities, no effect of ethylene was found on colour change and total carotenoid (Krajayklang et al., 1999; Fox et al., 2005). Therefore, for benefit on colour changes by reducing harvest times and labour costs, the ethylene treatments should be applied on the chilli plant.

During storage, colour of chillies and peppers can change depending on storage temperature and time. Bell peppers lost 20 % of carotene after storage for 7 days at 21 °C (Matthews et al., 1975) which is similar to results found by Lon Kan et al. (2007) who showed a decrease of β-carotene concentration in Datil hot pepper during storage at 20 °C. Jalapeño slices lost 32 and 13 % of β-carotene in air and MAP respectively when stored for 12 days at 4.4 °C plus an additional 3 days at 13 °C (Howard & Hernandez-Brenes, 1998).

1.2.4 Firmness

Firmness is an important quality criterion for fruit. Firmness of chillies and peppers can be measured by different methods such as measuring a puncture force through the stem end, centre, and tip of the pod (Biles et al., 1993; Avalos Llana & Sgroppo, 2009), measuring the force which can deform the whole fruit (Gonzalez et al., 2005) or measuring the shear force from pepper rings (Howard et al., 1997).

During maturation, firmness of 'New Mexico 6-4' peppers increased dramatically from 20 - 60 days after flowering (DAF) then decreased significantly until 103 DAF (Biles et al., 1993). Lurie et al. (1986) showed a decrease of firmness in pepper as colour changes from green to red and correlating to high water loss in red pepper.

During storage a decrease of firmness in Habanero was found at 7 °C and fruit became soft during shelf life at 22 °C (Gonzalez et al., 2005). To maintain firmness of chillies during processing, Jalapeño fruit or rings are pre-heated at 50 - 60 °C for 60 and 40 min respectively to inactivate enzymes such as pectinesterase and polygalacturonase which can cause softening. Ca (i.e. $CaCl_2$) adding is required in a preheating solution to form an ionic crosslink with pectin molecules, which can result to firm texture (Howard et al., 1997; Howard & Hernandez-Brenes, 1998; Villarreal-Alba et al., 2004).

1.2.5 Respiration rate and ethylene production

Chillies and peppers are classified as non-climacteric fruit (Saltveit, 1977; Lurie et al., 1986; Biles et al., 1993) because they show no respiration and ethylene production rises during fruit ripening (Kays, 1991; Kader, 2002a). For example

'Changjiao' hot pepper and bell pepper have been reported as non-climacteric (Saltveit, 1977; Lu et al., 1990; Thang, 2007). However, some disagreement has been found in other chillies and peppers which show climacteric rises such as in 'Chooraehong' hot chilli, red bell pepper, New Mexican peppers, and Habanero, particularly when fruit were harvested at immature or breaker maturity or monitored while attached to the chilli plants (Batal & Granberry, 1982; Gross et al., 1986; Lurie et al., 1986; Biles et al., 1993; Villavicencio et al., 1999; Krajayklang et al., 2000; Villavicencio et al., 2001; Thang, 2007). Meanwhile ethylene production has been found to increase later at the breaker maturity and peaked at the bright red colour stage in hot peppers (Krajayklang et al., 2000; Barrera et al., 2008), or even during senescence in peppers (Lurie et al., 1986). Exposure to exogenous propylene or ethylene did not induce climacteric rises of respiration rate and ethylene production of bell pepper or green or mature chillies (Saltveit, 1977; Krajayklang et al., 2000), which can indicate that these chillies are non-climacteric. Overall, it seems that both climacteric and non-climacteric patterns can be found in chillies and peppers as some chillies and peppers fruit show climacteric rise at immature stage, colour turning stage or during fruit development on the plants, but an increase of ethylene production at later stage may coincide to the onset of senescence.

Low temperature reduces metabolic rate (e.g. respiration and ethylene production) (Wills et al., 2007). Therefore storage of chilli and pepper fruit at low temperature (10 °C) and O_2 (1.5 %) condition can suppress respiration rate (Rahman et al., 1995; Avalos Llana & Sgroppo, 2009).

1.3 Phytochemical compounds

1.3.1 Capsaicinoids

Capsaicinoids are a group of compounds which relate to heat and pungency of chilli and pepper fruit. Concentration of capsaicinoids varies depending on chilli variety and cultivar (Suzuki & Iwai, 1984; Govindarajan, 1986). Overall, total capsicinoids (e.g. red pepper) are mainly composed of capsaicin (69 %) and dihydrocapsaicin (22 %), but also include the minor capsaicinoids nordihydrocapsaicin (7 %), homocapsaicin (1 %) and homodihydrocapsaicin (1 %) (Bennett & Kirby, 1968). Capsaicinoids are synthesized by the capsaicin gland in the placenta, the white tissue

that runs down the middle and along the sides of chillies or peppers (Iwai et al., 1979; Rowland et al., 1983; Zamski et al., 1987; Thiele et al., 2008; Broderick & Cooke, 2009) (Figure 1.4). Heat compounds can be derived from the phenylpropanoid pathway to vanillylamine and to capsaicin (Suzuki & Iwai, 1984; Sukrasno & Yeoman, 1993; Mazourek et al., 2009) (Figure 1.5).

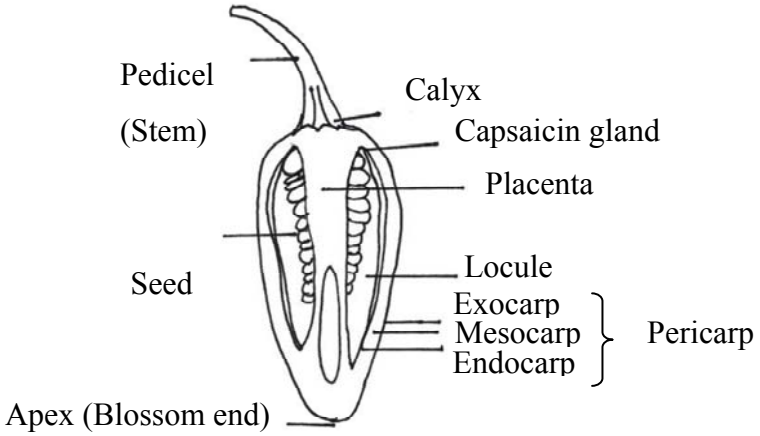


Figure 1.4 Cross section of chilli indicating individual part of fruit.

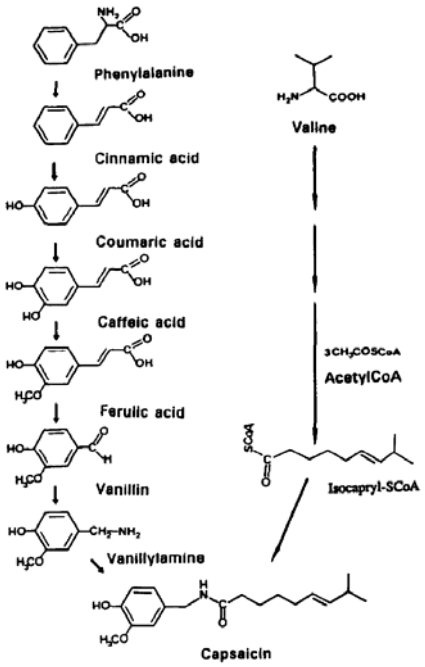


Figure 1.5 The capsaicin biosynthesis pathway (Sukrasno & Yeoman, 1993).

Application of nitrogen to chilli plants led to an increase of capsaicinoid content in both Habanero and Jalapeño fruit, which can suggest that amino acids, precursors for capsaicinoid biosynthesis are controlled by the availability of nitrogen (Monforte-

Gonzalez et al., 2010), while potassium fertilisation did not alter capsaicinoid content in Jalapeño and led to a reduction of capsaicinoids in Habanero (Johnson & Decoteau, 1996; Monforte-Gonzalez et al., 2010). Application of N, P and K was associated with an increase of pungency in Padrón pepper which correlated to a reduction of phenolic content (Estrada et al., 1998). This suggests that there is competition between lignin and capsaicinoid synthesis for a supply of intermediates (phenolic sources) during fruit maturation (Hall et al., 1987; Sukrasno & Yeoman, 1993). In addition, water stress treatments increased capsaicinoid content in pepper (Estrada et al., 1999b) which may explain that additional substrates for capsaicinoid synthesis may be sourced from cell wall metabolism under stress condition (Holden et al., 1987).

Pre-harvest factors have been reported to affect capsaicinoids in chillies and peppers. Fruit from lower nodes have higher pungency than fruit from higher nodes which can be explained by lower competition by other fruit on the plant for substrates, in the case of at lower nodes (Zewdie & Bosland, 2000). In contrast, Estrada et al. (2002) showed higher capsaicinoid concentration in the apical fruit than in the basal fruit suggesting that light exposure may stimulate capsaicinoid formation in the apical fruit (Iwai et al., 1979). The contrasting results of these two pieces of research demonstrates the variation of capsaicinoids that can be observed from different growing locations (Harvell & Bosland, 1997). Plant to plant variation within the same plot or fruit to fruit variation within one plant has been reported previously in chilli (Harvell & Bosland, 1997; Kirschbaum-Titze et al., 2002b; Mueller-Seitz et al., 2008). Growing season and environment has been reported as an important factor affecting pungency of chillies and peppers, for example pepper fruit grown in hot weather showed higher capsaicinoid content than fruit grown in cold weather (Harvell & Bosland, 1997; Estrada et al., 1999a). Due to the variation and inconsistencies of capsaicinoids observed previously in each chilli species, the capsaicinoid content is not used as chemotaxonomic indicator for *Capsicum* species (Zewdie & Bosland, 2001).

During fruit maturation, capsaicinoid concentration in most chillies and peppers increased and then decreased at fully mature stage in some cultivars (Iwai et al.,

1979; Estrada et al., 1997; Contreras-Padilla & Yahia, 1998; Estrada et al., 1999b; Estrada et al., 2000; Gnayfeed et al., 2001; Jha et al., 2001; Materska & Perucka, 2005; Conforti et al., 2007; Menichini et al., 2009; Pandey et al., 2010). Loss of capsaicinoids coincided with an increase of peroxidase enzyme acitivity (Contreras-Padilla & Yahia, 1998; Estrada et al., 2000).

During storage, capsaicinoid content was retained at low storage temperature in both fresh chilli and chilli powder (Gonzalez et al., 2005; Wang et al., 2009). Gonzalez et al. (2005) demonstrated a consistent capsaicinoid concentration during storage for Habanero at 7 °C for 20 days, after which concentration tended to decrease after moving to room temperature. However little information is available on capsaicinoid concentration in fresh chilli and pepper in other varieties and cultivars during storage.

1.3.2 Vitamin C

Chillies and peppers are recognised as a good source of vitamin C as most chilli and pepper fruit contain vitamin C content over of the recommended daily intake (RDI) of vitamin C (90 mg per day for males and 75 mg per day for females). Ascorbic acid is easily oxidised to dehydroascorbic acid (DHA) in the presence of O_2, but they both retain vitamin C activity (Howard et al., 1994; Howard, 2006). However, only ascorbic acid has been reported in most research because DHA concentration detected in most chilli fruit is less than 1 mg.100g^{-1} (Wimalasiri & Wills, 1983).

Vitamin C concentration in chilli and pepper fruit varies depending on cultivar (Howard et al., 1994; Mozafar, 1994; Osuna-Garcia et al., 1998; Howard et al., 2000; Howard, 2006; Deepa et al., 2007). Vitamin C is mainly found in the chloroplast (Mozafar, 1994; Conklin, 1998; Asensi-Fabado & Munne-Bosch, 2010), also may be transported from leaves to other parts of plant (Mozafar, 1994). In general, growing conditions affect nutritional composition in fruit, for example an increase of vitamin C concentration was found in citrus (Winston & Miller, 1948), kiwifruit (Remorini et al., 2007), lettuce (Grimstad, 1984), parsley (ÅBerg, 1949) and starfruit (Averrhoa carambola) (Zabedah et al., 2009) when fruit were exposed to light. This effect can be explained by the increase in sugar (e.g. glucose and sucrose etc.) produced from

photosynthesis, which is a precursor for vitamin C synthesis (Harris, 1977; Mozafar, 1994; Lee & Kader, 2000). In contrast, higher vitamin C was found in guava (*Psidium guajava*) (Asrey et al., 2007) and cherry tomato (*Solanum lycopersicum* var. cerasiforme) (Gautier et al., 2005) fruit located at lower nodes than fruit from higher or distal nodes, which was suggested to be a result of less competition for assimilate of fruit at lower nodes. In this research, a correlation between sugar and vitamin C accumulation was not found (Gautier et al., 2005).

In order to understand effects of fruit competition and assimilate availability, cultural practises such as pruning or thinning have been used to investigate effects on vitamin C (Lee & Kader, 2000). For example higher vitamin C concentration was found in peach fruit (*Prunus persica* Batsch) from commercial crop load than those from low crop load (Buendia et al., 2008), while crop load did not affect ascorbic acid in Jonagold apple (Stopar et al., 2002), cherry tomatoes (Gautier et al., 2005) and starfruit (Zabedah et al., 2008). These observed results are useful for growers to realize the important of pre-harvest factors on nutritional composition in fruit. However no research of these factors has been done on chemical composition in chillies and peppers.

Maturity at harvest is an important factor for vitamin C content in fruit (Lee & Kader, 2000); in chillies and peppers, ascorbic acid concentration (per g fresh weight) increased during fruit maturation (Rahman et al., 1978; Howard et al., 1994; Osuna-Garcia et al., 1998; Howard et al., 2000; Marín et al., 2004; Howard, 2006). However when vitamin C concentration has been reported per dried weight, ascorbic acid was highest at turning colour stage or mature green stage (Markus et al., 1999; Fox et al., 2005; Navarro et al., 2006; Deepa et al., 2007).

During storage, vitamin C is influenced by storage temperature. Vitamin C in bell pepper decreased by 10 and 25 % during storage for 10 days at 10 and 20 °C respectively, but no effect of polyethylene bags was found on ascorbic acid concentration (Watada et al., 1987). However green sweet bell pepper lost almost 50 % of vitamin C during storage at 7 °C for 45 days (Watada et al., 1987). In contrast, Wang (1977) and Tonelli et al. (1981) showed an increase of vitamin C in peppers

during storage at 13 °C and ripening at 20 °C due to changing of starch to sugar during the ripening process, which may increase vitamin C accumulation. Modified atmosphere packaging (MAP) has been reported to delay vitamin C degradation during storage with a combination of storage at low temperature in both whole and fresh cut chilli and pepper (Hernandez & Howard, 1996; Howard & Hernandez-Brenes, 1998; Sakaldas & Kaynas, 2010), while no effect of controlled atmosphere (5-20 % CO_2) was found on vitamin C content in bell pepper during storage at 2 and 8 °C for 6 - 12 days (Cappellini et al., 1984). A loss of vitamin C has been reported when chillies and peppers are washed with chlorinated water so the recommendation is to wash fruit with 50 - 100 $\mu g.ml^{-1}$ hypochlorite for 20 min, which can control microbial contamination with no effects on the quality of bell pepper (Nunes & Emond, 1999).

1.3.3 Antioxidant activity (AOX) and total phenolic content (TPC)

Chillies and peppers are known as a good source for antioxidant. Antioxidant activity (AOX) is contributed from ascorbic acid, carotenoid, phenolic compounds (TPC) such as capsaicinoids, and flavonoids (Howard et al., 2000; Materska & Perucka, 2005; Howard, 2006); therefore changes of AOX should relate to changes of these compounds. A correlation between AOX and TPC has been previously reported in several research reports (Howard et al., 2000; Fox et al., 2005; Conforti et al., 2007; Deepa et al., 2007; Sun et al., 2007). High correlations (R^2 = 0.78 - 0.89) between AOX and TPC were found in bell pepper (Fox et al., 2005; Sun et al., 2007) while weak correlation or no correlation was found in some chilli and pepper cultivars (Howard et al., 2000; Conforti et al., 2007; Deepa et al., 2007; Serrano et al., 2010). These results indicate that TPC is a major contributor to AOX in bell pepper but may not contribute to AOX in other chilli and pepper cultivars. As the chemical composition of chilli and pepper varies depends on variety; the AOX may be contributed by different compounds.

AOX has been reported to increase with fruit maturation: red chilli had higher AOX than green fruit (Howard et al., 2000; Fox et al., 2005; Materska & Perucka, 2005; Conforti et al., 2007; Deepa et al., 2007; Sun et al., 2007; Serrano et al., 2010) while phenolic compounds (TPC) in chillies and peppers, i.e. capsaicinoids, and

flavonoids, varied during fruit ripening depending on cultivar (Estrada et al., 2000; Howard et al., 2000; Fox et al., 2005; Materska & Perucka, 2005; Navarro et al., 2006; Conforti et al., 2007; Deepa et al., 2007; Menichini et al., 2009). For example TPC in Habanero and Padrón peppers decreased during maturation (Estrada et al., 1997; Menichini et al., 2009). Little information has been reported of effects of other pre-harvest factors on AOX and TPC particular in chillies and peppers.

1.4 Physical damage

1.4.1 Skin cracking or splitting

Major physical defects found in many fruit such as apple, cherry, tomato, chilli and pepper are cracks, scars or splits on fruit skin. Fruit with severe cracking can lead to a commercial loss in market (Bakker, 1988; Byers et al., 1990; Sekse, 1995; Aloni et al., 1998; Aloni et al., 1999; Demirsoy & Demirsoy, 2004; Dorais et al., 2004; Opara et al., 2010). Cracking can occur in both intact fruit on the plant and detached fruit during handling and storage (Mohsenin, 1972; Aloni et al., 1998; Aloni et al., 1999). The severity of cracking on fruit skin increases as fruit ripen.

Cracks present on fruit skin can change the structural integrity and reduce mechanical strength (Opara et al., 2010). In addition, crack areas are likely open wounds that could accelerate higher water loss, shrivel development and higher contamination by fungi and moulds (Reynard, 1951; Goode et al., 1975; Meyer, 1994). Overall, shelf-life of cracked fruit is shorter than non-cracked fruit. Skin cracking (i.e. cuticular cracking or lenticel cracking) is defined as fractures on fruit skin and appears only in the cuticular layer while splitting or flesh cracking is defined as when the cracks break into internal flesh (Opara et al., 2010). Skin cracking that can be seen by eyes or microscope begins to rupture at lenticels which are expected to be a weak point of the skin (Teaotia & Singh, 1970).

Cracking symptoms begin to appear when fruit attain their full size. Mature fruit tend to be more cracked than immature fruit (Aloni et al., 1999; Dorais et al., 2004; Opara et al., 2010). Fruit with rapid growth rates which generally occurs in low crop loads are likely to crack in tomato (Bakker, 1988; Peet, 1992; Dorais et al., 2004) and cherry (Measham et al., 2012). Characteristics of fruit skin also affect cracking. Fruit

with stronger and more elastic cuticular membranes are less susceptible to cracking (Peet, 1992; Sekse, 1995; Demirsoy & Demirsoy, 2004; Matas et al., 2004).

Irregular water supply including unexpected rainfall or late irrigation before harvest can cause high soil moisture and excess water uptake in fruit, which leads to cell enlargement and high hydrostatic pressure and reduces cell wall strength and skin elasticity (Peet, 1992; Sekse, 1995; Aloni et al., 1998; Dorais et al., 2004).

Growing conditions such as high RH (99-100 %) and temperature can cause severe cracking in fruit e.g. apple, tomato and pepper (Verner, 1935; Peet, 1992; Aloni et al., 1998; Moreshet et al., 1999). Fruit expansion and shrinkage due to temperature swings during fruit development also cause cracking on fruit skin (Moreshet et al., 1999). Cracking was mostly found in fruit exposed to sun which may lead to an inelastic cuticle such as in bell pepper (Aloni et al., 1999), tomato (Dorais et al., 2004) and some apple cultivars e.g. 'James Grieve' and 'Beauty of Bath' (Tetley, 1930; Knuth & Stosser, 1987; Opara et al., 2010). However, some apple cultivar such as 'York Imperial' show a high number of cracked fruit on shaded side (Shutak & Schrader, 1948). From this review, cracking on fruit skin is mainly related to fruit growth rate and growing condition and may differ in each fruit type or variety as they have different skin structures. It would be useful to growers if cracking can be controlled.

1.4.2 Chilling injury

Chilling injury is a disorder observed when fruit (particularly tropical and subtropical) are stored at low temperature (Kader, 2002b; Wills et al., 2007). Chillies and peppers are susceptible to chilling injury when fruit are stored below 7 °C (Moline & Hruschka, 1977; Lin et al., 1993b; Gonzalez-Aguilar, 2004; Lin, 2005; Lim et al., 2007; Lim et al., 2009). In general, chilling injury symptoms in chillies and peppers include surface pitting, water soaked areas, decay and discolouration of seed cavity as well as an increase of respiration rate, ethylene production and electrolyte leakage (Paull, 1990; Gonzalez-Aguilar, 2004). The severity of chilling injury depends on variety, maturity at harvest and storage period (Thompson, 1979; Sullivan & Bramlage, 2000; Lim et al., 2009; Lim & Woolf, 2010). For example, in

one study Hungarian wax was the most susceptible variety in which scald symptoms appeared after 4 days at 2.5°C while pitting in Serrano was only observed after 23 days at 2.5°C (Sullivan & Bramlage, 2000). Skin pitting as a surface depression under microscope was more severe in chilling sensitive cultivar of hot pepper (Lim & Woolf, 2010). Bell pepper harvested at breaker stage tended to be the most susceptible to chilling injury after storage at 1 °C for 2 weeks but no chilling injury symptoms were observed in bell pepper harvested at the red ripe stage (Lim et al., 2007). Similar results were found in greenhouse grown 'Bison' and 'Doria' pepper stored at 1°C for 1 or 2 weeks where chilling injury occurred in mature green peppers but not in ripe peppers. High CO_2 and C_2H_4 productions were found in mature green and breaker stage fruit exposed to chilling temperature (Lin et al., 1993b; Lim et al., 2007). Chilling injury symptoms in some peppers are observed when fruit are moved to room temperature. For example Lin (2005) found an increase of decay in sweet pepper when fruit are moved to room temperature after storage at 1 and 2.5 °C for 4 weeks. Application of an antioxidant e.g. diphyenylamine (DPA) by dipping or injecting into the seed cavity before storage at low temperature (1°C), can reduce chilling injury in green bell peppers (Purvis, 2002).

1.5 Postharvest storage treatments

Physiological changes after harvest can reduce marketability of chilli fruit. Postharvest treatment can be applied to delay these changes and prolong storage life of chillies.

1.5.1 Low temperature storage

Postharvest temperature is an important factor that influences quality of fresh horticultural commodities. Rate of deterioration (i.e. respiration and ethylene production etc.) increases about 2 - 3 fold for each 10 °C increase in product temperature, but storing fruit at too low temperature can cause chilling injury in some fruit (Thompson, 2002). Temperature management is the most important method for extending shelf-life of fresh produce. Optimum temperature for bell peppers and Paprika is 7 - 10°C, while 5 - 10° is a suitable temperature for hot pepper and chillies (Thompson, 1979; Thompson, 2002). However, most previous

research studies utilised the combination of temperatures and packaging and a few research studied on low temperature only.

1.5.2 Packaging

Fresh produce quality and freshness preservation can be assisted by packaging. To maintain quality and extend postharvest life of fresh fruit and vegetables, modified atmosphere packaging (MAP) is applied to control the level of CO_2 and O_2 in the atmosphere surrounding the commodity (Kader et al., 1989; Reid, 2002). The benefit of MAP are reducing water loss, respiration rate, ethylene production, and extending shelf-life of chillies (Govindarajan, 1985; Zagory & Kader, 1988). The recommended storage conditions for bell pepper are 2 - 5 % O_2 and 2 - 5 % CO_2 at 8 °C temperature while 3 - 5 % O_2 and 0 - 5 % CO_2 are recommended for fresh chilli when stored at 8 °C (Reid, 2002).

Many types of packaging have been reported in previous research. Habanero packed in perforated polyethylene bags can be stored for 20 days at 7 °C (Gonzalez et al., 2005). Hot peppers (*C. frutescens*, L.), packed in microperforated high density polyethylene (HDPE) bags and stored at 10 °C for 25 days showed only 4 % decay while fruit packed in HDPE bags and stored at 5 °C for 30 days showed severe CI symptoms expressed as increased electrolyte leakage after transferring to 28 - 30°C for 1 - 5 days (Mohammed et al., 1993). Similar results were found in red bell pepper packed in polylactic acid (PLA) bags, low-density polyethylene (LDPE) and perforated LDPE bags and stored at 3 and 7.5 °C for 14 days. Fruit packed in PLA bags had less water loss and decay, fewer CI symptoms and lower counts of coliform bacteria than green peppers packed in LDPE and perforated LDPE bags. There were no differences in colour, firmness, and ascorbic acid content as influenced by packaging (Koide & Shi, 2007). Each packaging has different properties which can be selected to suit different kind of chillies or peppers.

In MAP, O_2 concentration should not be less than 3 % and CO_2 concentration should not be higher than 10 % during storage (Meir et al., 1995; Koide & Shi, 2007) otherwise fruit can be susceptible to anaerobic respiration. Previous research has reported on effects of O_2 and CO_2 concentrations on fruit quality. LDPE and PE can

induce modified atmospheres close to the optimal gas concentrations (3 % O_2 and 5 % CO_2), which were required to maintain quality of green chilli peppers (Lee et al., 1993) and green chillies (New Mexican type) (Wall & Berghage, 1996). Bussel & Kenigsberger (1975) showed a delay of weight loss and colour change in green bell peppers during storage at 25 °C in PVC film bags (14 - 16 % of O_2 and 0.5 - 3 % CO_2). Akbudak (2008) found a delay of colour changes and maturity in pepper (*C. annuum*, L. cv.'Yalova Charleston') packed in PE bags during storage for 30 days. Fresh-cut bell peppers packed in breathable bags (PD-961EZ) had better visual quality, less leaked juice and higher firmness than those vacuum packed and stored at 5 °C for 21 days (Gonzalez-Aguilar et al., 2004). When a wax coating is combined with packaging (LDPE bag), a delay of colour changes, weight loss, loss of firmness and decay was found in bell pepper stored at 10°C for up to 40 days (Gonzalez & Tiznado, 1993). Overall, O_2 and CO_2 concentrations could be controlled in the package in combination with storage temperature to prolong shelf-life of chillies and peppers.

1.6 Aims and research objectives

Quality of chillies and peppers decreases after harvest as a result of physiological and phytochemical changes. Shrivel occurrence and loss of firmness are a major concern on chilli quality attributes therefore postharvest treatment (such as cool temperature and high humidity) is required with the optimum conditions to maintain the quality and prolong storage life after harvest.

However, optimising postharvest storage of chilli fruit is not enough to gain highest quality products in the market place if there is a significant variation in fruit size or quality of chillies and peppers found at harvest, which will limit final fruit quality. This variation may come from pre-harvest factors such as time of planting, position on plant, maturity at harvest or crop load, which have been reported in other fruit but with little information available for chillies and peppers. Developing knowledge on the factors that influence chilli variability will be valuable for chilli growers to deliver consistent product and potentially develop management techniques to manipulate the fact to maximise factors which are desirable in the market place.

The objectives of this research were:

- To determine an optimum storage temperature that can maintain quality and prolong storage life of three chilli varieties (Habanero, Jalapeño and Paprika)
    - To define physiological and quality changes (e.g. respiration rate, firmness, colour change, chilling injury and phytochemical compounds) of three chilli varieties during storage at these temperatures
- To define factors affecting to shrivel of Jalapeño during storage
    - To determine the route of water loss in Jalapeño
    - To compare water loss between cracked and non-cracked Jalapeño
    - To define factors influencing water vapour permeance (P'_{H_2O}) of Jalapeño
    - To predict rate of water loss in Jalapeño at different condition scenarios
- To develop understanding of the factors which contribute to variability in chilli quality at harvest
    - To compare quality of Jalapeño which were planted in commercial and controlled glasshouses
    - To define size, shape, colour and phytochemical compounds of Jalapeño during fruit maturation
    - To determine effects of pre-harvest factors (times of planting, positions on plant, maturity at harvest, crop loads) on size, shape, colour and phytochemical compounds of Jalapeño

CHAPTER 2

Materials and methods

2.1 Introduction

The aim of this project was to understand the physiological and biological changes of some varieties of chillies (*Capsicum* spp.) during maturation and after harvest during the postharvest period so as to obtain premium and uniform fruit while retaining a full nutritional complement. Enhancing quality and maintaining postharvest life would be useful for chilli growers to increase market opportunities in both domestic and international markets.

2.2 Plant management

In 2007 - 2008**,** Habanero (*C. chinense*), Jalapeño (*C. annuum*) and Paprika (*C. annuum*) which vary from non pungent Paprika to very pungent in Habanero were investigated. Three chilli varieties were supplied from a commercial glasshouse (Orcona Chillis 'N Peppers) located at Napier in New Zealand.

In 2008 - 2009 the Jalapeño variety was selected for further study in a commercial glasshouse. Jalapeño seeds (F1) (*C. annuum* cv. Conchos), widely grown in USA and Mexico, were supplied from Johnny's Selected Seeds (Winslow, Maine, USA). Jalapeño seeds were germinated on trays and placed on a heating table with plastic covering, where temperature was monitored at 18 °C. After 1 month, Jalapeño seedlings were transferred to black buckets (10 L) with drip irrigation 3 times a day. Fertilization i.e. N and K was applied during fruit development. Temperature in glasshouse was monitored between 18 - 28 °C during day time and 7 - 14 °C overnight.

Jalapeño plants in Napier were randomly divided into 6 blocks with 5 plants per block in case there was a variation on the location. Flowers were tagged at full bloom and subsequent measurements were taken as days from flowering. Temperature and humidity were recorded using TinyTag Ultra (Gemini) data loggers

(Energy Engineering Ltd., West Sussex, UK). Chilli fruit were harvested at weekly intervals from 1 to 9 weeks after flowering.

Due to the large variation in fruit size and limitation of fruit number in previous years, in the 2010 season Jalapeño plants were grown in a glasshouse at Plant Growth Unit, Massey University, Palmerston North, New Zealand (PGU glasshouse). In order to study physiological changes of Jalapeño fruit during maturation as influenced by seasonal effects, three lots of seeds were germinated at one month intervals starting from August to October 2009. Jalapeño seeds (F1) (*C. annuum* 'Conchos') from Johnny's Selected Seeds (Winslow, Maine, USA) were germinated in Grodan multi blocks size 50 cm (L) x 25 cm (W) x 4 cm (H) covered with medium vermiculite to prevent moisture loss and placed on a table which has water running at 20 - 22 °C with pH of water controlled at 5.8. Jalapeño seedlings were left for 1 month to develop adequate roots, before being transferred to 10 L black buckets with Daltons base mix (50 % C.A.N calcium and ammonium nitrate and Fines A Grade, a 30 % fibre (Pinus radiata cambium bark which has been shredded into fibres): 20 % Pacific pumice). The mix was pre-fertilised with 1 kg of serpentine super containing 16 % nitrogen (8.5 % nitrate nitrogen + 7.5 % ammoniacal nitrogen): 3.5 % phosphorus (soluble in neutral ammonium citrate and water): 10 % potassium (soluble in chloride free water): 2.4 % sulphur (sulphates form): 1.2 % magnesium per 100 L Daltons base mix.

The 90 Jalapeño plants (30 plants per month) were grown in 40 m^2 glasshouse (Fig. 2.1) with 30 plants per lot and 10 plants per row. After transferring to black buckets, the plants were tied to wires to avoid collapse. Plants were trained to two leaders to control fruit production and quality of the fruit and plants were allowed to grow until they reached the top wire (approximately 16 - 20 nodes). In each row, each plant was alternatively designated as either high crop load (H), in which the plant was allowed fruit to grow in every node; or low crop load (L), in which flowers were removed from the plant so that there was a fruit at every 4^{th} node (e.g. fruit at nodes 4, 8, 12, 16, and 20). Nodes were counted from the first node at the bottom of each plant (Fig. 2.2).

There were 2 solutions which were stock solution A (adding calcium nitrate 19.80 kg + potassium nitrate 13.16 kg into 200 L solution tank) and stock solution B (adding magnesium sulphate 9.94 kg + mono potassium phosphate 5.44 kg + Iron chelate 600 g + manganous sulphate 100 g + zinc sulphate 7 g + copper sulphate 6 g + boric acid 36 g + ammonium molybdate 1.6 g into 200 L solution tank).

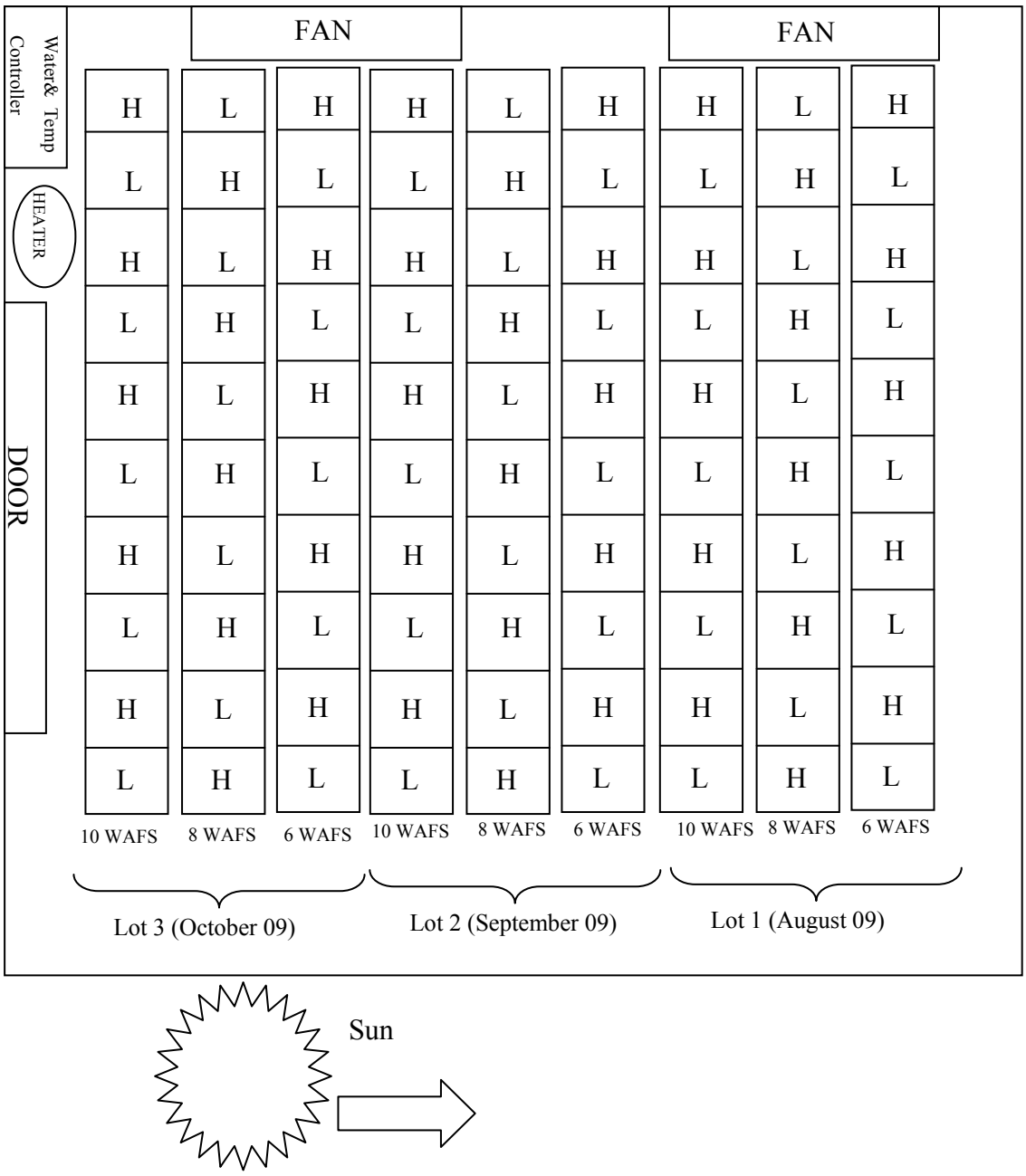


Figure 2.1 Layout of the Jalapeño plants in the glasshouse; H = high crop load and L = low crop load. Chilli fruit were harvested at 6, 8 and 10 weeks after fruit set (WAFS).

The medium solution A:B (1:1) was diluted with water 1:100 and supplied by drip irrigation 3 times a day from 8 am to 8 pm at 4 hour intervals (with no watering at night). All plants were sprayed approximately once a month with Attack™ (pyrethroid and organophosphate), Chess™ (pyridine azomethine), and Nuvos™ (dichlorvos). Whitefly and aphids were the only pests found in the glasshouse. The temperature of the glasshouse was set between 16 and 25 °C by heater and fan.

In 2009, full bloom chilli flowers were tagged weekly and maturity was defined as weeks after flowering (WAF) but approximately 50 % of fruit aborted in 2009. As this was not satisfactory in 2010 chilli fruit were tagged once fruit began to set and maturity of fruit was determined as weeks after fruit set (WAFS) for each individual fruit. Fruit were harvested at 6, 8 and 10 WAFS in order to obtain fruit at different maturity stages for subsequent physiological analysis.

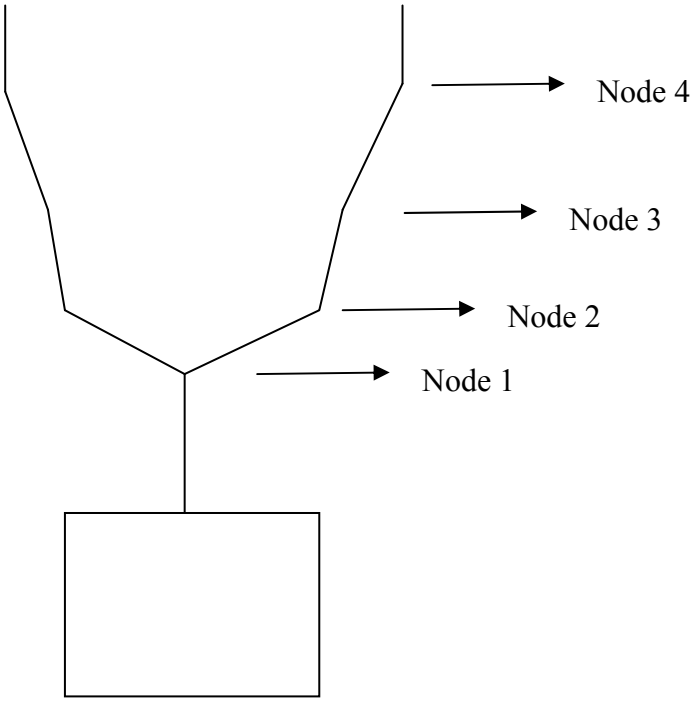


Figure 2.2 Basic sketch of plant indicating leaders and number of nodes.

A detail of chilli varieties, fruit sources used in this research was shown in Table 2.1.

Table 2.1 Varieties and fruit sources for each year of research.

Year	Varieties	Fruit source
2007 - 2008	Paprika, Jalapeño, Habanero	Orcona Chillis 'N Peppers, Napier, NZ
2008 - 2009	Jalapeño	Orcona Chillis 'N Peppers, Napier, NZ
2009 - 2010	Jalapeño	Plant Growth Unit, Massey University, NZ

2.3 Fruit handling and preparation

2.3.1 Experiments in 2007 - 2008

Commercially produced fruit of the three varieties were harvested and sent to Palmerston North on the following day. In this year, fruit were selected by the grower as they used size and colour as commercial maturity.

On arrival at Palmerston North, fruit free from decay or damage were selected and washed with 100 ppm chlorinated water (Janola bleach). Fruit were then air dried and randomly allocated into groups of 10 fruit weighing approximately 280 - 350 g. Each group was placed into a 25 cm × 30 cm × 35 μm low density polyethylene (LDPE) bag containing six 5 mm diameter holes. Bags were stored at 0, 4, 8, 12, and 20 °C respectively as treatments with three bags per variety. Total bags were 3 bags × 3 varieties × 5 temperatures = 45 bags with 150 fruit per variety. Physiological and quality evaluations were measured at weekly intervals.

2.3.2 Experiments in 2008 - 2009

Jalapeño (*C. annuum* 'Conchos') fruit were grown commercially in a glasshouse (Orcona Chillis 'N Peppers) located at Napier. Chilli fruit were harvested at different maturity stages defined by weeks after flowering (WAF) (from 2 - 9 WAF). Fruit were transported to the laboratory in Palmerston North within 3 hours of harvest where weight, shape and colour were measured for individual fruit. Each fruit was

cut into two halves from stem end to apex. One half was dried in an oven at 60 °C and later kept in a desiccator for capsaicinoid measurement while the other half was frozen in liquid N_2 and stored at -70 °C for other chemical analysis.

Cracked and non-cracked Jalapeño fruit were harvested also and used for determination of water loss and water vapour permeance of each anatomical part (i.e. calyx and skin) and compared with the whole fruit.

2.3.3 Experiments in 2009 - 2010

Jalapeño (*C. annuum* 'Conchos') fruit grown in Plant Growth Unit at Massey University, Palmerston North from sequential plantings (August - October) were harvested from different positions on plant, maturity stages (6, 8, and 10 WAFS) and crop load (high and low). Physical (weight, size, density and colour), physiological (respiration) and phytochemical attributes (ascorbic acid, capsaicinoid, antioxidant activity (AOX) and total phenolic concentration (TPC) were analysed.

2.4 Physiological and quality evaluation

2.4.1 Respiration rate

Respiration rate was measured by measuring accumulation of carbon dioxide (CO_2) within an enclosed container (Utto, 2001). Individual fruit were placed in a sealed glass container (2 L for Paprika, 500 mL for Jalapeño and 130 mL for Habanero). A 1 ml gas sample was taken immediately after closing containers and after a known period of time. Each sample was injected into a gas chromatograph with a miniature infrared CO_2 transducer (Analytical Development Company, Hoddesdon, UK) with O_2 - free N_2 as a carrier gas (flow rate 35 $mL.min^{-1}$). Output signals were analysed by integrator (Hewlett Packard, Model 3394A). Respiration rates were measured at 5 temperatures (0, 4, 8, 12, and 20 °C) with 30 individual fruit measured per temperature. Delay between sampling times was altered based on temperature of assessment with 1 hour for fruit at 20 °C and 2, 3, 6, and 12 hours for 12, 8, 4, and 0 °C respectively. Concentration of CO_2 generally remained below 0.5 % to avoid interference with respiration.

Respiration rate was calculated by Eq. 2.1;

$$r_{CO_2} = \frac{(V_{jar} - \frac{M_f}{\rho})(P_{CO_2}^{final} - P_{CO_2}^{initial})P^{total}}{R(T_f + 273.15)M_f t} \quad \textbf{Eq. 2.1}$$

where:

r_{CO_2} = respiration rate (mol.kg^{-1}s^{-1})

V_{jar} = volume of jar (m^3)

M_f = fruit mass (kg)

ρ = density of fruit (kg.m^{-3})

$P_{CO_2}^{final}$ = CO_2 concentration after certain period (%)

$P_{CO_2}^{initial}$ = CO_2 concentration immediately after closing container (%)

P^{total} = estimated total air pressure (Pa)

R = universal gas constant (8.3145 J.mol^{-1}K^{-1})

T_f = temperature (°C)

t = time (s)

2.4.2 Respiration rate model

Modelling of temperature dependence of the respiration rate (R_c) of each chilli variety was conducted by applying the Arrhenius equation:

$$R_c = A\exp\left[\frac{-E_a}{RT}\right] \quad \textbf{Eq. 2.2}$$

Where:

A = pre-exponential factor (mmol(CO_2)kg^{-1}s^{-1})

E_a = apparent activation energy (J.mol^{-1})

R = ideal gas constant (= 8.314 J.mol^{-1}.K^{-1})

T = temperature (K)

The apparent activation energy (E_a) and pre-exponential factor (A) were determined from the slope and intercept given by a linear regression of ln (R_c) vs (1/T) at each

time of measurement.

2.4.3 Water loss

Fruit were weighed to 0.001 g precision (Mettler-Toledo PG 503s, Medic Corporation Limited, NJ, US) and weight loss was calculated (Utto, 2001).

$$\text{Weight loss } (W_L) = \frac{(M_0 - M_t)}{t} \text{ (g.s}^{-1}\text{)} \qquad \textbf{Eq. 2.3}$$

Water loss was calculated and expressed as a percentage;

$$\text{Water loss (\%)} = \frac{(\frac{W_L}{18} - R_c)t}{M_0} \times 100 \qquad \textbf{Eq.2.4}$$

Where:

M_0 = Fruit mass at the beginning of the experiment (g)

M_t = Fruit mass during time of storage (g)

t = Storage time (s)

R_c = Rate of carbon loss from respiration rate (mol.s^{-1})

2.4.4 Colour measurement

Fruit surface colour was measured by reflectance spectrophotometer (CM-2600D, Konica Minolta, Albany, New Zealand) with 8 mm as the measurement area value (MAV). The device was set with the observer at 10°, illuminant source C and 100 % full UV. Reflectance was used with spectral component included (SCI). Colour was measured at three locations around the fruit shoulder. Measurements were read as the average L*, a* and b* values using Spectramagic NX software (CM-S100w, Konica Minolta, Albany, New Zealand) (Pranamornkith, 2009).

2.4.5 Firmness measurement

2.4.5.1 Compression test

Pericarp of chilli was prepared by cutting tissue into 1.5 × 1.5 cm squares. The compression test was undertaken using a texture analyser (TA-XT2i, Stable Micro System, Godalming, UK). A 5 mm diameter flat end probe was used to compress into the inner side of the sample at 5 mm.s^{-1} to 50% strain (Jansasithorn et al., 2010). Measurements were conducted at room temperature with 20 slices measured per

treatment. Peak force during penetration was recorded.

2.4.5.2 Tensile test

The 5 mm thickness fruit rings were cut from the fruit equator with a razor blade to get 3 rings per fruit. Seeds and placenta were removed before measurement. The ring was mounted vertically on tensile probes (Fig. 2.3) and the probes moved apart at 50 $mm.min^{-1}$ speed until the ring ruptured (Jansasithorn et al., 2010). Rings ruptured at the side that touched the probe and the peak force was recorded. The tensile test was measured in three chilli varieties.

Figure 2.3 Tensile test of Jalapeño rings.

2.5 Physical properties

2.5.1 Fruit density

Density of chilli fruit was measured by immersing the entire fruit into water (Utto, 2001). The displacement weight of water (density = 1 $g.cm^{-3}$) was measured as volume and density was calculated by Eq. 2.5:

$$\rho = \frac{M}{V} \qquad \textbf{Eq. 2.5}$$

Where:

V = fruit volume or the displacement weight of water (mL)

M = fruit mass (g)

ρ = density of fruit ($g.cm^{-3}$)

2.5.2 Surface area

Surface area of fruit was measured by image processing (ImageJ program, Image processing and analysis in Java) of a scanned image of a flattened cast of the fruit. In this case, 200 dpi (dots per inch) was set on the scanner. Fruit casts were created by

dipping fruit into egg white then covering with white tissue paper. Once dried, casts were cut in order to separate tissue from the chilli fruit, flattened and scanned with a black background. Previously, a surface area calibration curve was created using a data set of known surface area paper (Fig. 2.4).

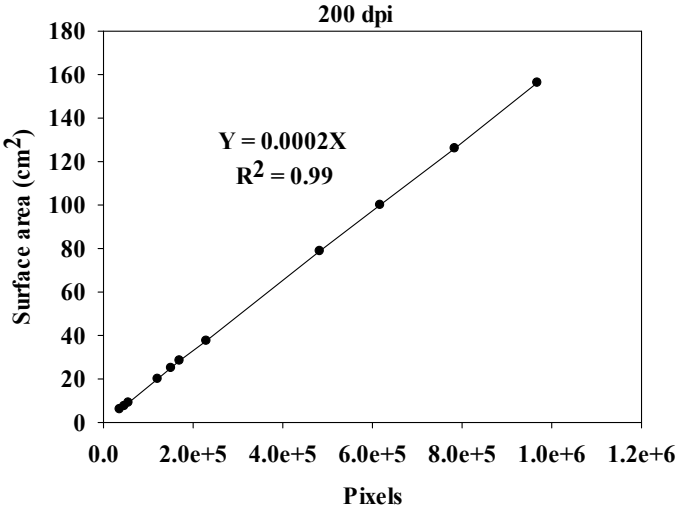


Figure 2.4 Calibration curve of pixels and known surface area.

2.6 Phytochemical analysis

Antioxidant activity (AOX), total phenolic concentration (TPC), capsaicinoids, and ascorbic acid were measured in Jalapeño during both maturation and subsequent storage at 8 or 20 °C. Fruit were halved longitudinally; one half was stored at -70 °C for ascorbic acid, chlorophyll, carotenoid, antioxidant activity (AOX) and total phenolic concentration (TPC) measurement and the other half was oven dried at 60 °C and stored for capsaicinoid measurement. Fruit were measured individually to capture data on fruit to fruit variation.

2.6.1 Antioxidant activity using ferric reducing antioxidant power (FRAP) assay

Before analysis, samples were freeze-dried and ground in a coffee grinder with liquid N_2 to create a powder. Ten milligrams (10 mg) of powder was extracted with 10 mL of extraction solvents (water or 50 % ethanol). The extracts were mixed well and left at 20 °C overnight. The FRAP method determines the capacity of the sample to reduce ferric ions (Benzie & Strain, 1996). Diluted FRAP reagent was freshly prepared on the day of analysis. The FRAP reagent consisted of 1:1:10 (v/v) of 10 mmol.L^{-1} TPTZ

(2,4,6-tripyridyl-*s*-triazine) in 40 mmol.L^{-1} hydrochloric acid; 20 mmol.L^{-1} ferric chloride and 300 mmol.L^{-1} acetate buffer at pH 3.6. Chilli extract (25 µL) was added to the FRAP reagent (275 µL) and absorbance at 595 nm was measured by Ultra Microplate Reader (EL_X 808, Bio-Tek Instrument INC, USA) after incubation at 37 °C for 30 min. Three replicates were measured per harvest maturity. A standard curve was created using a range of concentrations between 200 - 2000 µmol.L^{-1} of $FeSO_4 \cdot 7H_2O$ (Sigma Aldrich, NZ). Results were reported as µmol Fe (II) per litre of aqueous extract. Method verification was developed and described in chapter 7.

2.6.2 Ascorbic acid concentration

Ascorbic acid was extracted from frozen half chilli ground to powder using a coffee grinder and liquid N_2. Chilli powder was weighed (500 mg) into 15 mL plastic tubes. A modified ascorbic acid extraction was used (Morrison, 2003) in which potassium acetate (KOAc) (Merck, NZ) acidified to pH 3 with formic acid (1 ml) was added. The sample was mixed thoroughly with a Vortex mixer (Mode VM-100, Digisystem Laboratory Instruments Inc, Taiwan) and incubated at 4 °C on a Junior Orbit shaker (Labline, Vadodara, India) at 300 rpm for 1 hour. The sample was then centrifuged for 10 min at 3000 rpm at 15 °C (Heraeus Mutifuge 1S-R Centrifuge, Thermo Fisher Scientific, MA, USA) and the supernatant was filtered through a RC-membrane single use filter to a HPLC vial (Dionex Vial kit 1.5 ml/Slit septum). A Dionex HPLC instrument equipped with a P680 HPLC pump, ASI-100 automated sample injector and Thermostatted Column Compartment TCC-100 was used for analysis of ascorbic acid. A UVD 340U PDA detector was set at 254 nm. A C-18 column (Luna® 5 µm C18 100 Å, LC Column 150 x 4.6 mm) (Phenomenex, CA, USA) was used with SecurityGuard™ ULTRA cartridges for C18 column (Phenomenex, CA, USA). HPLC operation conditions are shown in Table 2.2.

Flow rate was 1.5 mL.min^{-1} and the HPLC was equilibrated at the initial condition (95 % solution A: 5 % solution B) for 7.5 min before injecting samples. The column was stored in 100 % MeOH before analyses.

Table 2.2 The ratio of mobile phase in HPLC operation conditions for of ascorbic acid measurement.

Time (min)	Solution A 0.1 M KOAc (pH 5 with formic acid)	Solution B 50 % Acetonitrile
0.0 - 3.0	95	5
3.0 - 3.5	84	16
3.5 - 5.5	0	100
5.5 - 6.0	0	100
6.0	95	5

Ascorbic acid was prepared as a standard in 0.1 M KOAc adjusted to pH 3 with formic acid at concentrations of 0, 10, 50, 100, 500, 700 $mg.L^{-1}$. The example of ascorbic acid peak was shown in Fig. 2.5 and the calibration curve of standard was used to evaluate the amount of vitamin C ($mg.g\ FW^{-1}$) in chilli samples.

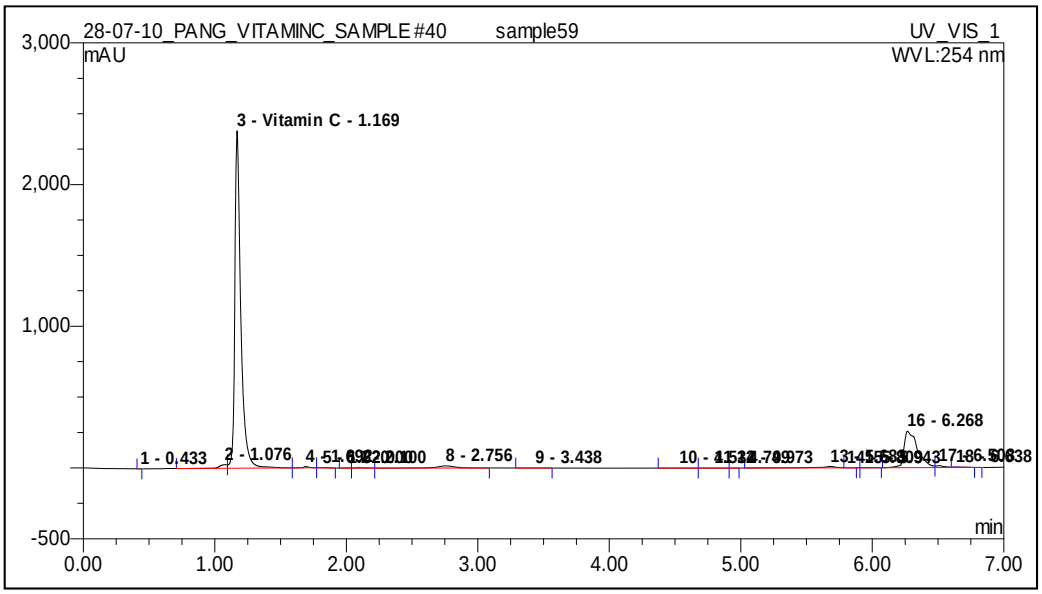


Figure 2.5 Example of HPLC chromatography of ascorbic acid peak in Jalapeño. Ascorbic acid concentration was calculated from comparing the retention time of the peak to those of standard compounds.

2.6.3 Determination of total phenolic concentration (TPC) by the Folin-Ciocalteu assay

Ten mg of freeze-dried sample was extracted with 10 ml of water or 50 % ethanol. Extracts were mixed well and left at 20 °C overnight. A modified Folin-Ciocalteu assay was used (Molan et al., 2008) in which 250 µL of 2 % Na_2CO_3 in water (Sigma Aldrich, NZ) was mixed with 12.5 µL of chilli extract in 96 well microplates. Folin-Ciocalteu phenol reagent (12.5 µL) was added; plates were shaken to mix and left at 25 °C for 30 min before reading absorbance at 650 nm. Three replicates were measured per harvest maturity. A standard curve was prepared using gallic acid (Sigma Aldrich, NZ) solution (100 - 1000 $\mu g.mL^{-1}$). The total phenolic concentration (TPC) of the extract was expressed in gallic acid equivalents (GAE) in $mg.gDW^{-1}$. Method verification is described in chapter 7.

2.6.4 Capsaicinoid concentration

Halve dried chilli was ground in a coffee grinder and kept in a 15 mL plastic tube before analysis. The capsaicinoid measurement method followed Collins et al. (1995). For capsaicinoid extraction, acetronitrile (AnalaR, BDH laboratory supplies, Poole, UK) was used as a solvent. A ratio of 10:1 of solvent: chilli powder was used. The 15 ml plastic test tubes were capped and placed in an 80 °C water bath for 4 hours and manually shaken hourly. Samples were then cooled to room temperature and centrifuged, followed by the supernatant being filtered through a RC-membrane single use filter to an HPLC vial (Dionex Vial kit 1.5 ml/Slit septum, USA). Vials were kept at 5 °C until analysis. A Dionex HPLC instrument equipped with a P680 HPLC pump, ASI-100 Automated sample injection and Thermostatted Column Compartment TCC-100 was used. A fluorescence detector (RT-2000) was set with excitation at 280 nm and emission at 338 nm. A C-18 column (150 × 4.6 mm) was used with a pre-column guard cartridge to prevent contamination. The HPLC operating conditions were set for a 20 min operation with 1 $ml.min^{-1}$ flow rate as shown in Table 2.3.

Table 2.3 The ratio of mobile phase in HPLC operation conditions for of capsaicinoid measurement.

Time (min)	Solution A 100 % Methanol	Solution B 10 % Methanol
0.0 - 10.0	57	43
10.0 - 20.0	68	32

A calibration curve was prepared from 8-methyl-n-vanillyl-6-nonanamide (capsaicin) and 8-methyl-n-vanillyl-nonanamide (dihydrocapsaicin) (Sigma-Aldrich New Zealand Ltd., Auckland, NZ) from 0 - 100 $mg.L^{-1}$ ($\mu L.L^{-1}$) in 100 % methanol. Chromeleon software (Dionex, Chromatography Data System, MA, USA) was used to operate and analyse sample peaks (Fig. 2.6). Method verification was developed and described in chapter 7.

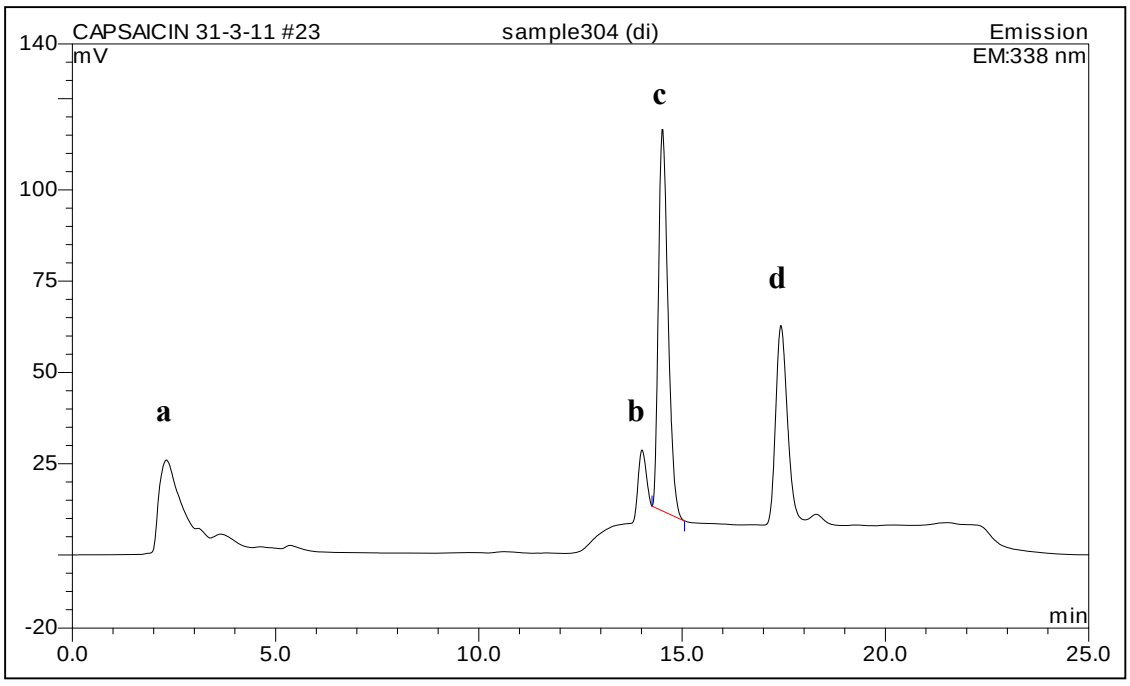


Figure 2.6 Example of HPLC chromatography separating pigment (a), nordihydrocapsaicin (b), capsaicin (c), dihydrocapsaicin (d) in Jalapeño. Capsaicinoid concentration was calculated from comparing the retention time of the peak to those of standard compounds.

2.6.5 Chlorophyll and carotenoid contents

A 200 mg sample of fresh chilli powder was extracted by adding 1 ml of acetone: methanol (7:3) + 200 mg $CaCO_3$ and thoroughly mixing in a vortex following Pranamornkith (2009). Afterwards, chilli extracts were centrifuged at 14000 rpm for 2 min (Eppendorf Minispin, Global Science, Auckland, NZ**)**. The supernatant was separated to a clean 15 ml tube covered in foil. Extraction was repeated by adding 1 ml of acetone: methanol (7:3). The supernatants were combined. Extraction was continued until the tissue became colourless. This supernatant was stored in the dark at 4 °C until the next step of the process. Pigments were isolated by adding 4 ml of diethyl ether and 4 ml of water, and then mixed gently. When the solutions were separated, the upper phase, that contained pigments, was removed to a labelled glass vial covered with foil. On occasions, addition of more water was required in order to achieve phase separation. Diethyl ether (2 mL) was added to the remaining solvent and mixed well. The upper phase was removed and combined into the previous vial. The ether phase was dried under N_2 using a water bath at 35 °C. The dried phase was resuspended in 1 ml of ethyl acetate (AnalaR, BDH laboratory supplies, England). A 950 µl of chloroform (AnalaR, BDH laboratory supplies, England) was added to 50 µl volume of the resuspended sample. The sample was measured at 480, 648 and 666 nm by spectrophotometer (UV-160A UV-Visible Recording Spectrophotometer, Shimadzu, Japan). The Wellburn equation (in chloroform) was applied to obtain in $\mu g.ml^{-1}$ of chlorophyll a (C_a), chlorophyll b (C_b), and total carotenoids (C_{x+c}) contents (equation 2.6 - 2.8) (Wellburn, 1994).

$$C_a = 10.91A_{666} - 1.2A_{648} \quad \textbf{Eq. 2.6}$$

$$C_b = 16.36A_{648} - 4.57A_{666} \quad \textbf{Eq. 2.7}$$

$$C_{x+c} = (1000A_{480} - 1.42C_a - 46.09C_b)/202 \quad \textbf{Eq. 2.8}$$

2.7 Statistical analysis

All data were analysed by analysis of variance (ANOVA) using the SAS statistics program version 9.1 (SAS Institute, Cary, NC, US). Comparison of means was performed by using least significant difference values (LSD) to evaluate significant differences at P = 0.05.

CHAPTER 3

Influence of storage temperatures on postharvest physiological and phytochemical changes of three chilli varieties

3.1 Introduction

Factors such as time of harvest, harvest method and maturity at harvest can affect fruit quality at harvest (Smith et al., 2006). After harvest, quality of fresh produce decreases as a result of physiological and phytochemical changes (Wills et al., 2007). Temperature management is considered as the most important method for extending the shelf-life of fresh produce (Wills et al., 2007). Rate of deterioration increases about 2 fold for each 10 °C increase in product temperature (Thompson, 2002). Respiration rate is considered to be a useful indicator of metabolic activity in fresh produce. Temperature is the main factor affecting respiration rates (Wills et al., 2007). Respiration rates of capsicums and chillies increase with increased temperature (Chen et al., 2000; Utto, 2001). Respiration rates of selected chilli varieties and cultivars are shown in Table 3.1.

Table 3.1 Postharvest respiration rate of chillies from previously published works.

Cultivar	Temperature (°C)	Production rate ($nmol.kg^{-1}s^{-1}$)	Source
'Changjiao'	25	499	Lu et al. (1990)
'Chooraehong'	20	947	Gross et al. (1986)
Paprika ('PS72285')	22	667	Krajayklang et al. (2000)
Cayenne	15	320	Utto (2001)

Chillies and peppers are non-climacteric fruit (Saltveit, 1977; Lurie et al., 1986; Lu et al., 1990; Biles et al., 1993), although 'Chooraehong' hot pepper was reported as climacteric (Gross et al., 1986). Chillies and peppers produce CO_2 at higher rates during fruit development while CO_2 productions decrease in fully developed fruit

(Biles et al., 1993; Villavicencio et al., 1999, 2001; Barrera et al., 2005; Thang, 2007; Barrera et al., 2008).

Generally, low temperature storage conditions have been used to maintain quality (such as appearance, texture and nutritional attributes) and extend shelf-life of fresh fruit and vegetables (Paull, 1999), but if the temperature is too low it can cause chilling injury, deterioration and quality loss. For chilli and pepper, the optimum temperature for postharvest storage is considered to be 7 - 13 °C and fruit can be stored for 3 - 4 weeks depending on cultivar (Thompson, 1979; Gonzalez-Aguilar, 2004) although hot chillies can tolerate temperatures of 5 - 10 °C (Thompson, 1979; Lin et al., 1993b; Lim et al., 2007). Most previous research related to chilli and pepper storage has focused on the role of packaging or a combination of modified atmosphere packaging and temperature to establish optimal storage conditions (Gonzalez & Tiznado, 1993; Lee et al., 1993; Mohammed et al., 1993; Meir et al., 1995; Wall & Berghage, 1996; Gonzalez-Aguilar et al., 2004). Only a few studies have been made on defining optimum storage temperature in specific chilli cultivars.

This chapter principally focused on establishing the quality changes of three New Zealand grown chilli varieties as a function of storage temperature. Respiration rate, texture, colour, chilling injury and chemical composition, including capsaicinoids and ascorbic concentration in fresh chillies were assessed as indicators of physiology and quality. In general, most growers use size or colour as their maturity indices of chillies and peppers. An understanding of the effects of storage temperature and variation of maturity at harvest on subsequent change of quality attributes in storage would be useful for growers and chilli marketers and potentially assist manipulation of the crop to fit consumer needs. In this work, Habanero, Jalapeño and Paprika, harvested at commercial maturity were stored at different temperatures; quality changes during storage were measured to understand effects of temperature.

3.2 Materials and methods

Three chilli varieties (Habanero, Jalapeño, and Paprika) were supplied from a commercial chilli grower (Orcona Chillis 'N Peppers) located at Napier, New Zealand. Fruit were harvested by the grower using commercial maturity indices of size and colour and sent to the postharvest laboratory in Palmerston North within 24 hours of harvest. Fruit free from decay or damage were selected and washed with 100 ppm chlorinated water for 15 min to reduce surface contamination. Fruit were air dried and 10 fruit were packed into 25 cm × 30 cm × 35 μm perforated low density polyethylene (LDPE) bags with six 5 mm diameter holes per side of bag. Three bags of each variety were stored at 0, 4, 8, 12, or 20 °C as treatments. Respiration rate (section 2.4.1), firmness (section 2.4.4) and chilling injury symptoms were measured weekly. Respiration rate model was developed by applying the Arrhenius equation (section 2.4.5.2).

A separate experiment was conducted to determine the phytochemical changes in Jalapeño during storage. Fruit were harvested at 6 and 8 weeks after fruit set (WAFS), phytochemical compounds e.g. ascorbic acid (section 2.6.2) and capsaicinoids (section 2.6.4) were measured from fruit during storage for 21 days at 8 and 20 °C.

All data were analysed by analysis of variance (ANOVA) using SAS statistics program version 9.1 (SAS Institute, Cary, NC, US). Comparison of means were performed by using least significant difference (LSD) to evaluate significant differences at $P = 0.05$.

3.3 Results and discussions

3.3.1 Respiration rate

Temperature is one of the most important factors maintaining the postharvest quality of chillies and peppers. Storage of a fresh commodity at low temperature suppresses respiration rate directly, but chilling injury can occur at low temperatures (Platenius, 1942; Paull, 1999; Seefeldt et al., 2012). At low temperature (0 - 12 °C), respiration rates of three chilli varieties were observed to be consistent across the postharvest period (28 to 35 days) and increased significantly ($P < 0.05$) when fruit were

removed from low temperatures (4 and 8 °C) to 20 °C for 7 days (Fig. 3.1A - C). Hence an average respiration rate during storage can be used to describe the respiration rates at each temperature (Fig. 3.1D). This was similar to Avalos Llana & Sgroppo (2009) who showed that respiration rate of 'Cherry' peppers was constant during 8 days at 10 °C. In addition, respiration rates of fruit which were transferred to 20 °C after storage at low temperature were similar to respiration rates of fruit stored constantly at 20 °C particularly in Jalapeño and Paprika. This can indicate that storage at low temperature may delay the physiological changes and then showed normal response once fruit were transferred to high temperature.

Respiration rates of Habanero increased from 44 nmol.kg^{-1}s^{-1} at 0 °C to 256 nmol.kg^{-1}s^{-1} at 20 °C (Fig. 3.1D). A 6 - 15 fold increase in respiration with increases in temperatures from 0 to 20° C was found for Jalapeño and Paprika. This was similar to respiration rates of Datil peppers (i.e. 'Wanda' and 'Super Datil Pepper') reported previously, that also increased from 126 to 631 nmol.kg^{-1}s^{-1} with an increase of storage temperatures from 5 to 20 °C (Lon Kan et al., 2007)

Overall, respiration rates of Jalapeño were lower than those of Habanero and Paprika during storage at different temperatures (Fig. 3.1D). Comparison between respiration rate at different temperatures with previous research showed that Jalapeño stored at 0 - 20 °C had similar rates to pepper during storage at different temperatures (Gonzalez-Aguilar, 2004) while Habanero and Paprika showed higher rates. This were similar to those of Cayenne chilli stored at 15 °C (Utto, 2001), Datil pepper stored at 20 °C (Lon Kan et al., 2007) and Paprika stored at 22 °C (Krajayklang et al., 2000) (Table 3.1). Respiration rate of chillies and peppers varies depending among varieties and storage temperatures particularly in Paprika which showed high respiration rate at high temperatures.

In this experiment, no climacteric pattern of CO_2 production was found in any of the three chilli varieties during storage (Fig. 3.1). Respiration rates of some peppers such as 'Tabasco', 'Camelot', 'Papri Queen' and 'Aries' showed a climacteric peak of CO_2 during ripening on the plant but this peak was not observed when these fruit ripened off the plant (Villavicencio et al., 1999, 2001; Thang, 2007). However, the

rise of CO_2 production reported in some peppers were seen only when fruit were harvested at immature or breaker maturity (Villavicencio et al., 1999; Thang, 2007). It is possible that fruit in this research were harvested at mature stage so no climacteric peaks were found. A more likely explanation is these varieties were not climacteric as shown for some other cultivars (Biles et al., 1993; Barrera et al., 2005; Barrera et al., 2008).

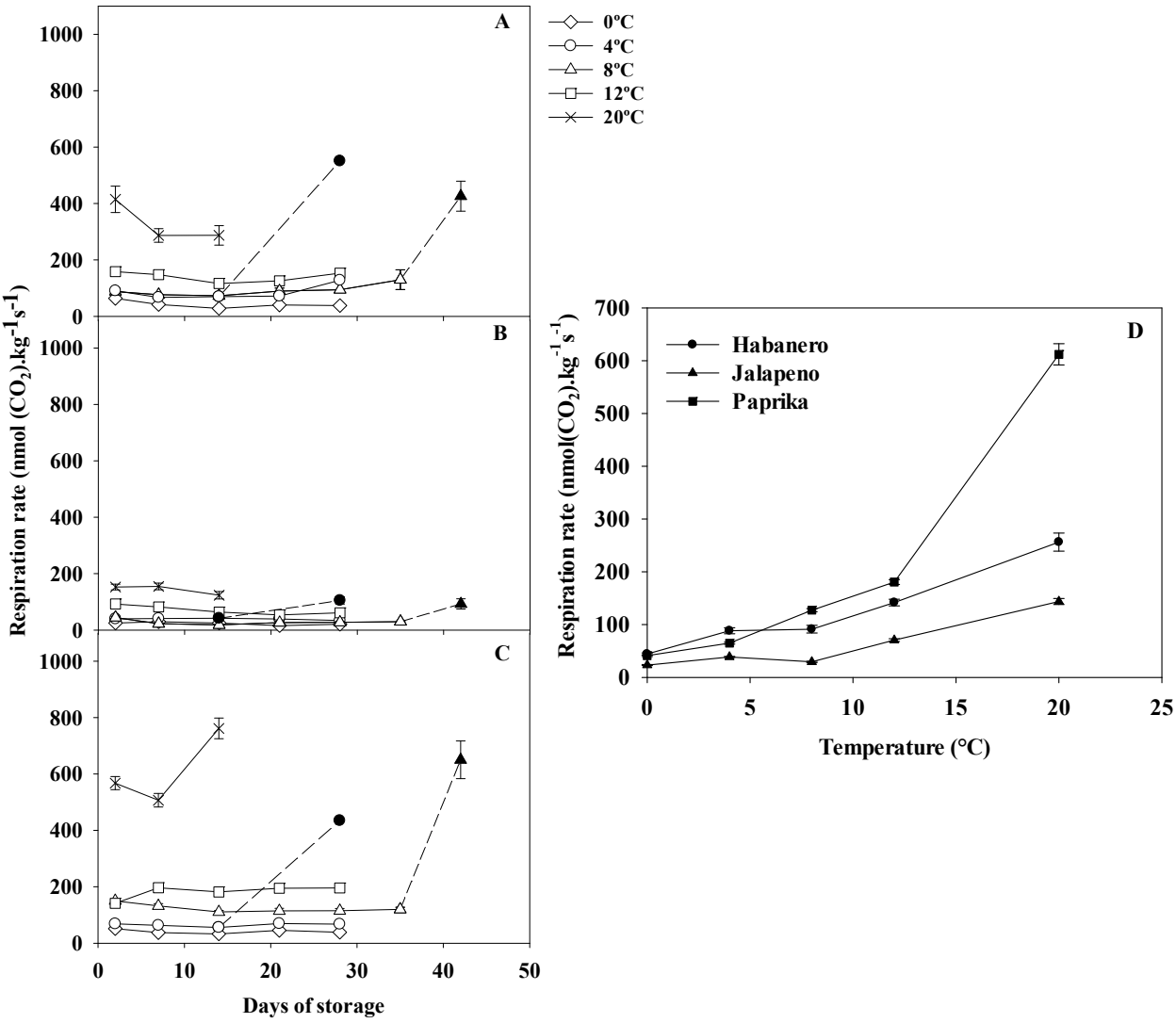


Figure 3.1 Postharvest respiration rates of Habanero (A), Jalapeño (B), and Paprika (C) fruit maintained at a range of storage temperatures from 0 to 20 °C. Each data point represents the average of 30 individual fruit. After chilli fruit were stored for 14 days at 4 °C and 35 days at 8 °C, fruit were transferred to 20 °C for 14 and 7 days respectively which were indicated with closed symbol and dashed line. (D) Average of respiration rates at individual temperature during four weeks of storage of three chilli varieties. Data represent means ± S.E. (*).

(*) Fig. 3.1, Fig. 3.3 and Table 3.2 from this chapter are included in the paper Jansasithorn, R., East, A.R., Hewett, E.W., Mawson, A.J. and Heyes, J.A. 2010. Temperature dependency of respiration rates of three chilli cultivars. *Acta Horticulturae*. 877:1821-1826.

Respiration rates of Jalapeño fruit from different harvests were compared during storage at different temperatures. Respiration rates of Jalapeño harvested early in the season were significantly higher than fruit from other harvests after 7 days (Fig. 3.2A). During early harvest, chilli plants are still developing therefore such fruit may have been metabolising at a much higher rate when compared with fruit from later harvest. Harvest times also influenced respiration rate in other products. Broccoli florets harvested at commercial maturity in early summer have higher respiration rate than those harvested in the late summer and this may relate to higher dry matter content, a precursor of respiration, in the early harvest florets (Seefeldt et al., 2012). Variation of respiration rate found in fruit from different harvest times could relate to different environmental conditions. In addition, harvest maturity of fruit used in this research was determined by a commercial grower and based almost entirely on fruit size and colour so undefined maturity indices for these chillies may also relate to this variation. Therefore, maturity indices defined by weeks after flowering or weeks after fruit set should be controlled to obtain less variability.

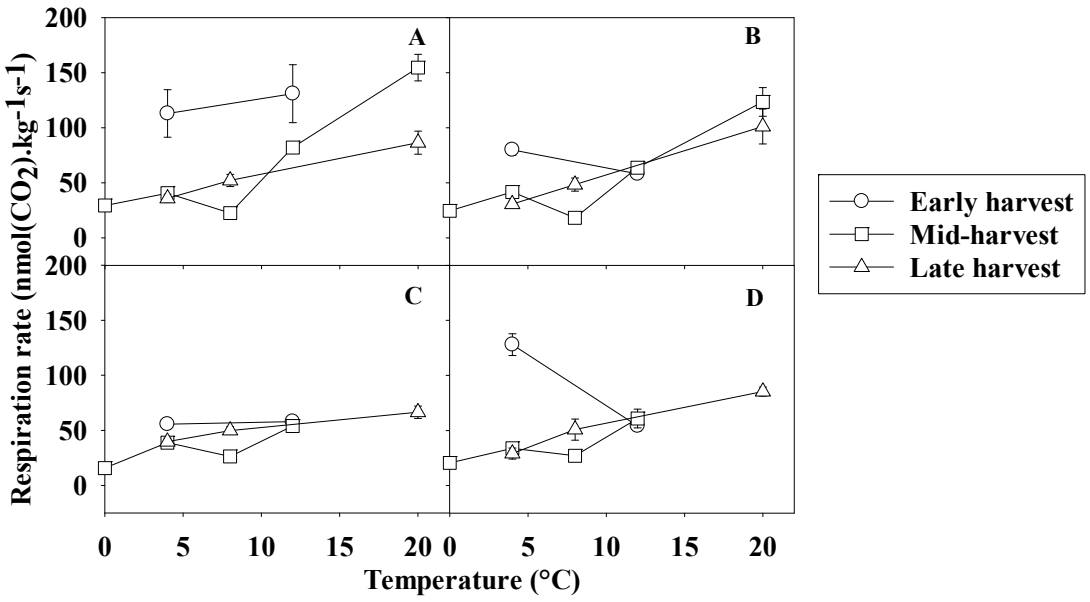


Figure 3.2 Respiration rate of Jalapeño harvested at early, mid and late season and stored at different temperatures (0 – 20 °C) for 7 (A), 14 (B), 21 (C) and 28 (D) days. Each data point represents the average of 30 individual fruit. Error bars indicate standard error of means.

3.3.1.1 Modelling of the temperature dependency on respiration rate of chilli varieties

Storage temperatures directly influence respiration rates of fresh produce and consequently rates of quality change. Temperature management is the most important method for extending postharvest life of fresh produce. Determining the dependence of respiration rate on temperature of three chilli varieties should assist in predicting rates of quality change and may aid packaging design. Modelling of respiration rate (R_c) of each chilli variety was conducted by applying an Arrhenius based equation (section 2.4.2) (Utto, 2001). Respiration rates of all three chilli varieties in relation to temperature were adequately modelled (Fig. 3.3; Table 3.2). Temperature dependence of chilli respiration rate significantly differed ($P < 0.05$) among chilli varieties. Previously, respiration of 'Cayenne' chillies was found to increase from 70 to 1340 nmol.kg^{-1}s^{-1} when temperature increased from 5 to 30 °C (Utto, 2001). These values were similar to Paprika in this research, which indicated that Paprika and Cayenne chilli were more sensitive to temperature change than Habanero and Jalapeño.

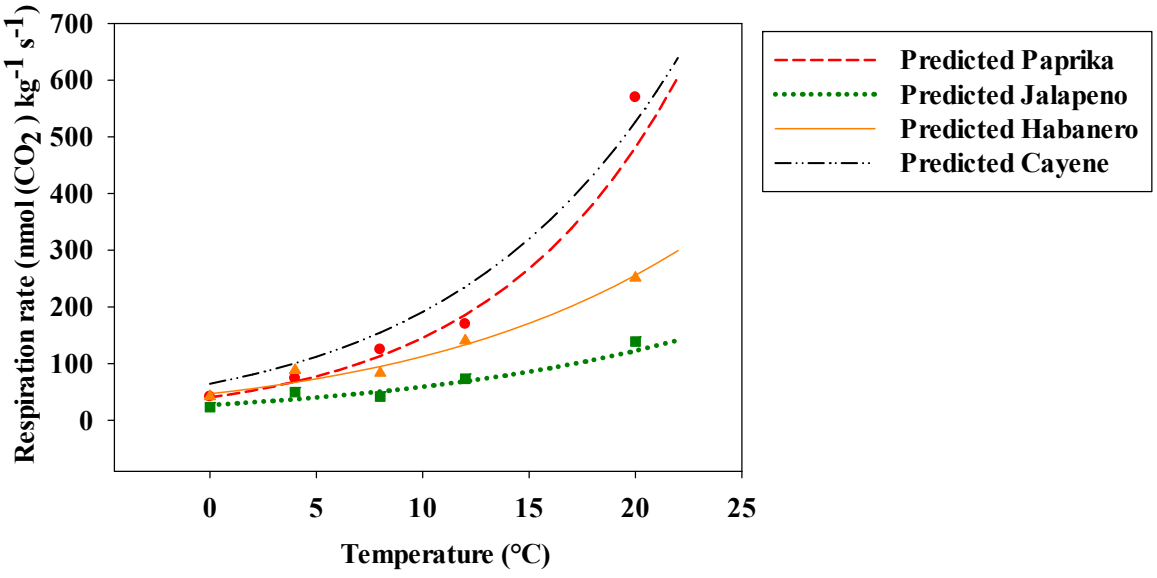


Figure 3.3 Average measured (solid symbol) and modelled (curve) respiration rate as a function of temperature for Cayene (Utto, 2001), Habanero, Jalapeño and Paprika (red, round symbol, dash line) chilli fruit (*).

Table 3.2 Fitted model parameters for respiration; pre-exponential factor (A) and activation energy (Ea) for Habanero, Jalapeño and Paprika comparing to previously reported Green Cayenne (*).

Varieties	A (nmol(CO_2) $kg^{-1}s^{-1}$)	E_a ($J.mol^{-1}$)
Habanero	3.07×10^{12}	56500
Jalapeño	1.22×10^{11}	50500
Paprika	2.48×10^{17}	82500
Green Cayenne (Utto, 2001)	1.47×10^{15}	69813

3.3.2 Firmness

One of the most important consumer acceptance factors is firmness (Harker et al., 1997a). Loss of firmness during storage of chillies and peppers mostly relates to water loss (Lurie et al., 1986; Harker et al., 1997a), unlike many fruit which showed ripening related texture changes. Firmness of Habanero, Jalapeño, and Paprika stored at 4 °C, 8 °C, and 20 °C was measured by compressive and tensile test, although Habanero pericarp was too thin to be measured by compressive test.

3.3.2.1 Compressive test

Compressive firmness of both Jalapeño and Paprika fruit decreased ($P < 0.05$) during storage at 20 °C (Fig. 3.4). Paprika fruit developed shrivel symptoms during stored at 20 °C over 14 days causing the pericarp to become extremely thin and immeasurable using the compressive test (Fig. 3.4B). Both varieties showed minimal loss of firmness at 4 °C and 8 °C. When fruit of both varieties that had been stored at 8 °C were transferred to 20 °C for 7 days to evaluate shelf-life, firmness decreased rapidly (Fig. 3.4). Previous research also found a decrease of firmness in Habanero measured as a force needed to deform the entire fruit during storage for 35 days at 7 °C which decreased rapidly once chilli fruit were moved to room temperature (Gonzalez et al., 2005) and similar results were found in whole peppers and fresh-cut pepper during storage at 10 °C for 8 - 14 days (Gonzalez-Aguilar et al., 2004; Toivonen & Stan, 2004; Vicente et al., 2005; Avalos Llana & Sgroppo, 2009). However, firmness of whole cherry peppers did not change during storage at 10 °C for 8 days which may

indicate that 10 °C is an optimum temperature for cherry peppers (Avalos Llana & Sgroppo, 2009).

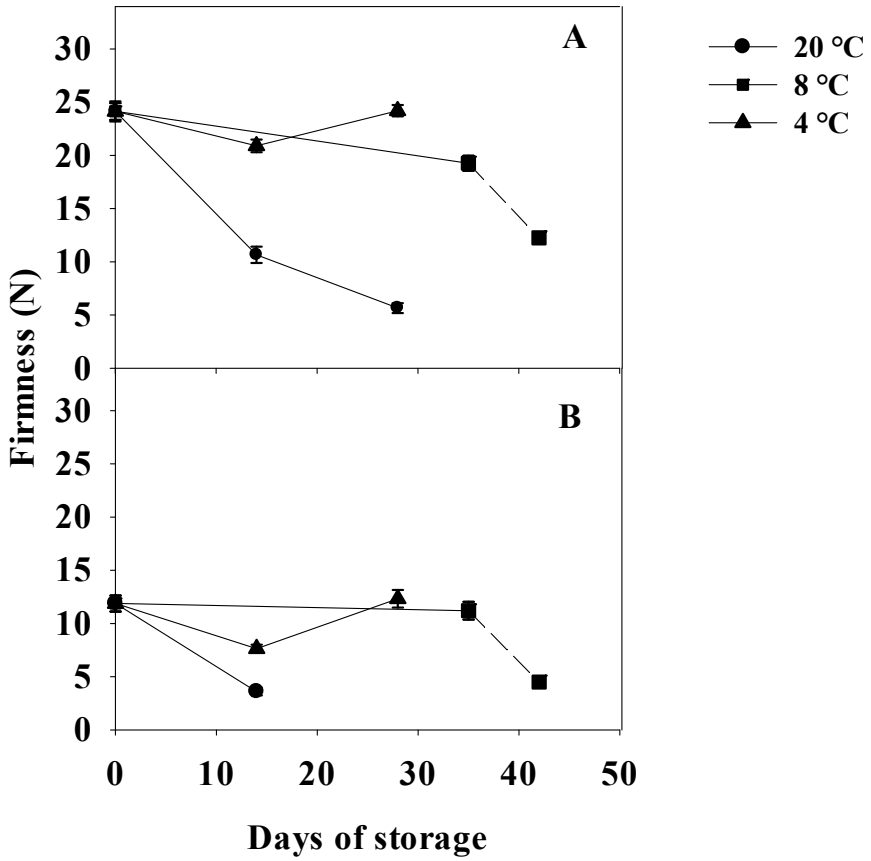


Figure 3.4 Firmness (maximum compressive force) of Jalapeño (A) and Paprika (B) stored at 20 °C (●), 8 °C (■), 4 °C (▲); and during shelf-life at 20 °C for 7 days after storage at 8 °C (dashed line). Data represent means ± SE; n = 20 (**).

All fruit lost their water during storage which showed higher water loss at high temperatures. When firmness was plotted against water loss during storage at each temperature, firmness of Jalapeño showed a consistent negative relationship to water loss during storage at 8 and 20 °C (Fig. 3.5A) while Paprika showed this correlation only at 20 °C (Fig. 3.5C). However, firmness of Jalapeño and Paprika stored at 4 °C did not show a relationship of firmness to water loss and no loss of firmness was found when fruit lost less than 10 % of their water (Fig. 3.5A and C). A linear relationship between firmness and water loss was found in Jalapeño (R^2 = 0.93) and Paprika (R^2 = 0.89) during storage (Fig. 3.5B and D). Therefore the loss of compressive firmness of chillies may be predictable from water loss data.

(**) Fig. 3.4 - 3.7 from this chapter are included in the paper: Jansasithorn, R., East, A.R., Hewett, E.W., Mawson, A.J. and Heyes, J.A. 2010. Relation of water loss to compressive and tensile texture attributes of three chilli cultivars. *Acta Horticulturae*. 880:503-509.

This result was similar to Lurie et al. (1986) who demonstrated a relationship between firmness (measured by compression test) and weight loss in bell peppers stored at 17 °C. Additionally Diaz-Perez et al. (2007) used the relationship between firmness and weight loss of bell pepper stored at 20 °C to predict the maximum permissible weight loss that could lead to the minimum acceptable firmness of pepper.

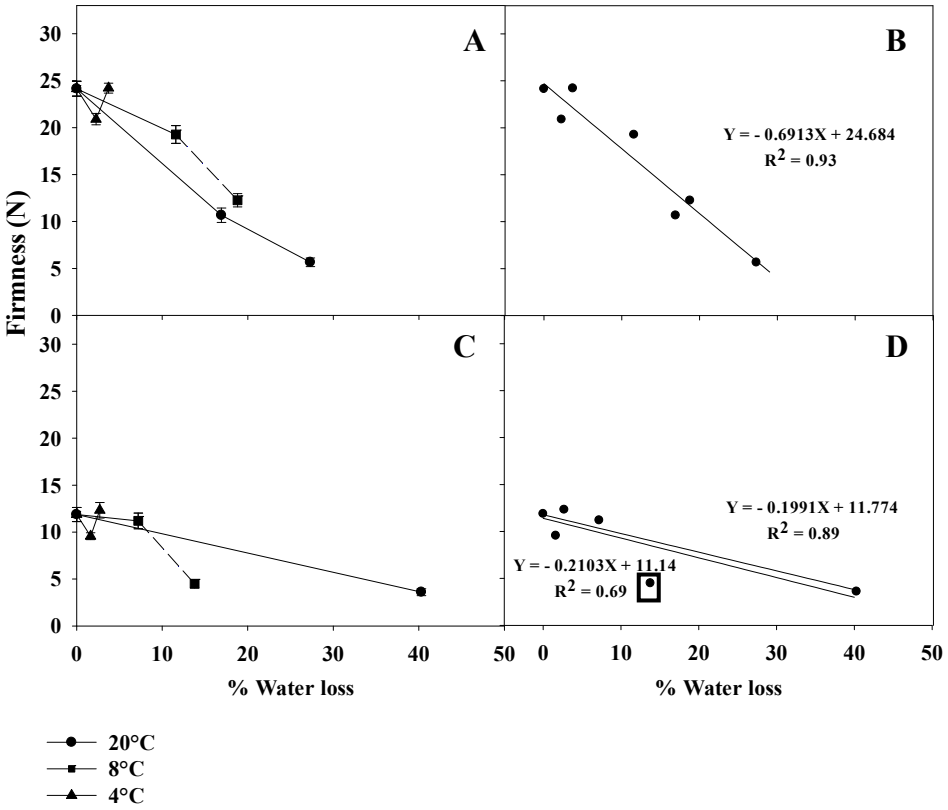


Figure 3.5 Relationship between compressive force and % water loss of Jalapeño (A) and Paprika (C) stored at 20 °C (●), 8 °C (■), 4 °C (▲); and during shelf-life at 20 °C for 7 days after storage at 8°C (dashed line). Data represent means ± SE; n = 20. The overall correlation between compressive force and % water loss of Jalapeño (B) and Paprika (D) are shown. For Paprika, the correlation was done in 2 occasions which included (R^2 = 0.69) and excluded (R^2 = 0.89) firmness of fruit which were moved to 20 °C after storage at 8 °C (black square) (**).

3.3.2.2 Tensile test

Clearly, water loss has a major influence on firmness of chillies. In order to test whether there is a more subtle influence on cell wall properties, a tensile test was used. In general, tensile tests are sensitive to the strength of the cell wall and/or of cell to cell adhesion (Harker et al., 1997a) and water loss can also influence rigidity of the tensile ring. A loss of tensile strength was found in all three varieties over time. The tensile strength decreased slightly faster at 20 °C than low temperatures (4 and 8 °C) in Habanero and Jalapeño (Fig. 3.6A and C). Meanwhile Paprika displayed a rapid decrease of tensile strength in fruit stored for 14 days at 4 °C (Fig. 3.6E). When fruit were removed to 20 °C for 7 days after storage for 35 days at 8 °C, a slight decrease of tensile strength was found in Habanero and Paprika (Fig. 3.6A and 3.6C).

When tensile strength of the three chilli varieties was plotted against water loss, they tended to decrease with an increase in water loss during storage at 20 °C (Fig. 3.6B, D and F). However, a major loss in tensile strength was clearly shown in Paprika stored at 4 and 8 °C which was not explained by water loss (Fig. 3.6F). This indicates that loss of tensile strength may relate to chilling injury. No evidence for this potential chilling injury was found in Jalapeño, but a slight amount was found in Habanero stored at 4 °C. When fruit were removed to 20°C, no significant changes ($P > 0.05$) of tensile strength were found in three chilli varieties (Fig. 3.6B, D and F).

Typical force-distance curves obtained during the tensile test for all three chilli varieties are shown in Fig. 3.7; the force of fruit measured at harvest increased in an exponential pattern and suddenly dropped at the point where the ring broke (Fig. 3.7A). The force-distance curves at harvest were similar to those found in apple and watermelon tissue, which showed a sharp peak in force until the tissue breaks apart abruptly (Harker et al., 1997b). These force curves indicate cell breakage on the fracture surface and represent a typical curve of the crisp characteristic of the relatively thin wall in fruit tissue. However, after 14 days of storage at 20 °C the force showed a more gradual increase prior to tissue failure (Fig. 3.7B). This was similar to soft fruit tissue, which may indicate that cell to cell separation in the tissue

was reducing the force required to break the rings (Harker et al., 1997a; Harker et al., 1997b).

The different distances in force-distance curves among three chilli varieties varied depending on inner diameter of rings which the probe travelled before the ring breakage. In the case of Jalapeño which has a thicker pericarp (Bosland & Votava, 2000), some of the force-distance curves showed several force peaks before final fracture. It can be explained that during measurements, it was observed that the excised rings initially fractured on the sides of the ring and later finally fractured at the point that touched the probe. This is consistent with the idea that outer epidermis was the strongest region of tissue.

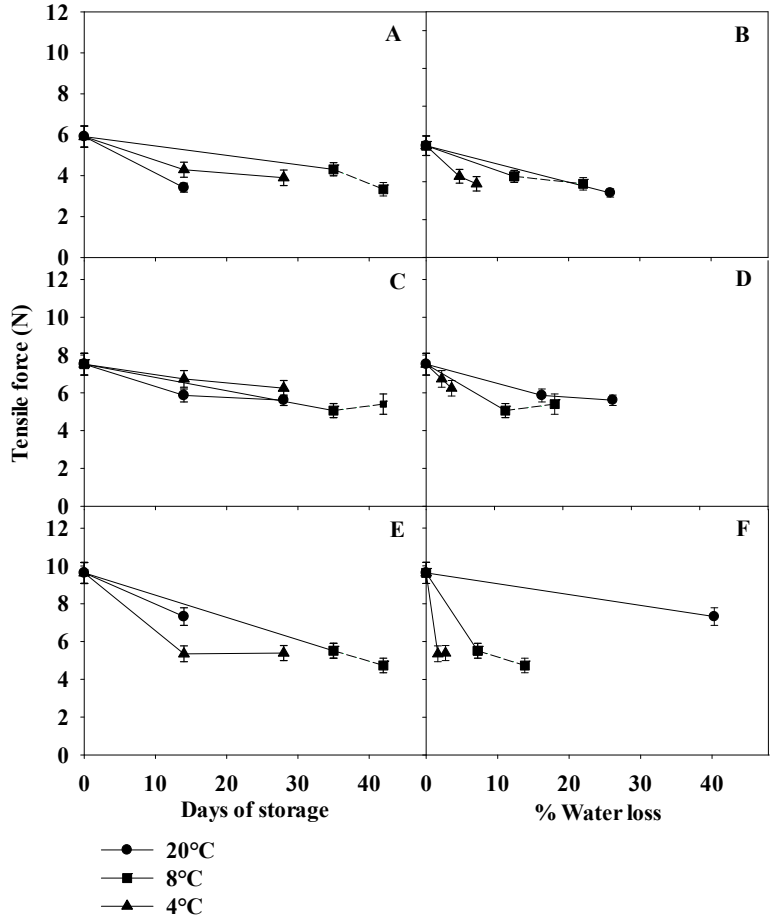


Figure 3.6 Changes of tensile force (break strength) in Habanero (A, B), Jalapeño (C, D), and Paprika (E, F) during storage. Results are plotted against storage time (A, C, E) or water loss (B, D, F) when chillies were stored at 20 °C (●), 8 °C (■), 4 °C (▲); and during shelf-life at 20 °C for 7 days after storage at 8 °C (dash line). Data represent means ± SE; n = 20 (**).

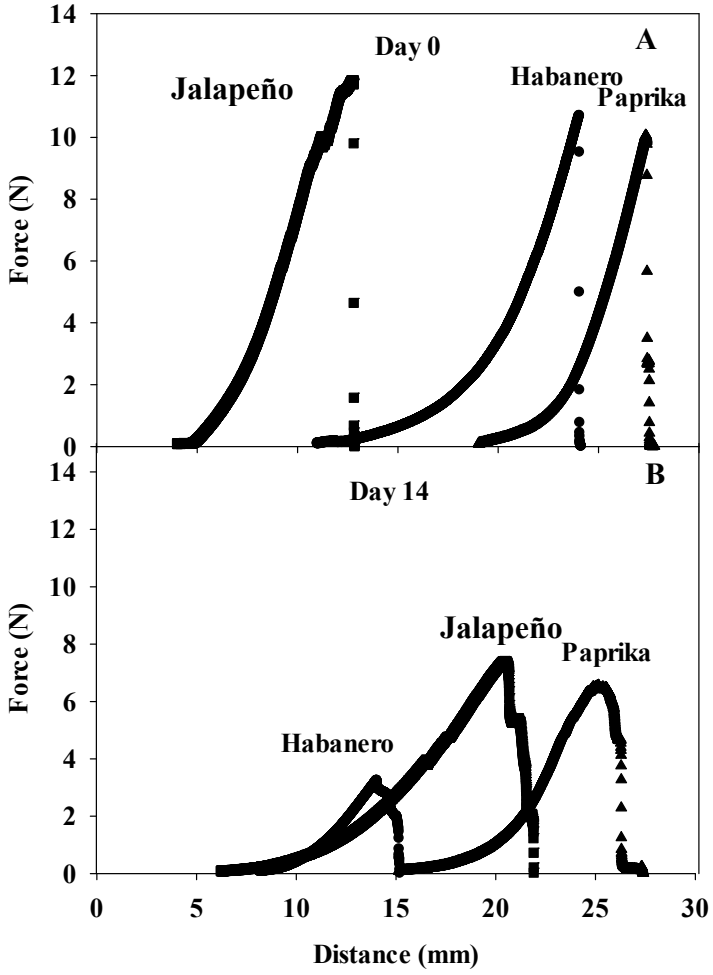


Figure 3.7 Force and distance curves during tensile test of Habanero, Jalapeño and Paprika at the initial day (A) and after 14 days (B) of storage at 20 °C (**).

3.3.3 Chilling injury

Chillies and pepper are susceptible to chilling injury following storage at temperature below 7 °C (Gonzalez-Aguilar, 2004). However, they can be kept at low temperature (5 °C) depending on cultivar, maturity at harvest, and storage period (Lin, 2005). Symptoms of chilling injury include surface pitting, water-soaked area, seed browning and microbial decay (Thompson, 1979; Lin et al., 1993a, b; Chae Shin et al., 2009; Lim et al., 2009; Cuadra-Crespo & del Amor, 2010). After fruit are transferred to room temperature, severe decay can develop as an effect of chilling injury (Barrera et al., 2005). In this research, chilling injury symptoms of three chilli varieties harvested at commercial maturity stage were compared when fruit were stored at a temperature regarded as optimum (8 °C) and low temperatures (0 and 4° C) and also after fruit were transferred to 20 °C for 7 days.

3.3.3.1 Habanero

During storage at 8 °C for 28 days, Habanero fruit retained marketable appearance but pedicel darkening was found (Fig. 3.8A). Pitting, pedicel and calyx darkening and pedicel separation as well as off-odour development were found in Habanero fruit stored at 4 and 0 °C (Fig. 3.8B, C) which occurred on 50 - 70 % of fruit (from a total of 30 fruit) after 28 days. When fruit stored at 8 °C were moved to 20 °C, shrivel of the calyx, pedicel and fruit skin were found in more than 80 % of fruit (Fig. 3.8D). However, fruit stored at 4 and 0 °C showed severe rotting around the calyx, apex and cavity after fruit was moved to 20 °C for 7 days (Fig. 3.8E and F) and no marketable fruit were found.

3.3.3.2 Jalapeño

Jalapeño fruit stored at 8 °C maintained acceptable appearance for 28 days of storage. All fruit remained green (Fig. 3.9A) and 20 % were shrivelled. There were no apparent chilling injury symptoms found in Jalapeño during storage at low temperatures (0 and 4 °C) for 28 days. Small pits on the skin and darkening of pedicel and calyx were found on around 50 % of total fruit (Fig. 3.9B, C). After 8 °C stored fruit were moved to 20 °C for a further 7 days, 30 % changed colour and more than 70 % showed shrivel symptoms on calyx, pedicel, and fruit skin (Fig. 3.9D). However, surface pitting, water soaking area, decay around calyces and pedicels, discolouration of seed cavities, and pedicel separation developed in most fruit (more than 90 %) when fruit were moved from low temperatures (0 and 4 °C) to 20 °C for 7 days (Fig. 3.9E, F).

3.3.3.3 Paprika

After storage of Paprika at 8 °C for 28 days, approximately 50 % of fruit showed shrivel symptom (Fig. 3.10A). No obvious chilling injury symptoms were found in Paprika during storage at low temperatures (0 and 4 °C). Only skin darkening occurred on the skin of some fruit (Fig. 3.10B and C). After fruit were moved from 8 to 20 °C for 7 days, more than 80 % of fruit showed severe shrivel and decay developed on 10 % of fruit (Fig. 3.10D) while fruit moved from low temperatures (0 and 4 °C) to 20 °C showed severe rotting which developed around the calyx and the fruit tip and pitting on skin surface appeared in all fruit (Fig. 3.10E and F).

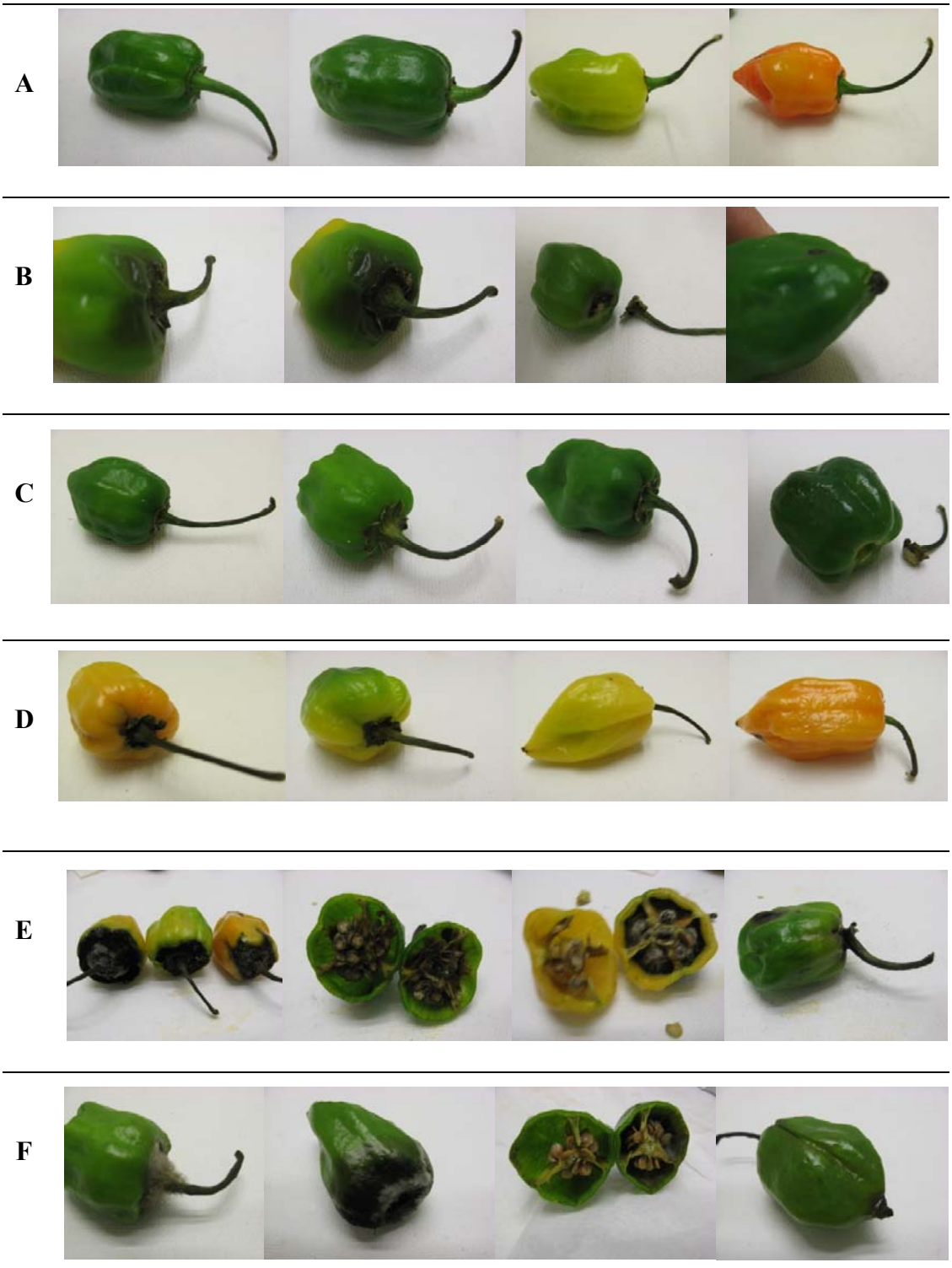


Figure 3.8 Habanero fruit during storage at 8 °C (A), 4 °C (B) and 0 °C (C) for 28 days and after fruit were moved from 8 °C (D), 4 °C (E) and 0 °C (F) to 20 °C for 7 days.

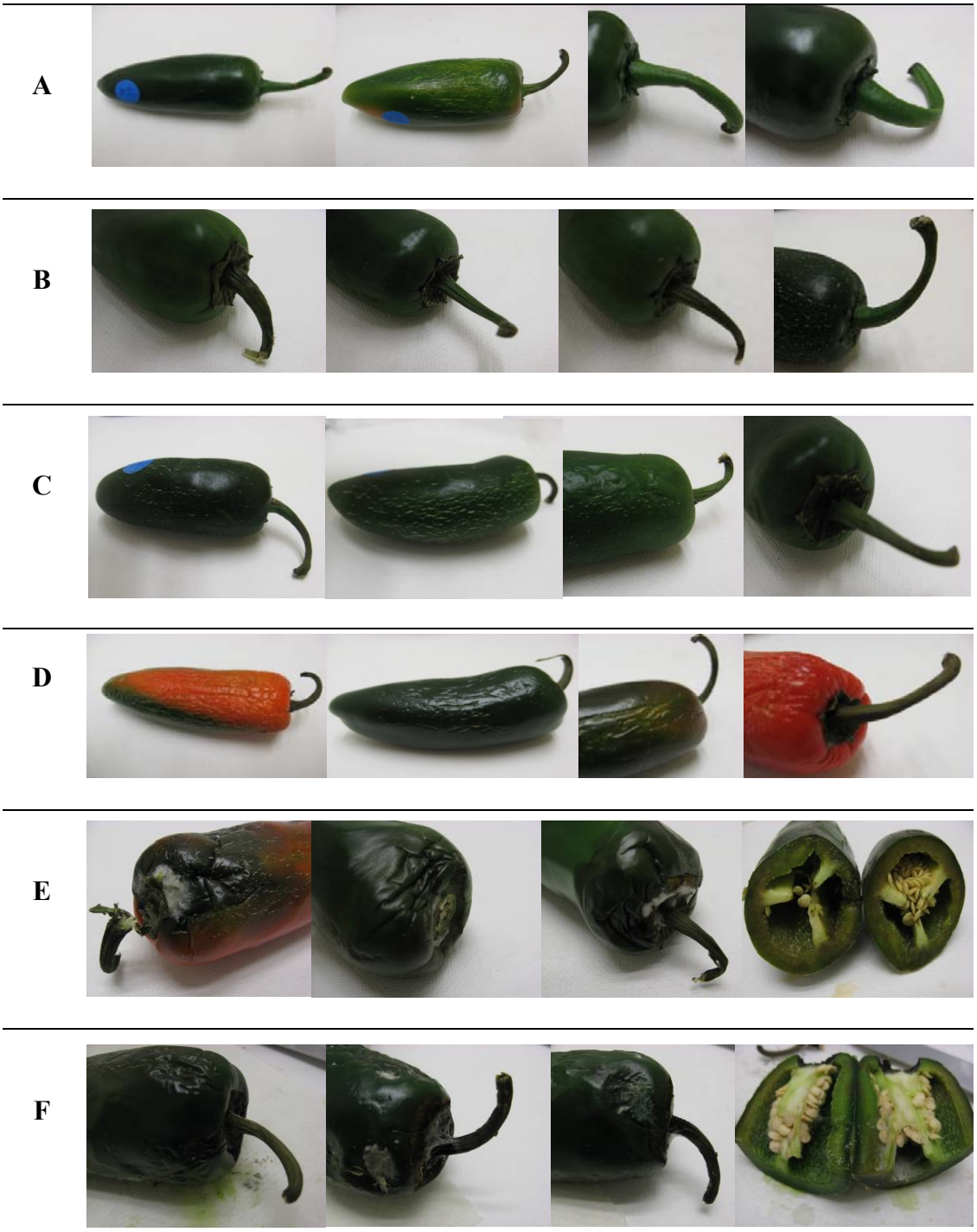


Figure 3.9 Jalapeño fruit during storage at 8 °C (A), 4 °C (B) and 0 °C (C) for 28 days and after fruit were moved from 8 °C (D), 4 °C (E) and 0 °C (F) to 20 °C for 7 days.

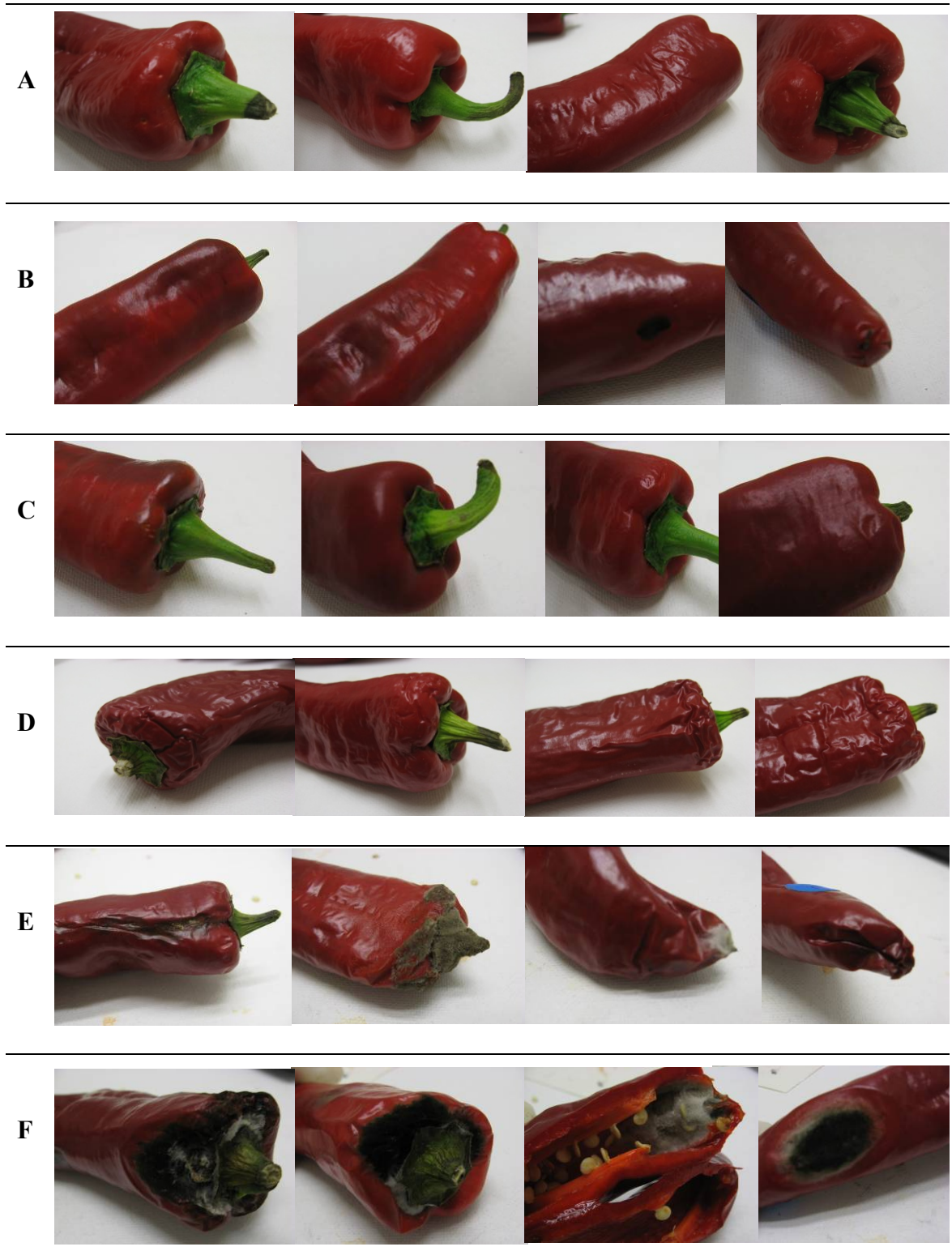


Figure 3.10 Paprika fruit during storage at 8 °C (A), 4 °C (B) and 0 °C (C) for 28 days and after fruit were moved from 8 °C (D), 4 °C (E) and 0 °C (F) to 20 °C for 7 days.

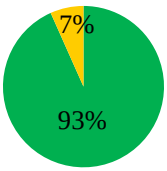
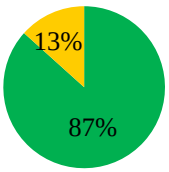
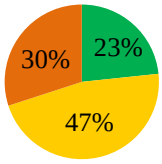
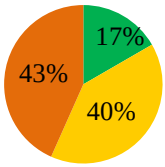
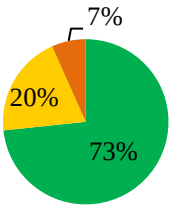
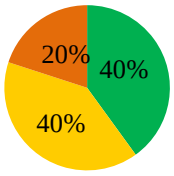
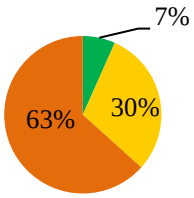
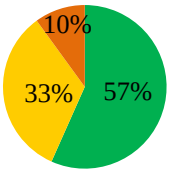
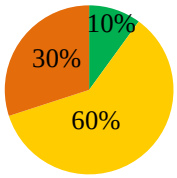
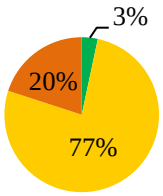
Overall, Habanero, Jalapeño, and Paprika did not show obvious chilling injury symptoms during storage at 0 and 4 °C but severe decay developed when fruit were moved to 20 °C (Fig. 3.8 - 3.10). These results mimic those of Ogata et al. (1968) who found that peppers stored at 1 °C for 2 weeks or 6 °C for 3 weeks rapidly deteriorated when fruit were moved to high temperature and Lin (2005) who found a rapid increase of decay in sweet pepper at room temperature after storage at 1 and 2.5 °C for 4 weeks. These results confirmed that chilling injury symptoms in most chillies develop at temperatures below 8 °C and become visible when fruit are returned to room temperature.

3.3.4 Colour changes

Maturity at harvest is mainly assessed by colour change in chillies and peppers (Wall & Berghage, 1996; Krajayklang et al., 1999; Krajayklang et al., 2000). Chillies and peppers harvested at an immature stage did not change colour to fully red during storage while fruit harvested at breaker or mature stage can develop colour during postharvest storage depending on variety and cultivar (Knavel & Kemp, 1973; Worku et al., 1975; Krajayklang et al., 1999; Krajayklang et al., 2000; Gonzalez et al., 2005). In the first year, three chilli varieties were harvested by the grower using maturity indices based mainly on fruit size.

During ripening, Habanero changes colour from green to orange or red. Habanero fruit stored at low temperatures (0 and 4 °C) remained green throughout storage for 28 days (data not shown). At higher temperatures (8 - 20 °C), colour of most chilli fruit (50 - 90 %) changed from green to yellow, or orange (Table 3.3). At 20 °C, the transition in colour from green to 30 % yellow or 63 % orange occurred within 14 days of storage while at 12 °C around 90 % of fruit changed colour from green to orange or yellow after storage for 28 days. Approximate 43 % of fruit stored at 8 °C for 35 days changed colour (Table 3.3). Once fruit stored at 8 °C were moved to 20 °C for 7 days, more than 90 % of fruit changed colour (Table 3.3). The ratio of orange fruit was higher than yellow fruit during storage at 20 °C but yellow fruit showed higher ratio during storage at 8 and 12 °C or when fruit were moved from 8 to 20 °C.

Table 3.3 Proportion of the skin colour (green, yellow or orange) of Habanero fruit during storage at 8, 12, and 20 °C. The same 30 fruit were observed through storage.

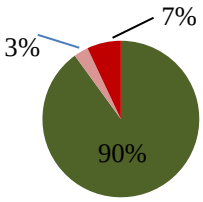
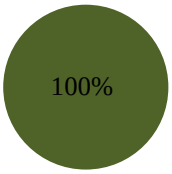
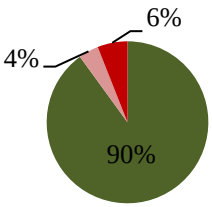
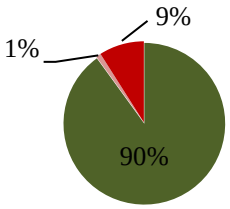
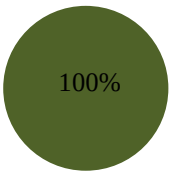
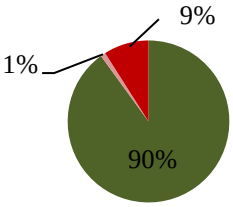
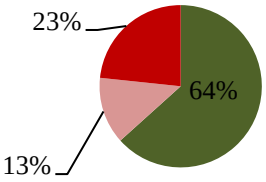
Days of storage	8 °C	12 °C	20 °C
3			
7	N/A	N/A	
14			
28/ 35			
42*			

*Fruit were moved from 8 °C to 20 °C as shelf-life condition for 7 days. N/A = Not available.

For Jalapeño chilli, colour changes from green to red with no transitional colour of yellow and/or orange. Like Habenero, Jalapeño fruit stored at 0 and 4 °C remained green during storage for 28 days (data not shown). At higher temperatures, 10 % of

fruit changed from green to red colour after 14 days at 20 °C or 28 days at 12 °C (Table 3.4). Fruit stored at 8 °C remained green during storage for 35 days and began to change colour when fruit were moved to 20 °C for 7 days (Table 3.4).

Table 3.4 Proportion of the skin colour (green, less than half red, more than half red*) of Jalapeño fruit during storage at 8 to 20 °C. The same 30 fruit were observed through storage.

Days of storage	8 °C	12 °C	20 °C
7	N/A	N/A	
14			
28/ 35			N/A
42*		N/A	N/A

*Fruit were moved from 8 °C to room temperature at 20 °C as shelf-life condition for 7 days. Fruit number which exhibited less than 50 % red colour defined as light red colour, fruit number which exhibited more than 50 % red colour defined as dark red. N/A = Not available.

Paprika was red at harvest, so all fruit remained red during storage at 8, 12 and 20 °C (data not shown).

Overall, low temperatures (0, 4 and 8 °C) tended to maintain immature fruit colour of Habanero and Jalapeño during storage while more than 90 % of Habanero, but only 10 % Jalapeño, developed red/yellow/orange colour at 20 °C when fruit were harvested at commercial maturity (Table 3.3 - 3.4). Previously, most chillies and pepper including Habanero and Mexican peppers developed their colour during storage at high temperature (Banaras et al., 2005; Gonzalez et al., 2005; Lon Kan et al., 2007), while storage at low temperature (4 - 8 °C) seemed to maintain colour during storage (Banaras et al., 2005; Gonzalez et al., 2005; Lon Kan et al., 2007; Raffo et al., 2008). Low temperature storage has been found to inhibit normal colour development. For example Habanero fruit stored at 7 °C for 35 days did not develop colour to the same degree as fruit stored at room temperature after they were removed from low to room temperature (Gonzalez et al., 2005). The results of Habanero colour in this research confirmed this observation suggesting that orange colour development is potentially inhibited by periods of low temperature storage.

In this research, fruit were harvested at commercial maturity as dictated by size. It is possible that most fruit of variable physiological maturities would influence consistency of colour changes occurring during storage. In the following seasons of research (section 2.3.2 and 2.3.3), tagging flowers at fruit set was conducted to control maturity at harvest. In 2009, Jalapeño fruit remained green when harvested before 5 WAFS and only a proportion of fruit harvested at 5 (27 %) and 6 (39 %) WAFS changed to red colour after 28 days at 20 °C (Table 3.5).

In 2010, all fruit harvested at 6 WAFS were green at harvest with just 3 % of fruit changing to red colour after 21 days at 8 °C, while 63 % turned red after 21 days at 20 °C (Table 3.5). Fruit harvested at 8 WAFS showed approximately 7 - 10 % of red fruit on the plant, which later changed to 25 and 100 % red colour during storage at 8 and 20 °C, respectively (Table 3.5). Lightness (L*) values increased after fruit initially changed to red colour while a* value increased to 15 - 28 as fruit became completely red. There was no significant difference ($P > 0.05$) of L* and a* values

between fruit harvested at 6 and 8 WAFS. Comparing between 8 and 20 °C, the a* value of chilli fruit stored at 20 °C was higher than fruit stored at 8 °C. However, there was no significant difference ($P > 0.05$) in L* and a* values between these two temperatures. Based on this work, maturity at harvest is an important factor for colour changes. Harvesting chilli at a suitable maturity index may result in development of fruit colour during postharvest storage. The ability to predict colour change would be useful for chilli growers to assist achieving the consumer requirement. For example for green Jalapeño, fruit should be harvested by 6 WAFS while for red Jalapeño fruit should be harvested after 8 WAFS. However, to identify actual maturity (i.e. WAFS) prior to the onset of colour is practically impossible. An alternative method to achieve red Jalapeño would be to harvest when fruit begin to change colour on the plant which these fruit can change to fully red colour after harvest while it would be more challenge for green Jalapeño as fruit could be harvested full size but not beyond 6 WAFS as these fruit are green at harvest which may suit for fresh market. However, these fruit may change to red colour during storage at high temperature (i.e. 20 °C) or fruit can be harvested by 6 WAFS and fruit will not change, colour but there might be a yield penalty as these fruit were small.

Table 3.5 Proportion of fruit colour, L* and a* value of fruit harvested at different maturity stages and stored at 8 and 20 °C for 21 or 28 days.

Year	Harvest maturity (WAFS)	Storage temperature (°C)	Storage time (days)	Red fruit at harvest (%)	Red fruit after storage (%)	Average colour of red fruit after storage	
						L	a* value
2009	3	20	28	0	0	N/A[1]	N/A
	4	20	28	0	0	N/A	N/A
	5	20	28	0	27	40.81	28.78
	6	20	28	0	39	40.97	23.95
2010	6	8	21	0	3	32.99	15.44
	6	20	21	0	63	38.28	24.83
	8	8	21	10	25	40.18	23.57
	8	20	21	7	100	37.58	27.92
					Year	*	NS
					Maturity	NS	NS
					Temperature	NS	NS

[1] N/A = Not available. * = Significant at 5 % levels.

3.3.5 Phytochemical composition of Jalapeño chilli during storage

Chillies and peppers contain many phytochemical compounds beneficial for human health including capsaicinoids and ascorbic acid. Maintaining pepper fruit under optimum postharvest storage conditions may delay a reduction of phytochemical content (Howard et al., 1994; Howard et al., 2000; Howard, 2006).

3.3.5.1 Total capsaicinoid concentration

Total capsaicinoids comprising of capsaicin and dihydrocapsaicin was measured in Jalapeño fruit harvested at 6 and 8 weeks after fruit set (WAFS) and during subsequent storage at 8 and 20 °C. There were no differences ($P > 0.05$) in capsaicinoid concentration between storage temperatures (Fig. 3.11A and B). Total capsaicinoid concentrations were consistent in Jalapeño fruit harvested at 6 WAFS during storage at both temperatures while a decrease ($P < 0.05$) of total capsaicinoid concentration was found in fruit harvested at 8 WAFS when fruit were moved to 20 °C after storage for 21 days at 8 °C. Similarly, storage temperature seemed not to influence capsaicinoid concentration of hot pepper and Paprika stored at 5 - 10 °C, but capsaicinoid decreased with time of storage (Kirschbaum-Titze et al., 2002a; Barrera et al., 2005; Gonzalez et al., 2005). In addition, there was no significant difference ($P > 0.05$) between maturity stages during storage at both temperatures, however high variation of total capsaicinoid concentrations was observed between each individual fruit. In contrast, previous research found capsaicinoid concentrations in Habanero and Piquin harvested at 6 WAFS to be higher than fruit harvested later (Contreras-Padilla & Yahia, 1998) while pungency of Paprika (in powder form) was not affected by maturity at harvest (Krajayklang et al., 2000)

3.3.5.2 Ascorbic acid concentration

Ascorbic acid in Jalapeño decreased ($P < 0.05$) with time of storage at 8 and 20 °C, but no difference ($P > 0.05$) of ascorbic acid concentration was observed between fruit stored at two temperatures (Fig 3.11C and D). Similarly, ascorbic acid in most chillies and peppers (both whole and fresh-cut forms) appear to decrease with time of storage at 5 - 10 °C over 8 - 45 days (Gonzalez et al., 2005; Akbudak et al., 2006; Raffo et al., 2008; Avalos Llana & Sgroppo, 2009; Ruiz-Cruz et al., 2010; Sakaldas & Kaynas, 2010). However, some peppers showed an increased or unchanged

ascorbic acid content during storage at 10 °C for 12 days (Sherafati et al., 2010) while Wang (1977) and Tonelli et al. (1981) found an increase of ascorbic acid content in sweet pepper during storage at 13 °C and ripening at 20 °C, which may be explained by the ripening process and weight loss during storage. At harvest, ascorbic acid concentration in mature fruit was higher ($P < 0.05$) than in younger fruit but there was no significant difference ($P > 0.05$) of ascorbic acid between these two maturities during storage except in fruit stored at 20 °C for 21 days, which showed higher ($P < 0.05$) ascorbic acid in immature fruit (Fig. 3.11D). Higher ascorbic acid has been reported previously in red Jalapeño (Howard et al., 1994; Howard, 2006) which may indicate that ascorbic acid accumulates during ripening on the plant (Howard et al., 1994; Lee & Kader, 2000).

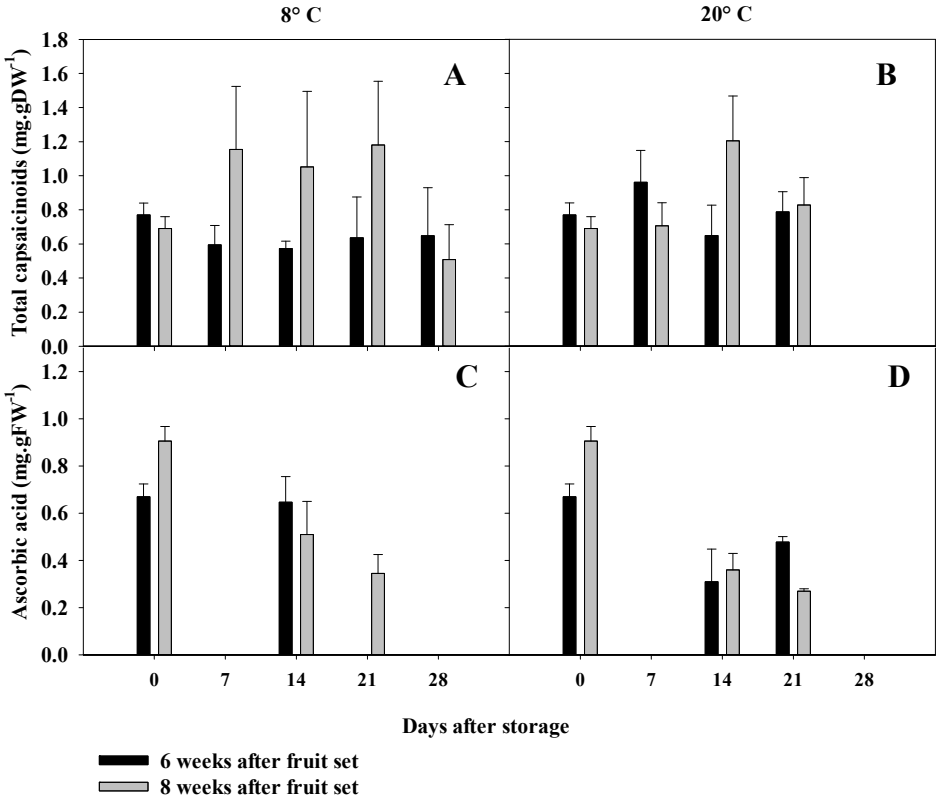


Figure 3.11 Phytochemical composition changes, total capsaicinoids (A and B) and ascorbic acid (C and D) concentrations of Jalapeño fruit harvested at 6 and 8 WAFS during storage for 0 – 21 days at 8 and 20 °C and when fruit were moved to 20 °C for 7 days. Data represent means ± S.E. (n = 3 - 6 fruit).

Overall, storage temperature did not show a high impact on phytochemical compounds in Jalapeño, while storage period showed an effect only on ascorbic acid, but not for total capsaicinoid which seemed to be consistent during storage. However, although harvest maturity was controlled, considerable of fruit to fruit variation in phytochemical concentrations was observed. Therefore, further work is required to understand the unknown interactions between pre-harvest factors such as time of planting, position on plant, fruit maturity and crop load which can affect the variation on phytochemical compounds and also other chilli qualities (i.e. size, shape, and colour).

3.4 Conclusion

The optimum storage temperature of Jalapeño and Habanero was 8 °C as fruit can be stored for 3-4 weeks and retain marketable quality (i.e. firmness, colour and phytochemical compounds) and appearance while Paprika lost their firmness, particularly tensile strength, during storage at 4 and 8 °C. Chilling injury symptoms of three chilli varieties during storage at low temperatures were visible when fruit are moved to 20 °C after storage at low temperatures (0 and 4 °C). A model of respiration rate as a function of temperature was developed. This model would be useful in developing modified atmosphere packaging of chilli fruit.

For commercial aspect, fruit could be harvested at full size, but should not be left on the plant beyond 6 WAFS for green Jalapeño at harvest or harvested at fully mature stage to obtain high vitamin C content, or harvested at a breaker stage to allow further colour development depending on market demand at any given time through the season.

Most likely, fruit in this experiment were harvested by size as a commercial maturity, high fruit to fruit variation was observed. Therefore, investigation factors such as maturity at harvest and other pre-harvest factors which contributed to fruit to fruit variability is required. As these knowledge can be helpful to potentially identify a method of plant management and fruit harvest to reduce this variability.

CHAPTER 4

Sources of water loss and cracking problems

4.1 Introduction

Water loss represents the majority of mass loss in horticultural produce, affects quality attributes and limits marketable life. In general, wilting, shrivelling and softening of tissue can be observed when fruit lose 5 - 7 % of fresh weight (Wills et al., 2007). Chillies and peppers are susceptible to water loss during postharvest handling and storage (Zsom et al., 2005). Water loss in chillies and peppers is influenced by postharvest storage conditions (e.g. temperature and humidity), fruit properties and skin structure.

Optimum storage conditions for green peppers range from 7 - 13°C at 90 - 95 % RH resulting in a shelf-life of 14 - 21 days (Thompson, 1979; Paull, 1999; Gonzalez-Aguilar, 2004). Weight loss is influenced by fruit properties such as surface area, shape, and fruit size. For example large fruit generally have a smaller surface area to volume ratio (SA/V) than small fruit. While large fruit lose more total weight (kg) due to possessing a larger surface area, proportionally, smaller fruit lose more weight (% weight loss) than large fruit due to larger surface area to volume ratio. Diaz-Perez et al. (2007) observed the water loss rate (%. day^{-1} kPa^{-1}) of bell pepper fruit to decrease with an increase of fruit size. The structure of skin surface including size, shape and number of stomata and lenticels and the thickness of the cuticle or epicuticular wax are also related to water loss. However, chillies and peppers lack stomata on the fruit surface (Lownds et al., 1993; Blanke & Holthe, 1997). Therefore transpiration and gas diffusion can occur via either the pedicel and calyx or the cuticle, pores and cracks on the fruit skin (Diaz-Perez, 1998; Banks & Nicholson, 2000; Bower et al., 2000; Diaz-Perez et al., 2007). While the epicuticular wax of chillies and peppers inhibits water loss, the composition is variable in each cultivar (Ben-Yehoshua, 1987; Lownds et al., 1993).

In this research, three chilli varieties (Habanero, Jalapeño, and Paprika) were selected to investigate relationships of water loss to their physical properties. In addition, routes of water loss from Jalapeño fruit as dictated by fruit structure were

studied through calculation of water vapour permeance. The structural nature of cracking, a common problem found in Jalapeño fruit, was investigated the effects on water loss. Water vapour permeance (P'_{H_2O}) of Jalapeño during storage at different temperatures was also studied to determine rates of water loss and when fruit develop wilting and shrivel symptoms in the postharvest environment. In addition, the experimental data were used to develop a model to predict water loss in chillies during storage at different conditions. The resulting model provides indications to chilli grower to reduce and prevent water loss occurring in chillies during handling and storage.

4.2 Materials and methods

Habanero, Jalapeño and Paprika were harvested at commercial maturity (as indicated by size from a commercial glasshouse (section 2.3.2). Size, shape, surface area (section 2.5.2) and % water loss (section 2.4.3) during storage at different temperatures (0 - 20 °C) were measured. Cracked and non-cracked Jalapeño fruit were harvested in another season to determine effect of cracking on water loss. Water vapour permeance (P'_{H_2O}) was calculated for data collected following the method of Maguire (Maguire et al., 1999). In addition, application of wax on individual fruit structures (calyx and pedicel or fruit skin) of Jalapeño was conducted to understand the effect of wax on water loss and route of water loss from Jalapeño fruit. Effects of temperature and maturity at harvest on P'_{H_2O} were also studied. Microscopy was used to investigate the structures of the cracking on fruit skin. Details of these methods are described below.

4.2.1 Water vapour permeance

Individual fruit were weighed by balance (0.001 g precision Model P503S, Mettler Toledo, Australia) and placed in an airflow cabinet (≈ 3 $m.s^{-1}$). The rates of weight loss from each fruit were determined at 0, 24, 48 and 72 h. Dry and wet bulb temperatures (thermister probes CM types, U bead, ± 0.2°C; Grant Instrument, Cambridge, U.K.) were recorded. Fruit surface temperature was determined by inserting a thermistor probe (FF type, U bead, ± 0.2 °C; Grant Instrument, Cambridge, U.K.) under the skin with a needle. All temperatures were recorded by

Grant Squirrel logger (1200 series Grant Instrument, Cambridge, U.K.). Respiration rate was also measured for water loss calculation. Fruit harvested from 5 - 7 weeks after fruit set were stored at 4, 8 or 20°C to determine temperature effects on P'_{H_2O}.

Water vapour permeance (P'_{H_2O}) characterises the barrier properties of the fruit surface to water vapour which explains the ease in which water vapour can diffuse from the fruit. Water vapour permeance (P'_{H_2O}) at each temperature was calculated by rearrangement of rate of water loss equations (Maguire, 1998).

$$P'_{H_2O} = \frac{r'_{H_2O}}{\Delta p_{H_2O} A} \qquad \textbf{Eq. 4.1}$$

where:

P'_{H_2O} = Water vapour permeance of the fruit surface (mol.s^{-1}m^{-2}Pa^{-1})

r'_{H_2O} = Rate of water loss (mol.s^{-1})

A = Surface area of fruit (m^2)

Δp_{H_2O} = The difference in partial pressure of water vapour between the environment ($p^e_{H_2O}$) and the fruit ($p^f_{H_2O}$) (Pa)

We can apply this equation (Eq. 4.1) either to the whole fruit or we could use to estimate water loss from each part of the fruit, for instance the permeance through the fruit skin (P'_s) would be a function of the surface area of the skin itself (A_s). It will be similar for the permeance through the calyx and pedicel (P'_c) where it would be a function of the surface area of the calyx and pedicel itself (A_c).

r'_{H_2O} (mol.s^{-1}) is calculated from (section 2.4.3);

$$r'_{H_2O} = W_L - R_c \qquad \textbf{Eq. 4.2}$$

Δp_{H_2O} is calculated by the following equation;

$$\Delta p_{H_2O} = p^f_{H_2O} - p^e_{H_2O} \qquad \textbf{Eq. 4.3}$$

Where:

$p^f_{H_2O}$ = The partial pressure of water vapour in fruit (Pa)

$p^e_{H_2O}$ = The partial pressure of water vapour in the environment (Pa)

$$p_{H_2O}^{f} = p_{H_2O}^{sat}(T_f) = 611\exp^{\left[17.27\left[\frac{T_f}{T_f+237.3}\right]\right]} \times a_w \quad \textbf{Eq. 4.4}$$

Where:

T_f = Temperature at fruit skin (°C)

a_w = Water activity (assumed to be 0.995)

$$p_{H_2O}^{e} = p_{H_2O}^{sat}(T_w) - \gamma(T_e - T_w) \quad \textbf{Eq. 4.5}$$

$$p_{H_2O}^{sat}(T_w) = 611\exp^{\left[17.27\left[\frac{T_w}{T_w+237.3}\right]\right]} \quad \textbf{Eq. 4.6}$$

where:

T_e = Temperature of environment (dry bulb temperature) (°C)

T_w = Wet bulb temperature (°C)

γ = Psychrometric constant (a value of 67 Pa.°C^{-1})

4.2.2 Microscopy analysis of cracked tissue

Skin of Jalapeño was stained by Berberin and Aniline blue and visualised with light microscopy in order to investigate the structure of cracking. Before staining tissue by Berberine and Aniline Blue, cracked and non-cracked fruit were prepared as follows.

4.2.2.1 Tissue preparation

Jalapeño skin was sectioned and immersed into FAA solution (Formalin (37 % formaldehyde) 10 mL: Alcohol (ethanol) 50 mL: Acetic acid 5 mL: water 35 mL). Next the tissue was placed in a vacuum to evacuate air and water from the tissue. Fresh FAA was added after suction and left for 24 hours at room temperature. FAA solution was substituted by 50 %, 75 %, 90 % and 100 % ethanol respectively and each solution was incubated for at least 1 hour. Once the tissue was immersed in 100 % ethanol, Histoclear (Thermo Scientific, USA) was added as a clearing agent to substitute the proportion of ethanol in the tissue until filled with 100 % Histoclear solution and left overnight at room temperature. Tissue was subsequently heated in an incubator at 42°C and wax chips were added into the vial and completely dissolved. Wax chips were continually added until the vial was full and the wax had completely substituted for Histoclear.

4.2.2.2 Tissue embedding

The tissue in wax form was heated until the wax was dissolved. Then the tissue was transferred to a plastic mould that contained previously added wax. The tissue was arranged in a section position and placed on an ice bath for solidification. When the wax was solid, the waxed sample was torn out from the mould and placed on a metal stub by using melted wax as an adhesive. The waxed sample was sectioned to thin pieces (approx. 5 μm) by microtome. These sections were placed on glass slides for staining.

4.2.2.3 Tissue Staining

Tissue staining was conducted by the method following Brundrett et al. (1988).Wax was removed from the tissue section by using a range of ethanol concentration from high to low and substituting ethanol with water. The tissue was stained by 0.1 % (w/v) Berberine hemi-sulphate in distilled water for 1 hour, followed by 0.5 % (w/v) Aniline blue in distilled water for 30 minutes at room temperature (Brundrett et al., 1988).

4.2.2.4 Microscopy

The section was observed by using a light microscope with UV illumination using excitation filters BP490, BP495, and UG-1 (BP330-385). Photographs of the tissue from the microscopy were taken by an Olympus camera connected to the microscope. Photographs of the tissue were taken in a few hours after staining.

4.3 Results and discussion

4.3.1 Physical properties

Understanding physical properties of each chilli variety can assist in determining the relationship of these factors to the rates of water loss observed in chillies. The average length, diameter, fresh weight, volume, density, and surface area of Habanero, Jalapeño, and Paprika were collected (Table 4.1). Paprika was longer than the other two varieties. Jalapeño had more weight and was denser than Paprika and Habanero.

Table 4.1 Physical properties of mature Habanero, Jalapeño and Paprika.

Varieties	Habanero	Jalapeño	Paprika
Length (cm)	2.9 ± 0.2[a]	8.0 ± 1.3	11.4 ± 0.3
Diameter (cm)	2.0 ± 0.1	3.3 ± 0.7	2.8 ± 0.1
Fresh weight (g)	3.5 ± 0.5	45.8 ± 1.2	32.4 ± 2.0
Volume (cm^3)	6.5 ± 1.1	48.1 ± 1.8	56.6 ± 3.3
Density ($g.cm^{-3}$)	0.58 ± 0.04	0.82 ± 0.02	0.57 ± 0.01
Surface area (cm^2)	12.7 ± 1.3	79.5 ± 2.2	78.9 ± 2.6
n	7 - 13	85 - 144[b]	27

[a] Data represent Mean ± SE
[b] Jalapeño fruit number from 3 years of studies

Fruit surface area can be estimated by a number of methods. In many cases, surface area of fruit with a regular shape can be estimated by mathematical calculation from their dimension describing a simplified shape e.g. spheres, for example apple (Clayton et al., 1995; Bovi & Spiering, 2002). However, this approach is not applicable for irregular fruit shape with individual differences like chilli and pepper. A scanned image of a flattened cast of the fruit and analysis by using an image processing (fully described in section 2.5.2) was used to determine surface area of chillies. Due to the difficulty of fruit surface measurement, a correlation of surface area and some easily measured parameters such as fruit weight and volume was developed. Previously, strong relationships between surface area and fruit mass or volume have been developed for apple (Clayton et al., 1995), cantaloupe, strawberry and tomato (Eifert et al., 2006). Diaz-Perez et al. (2007) use a similar approach to find a relationship between surface area and diameter in bell peppers. Linear correlations of fruit surface area to initial fresh weight or volume explained 73 - 85 % of the total variation in surface area of the three varieties (Fig. 4.1). The best correlation between surface area and fresh weight was found for Paprika (Fig. 4.1E). In this experiment, these correlations allow estimation of the fruit surface area of the three varieties of chillies to be determined by fresh weight or volume. Fresh weight would be more practical option to use as weight is easier to measure than volume.

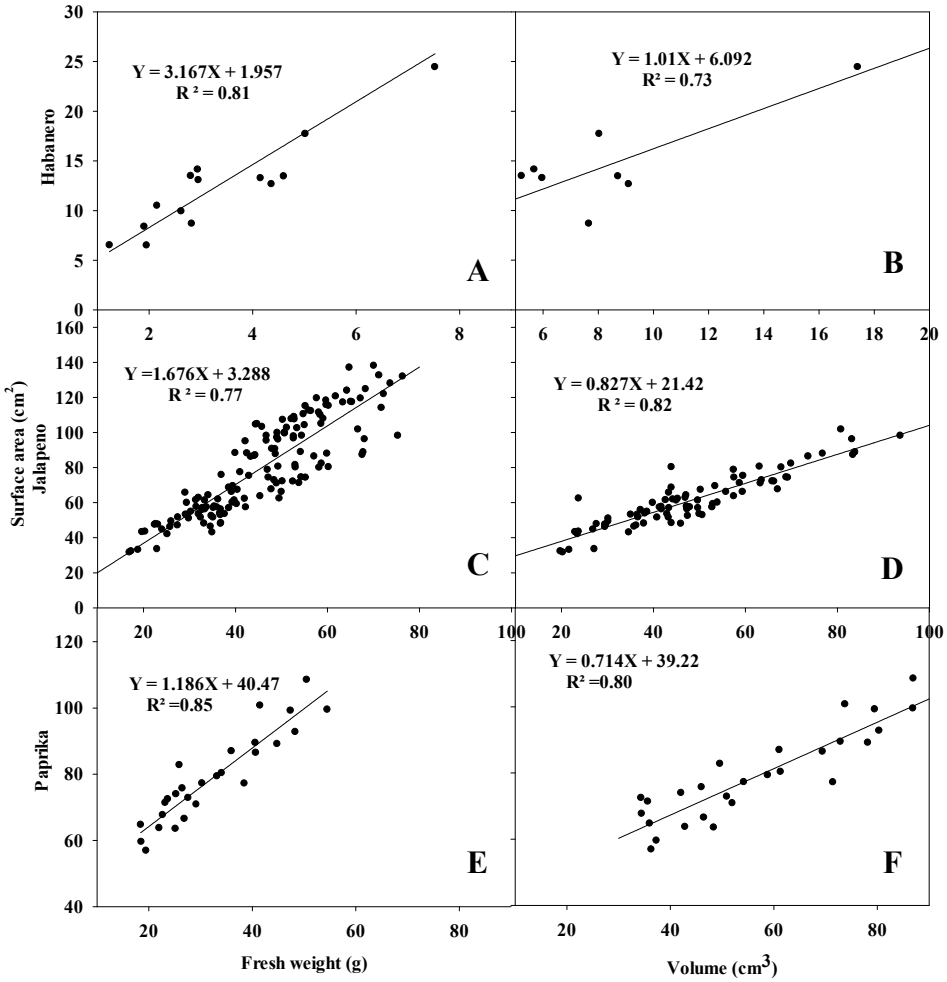


Figure 4.1 Relationship between fresh weight and volume to surface area (by image analysis – section 2.5.2) in Habanero (A - B), Jalapeño (C - D), and Paprika (E - F). Each data point represents an individual fruit. Surface area of Habanero and Paprika did not include pedicel and calyx while surface area of Jalapeño is included pedicel and calyx.

4.3.2 Water loss of three chilli varieties during storage at different temperatures

Three chilli varieties were packed in LDPE bags with holes (section 2.3.1) and stored at different temperatures from 0 - 20 °C. Water loss of Habanero stored at low temperatures (0 - 8 °C) ranged from 7 - 10 % after 28 days of storage. Water loss of Habanero stored at 12 °C and 20 °C increased ($P < 0.05$) to 16.6 % (after 28 days) and 25.9 % (after 14 days) respectively (Fig. 4.2A). Water loss of Jalapeño were affected ($P < 0.05$) by temperature. Water loss ranged from 4.7 % at 0 °C after 28 days to

27.3 % at 20 °C after 14 days (Fig. 4.2B). Paprika lost approximately 40 % of water loss during storage at 20 °C while fruit stored at other temperatures lost less than 20 % over the same time period (Fig. 4.2C). These results are similar to previous work: Lownds et al. (1994) found that water loss in some peppers increased with temperature, which is similar to apples and mushroom, indicating that an increase in temperature results in higher transpiration (Smith, 1933; Mahajan et al., 2008). However, water loss in each of the chilli and pepper varieties is different due to fruit maturity, cuticular permeability, cuticle thickness, number of pores or cracks on the skin (Lownds et al., 1994)

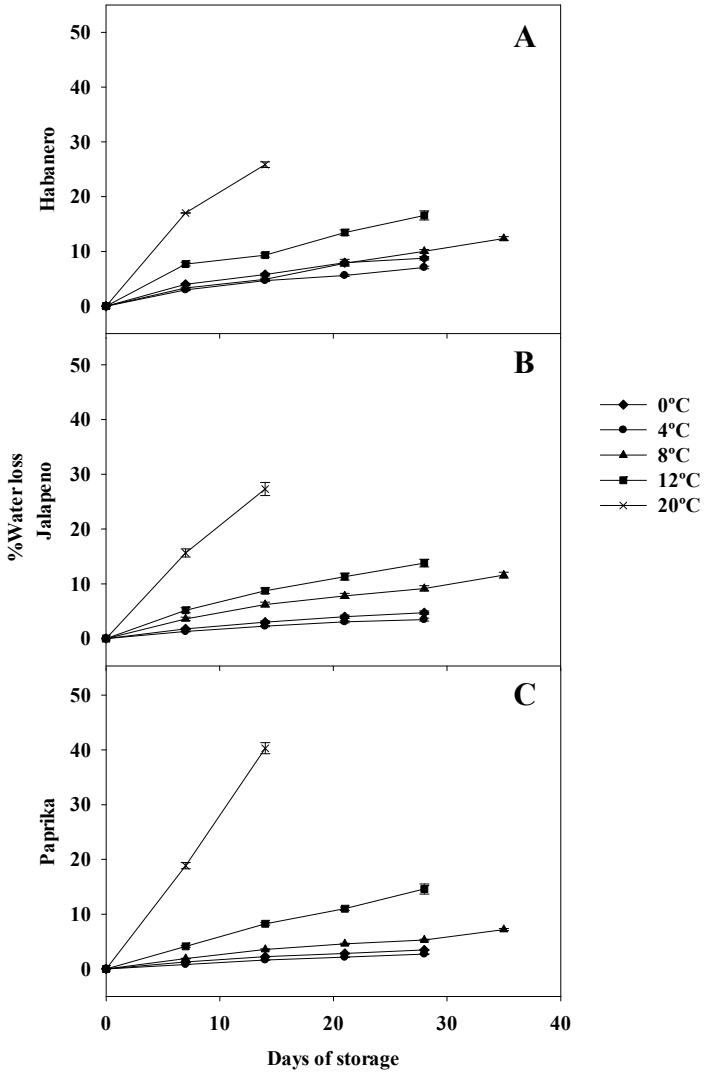


Figure 4.2 Water loss of Habanero (A), Jalapeño (B), and Paprika (C) during storage in LDPE bags at different temperatures from 0 - 20 °C. Data represent means ± S.E. (n = 30 fruit).

4.3.3 Relationship of rate of water loss to physical properties of three chilli varieties

A number of physical properties have previously been found to influence postharvest water loss of chilli and pepper (Lownds et al., 1993; Diaz-Perez et al., 2007). Burton (1982) indicated that water loss in fruit is a function of surface area. In this work, the relationship between rates of water loss ($\mu mol.s^{-1}$) of Habanero, Jalapeño and Paprika stored at 20 °C for 14 days and the surface area (as predicted from fresh weight, Fig. 4.1) were studied (Fig. 4.3). Water loss at 20 °C was proportional to the surface area of chilli fruit, particularly Habanero. Fruit with large surface area showed higher rate of water loss (Fig. 4.3). Water loss of Habanero was highly attributed ($R^2 = 0.91$) (Fig. 4.3A) to surface area while only 43 % of the variability was explained by surface area for Jalapeño (Fig. 4.3C). Positive correlations between rates of water loss ($mol.s^{-1}$) and surface area were also found in apple (Pantastico, 1975).

However, a negative correlation between weight loss ($\%.day^{-1}kPa^{-1}$) and the surface area has been observed in some peppers ('Keyston', 'NuMex R Naky' and 'Santa Fe Grande') (Lownds et al., 1993), but these correlations were not found in three chilli varieties in this research when rate of water loss were converted to % water loss ($\%.s^{-1}kPa^{-1}$) (data not shown). This can be explained that each chilli varieties had different fruit properties such as cuticle permeability and epicuticular wax, for example fruit with smaller surface area may have high cuticular permeability resulting in higher water loss (Lownds et al., 1993; Banaras et al., 1994). In addition, Diaz-Perez et al. (2007) showed that water loss ($\%.day^{-1}\ kPa^{-1}$) varied with the fruit weight of bell pepper; small sized fruit (< 50 g) showed a higher impact on water loss compared to large sized fruit (> 50 g), which showed no differences on water loss when fruit increased their weight indicating that fruit weight did not showed a major impact on water loss when fruit were getting larger. Sastry et al. (1978) suggested that surface area to volume ratio (SA/V) and surface area to fresh weight ratio (SA/FW) can be used to explain rate of water loss better than surface area alone. Previous research found a high SA/FW in pepper due to the fact that fruit are hollow (high SA but low FW) and a positive correlation was found between SA/FW and rate of water loss ($\%.day^{-1}kPa^{-1}$) (Lownds et al., 1993, 1994; Muhammad et al., 1994; Maalekuu

et al., 2005; Diaz-Perez et al., 2007; Guerra et al., 2011). However, in this research this trend was found only for Habanero (Fig. 4.3B; $R^2 = 0.34$) (Fig. 4.3D and F). This may be explained by the different characteristics of individual varieties. For example SA/FW ratio of Jalapeño is low (approximate 1.5) as fruit often fully contain with placenta while Habanero are always hollow.

In this work, fruit surface area of Habanero and Paprika were defined as only the fruit not including pedicel and calyx, as these two areas are relatively small in comparison to the whole fruit. In addition, picking scar or stem scar which is created at harvest can also influence water loss from fruit, as observed for tomato (Kader, 1996). No sealing of stem scar was conducted in this experiment. In order to estimate the error introduced by neglecting the surface area of the pedicel, calyx and stem scar, the y interception from the relationship between rate of water loss and predicted surface area can be used. Habanero showed a positive constant offset (Fig. 4.3A). If hypothetical a fruit of no surface area existed, this relationship suggests that weight loss still occurs which indicates that significant water loss occurs via pedicels, calyxes and picking scar. Utto (2001) also found a positive constant offset value from the relationship between rate of water loss and surface area indicating that water loss occurred even when fruit were totally waxed. Contrastingly a negative intercept was found for Paprika indicating that these fruit lose negligible weight via the pedicel, calyx or picking scar (Fig. 4.3E). This result may indicate that pedicels and calyxes of Paprika are less susceptible to water loss than those of Habanero. For Jalapeño, surface area estimation included pedicels and calyx but a small negative intercept ($P > 0.05$) was also found (Fig. 4.3C). Due to water loss can occur through all area parts, surface area of pedicel and calyx should be included for more accurate in the future work.

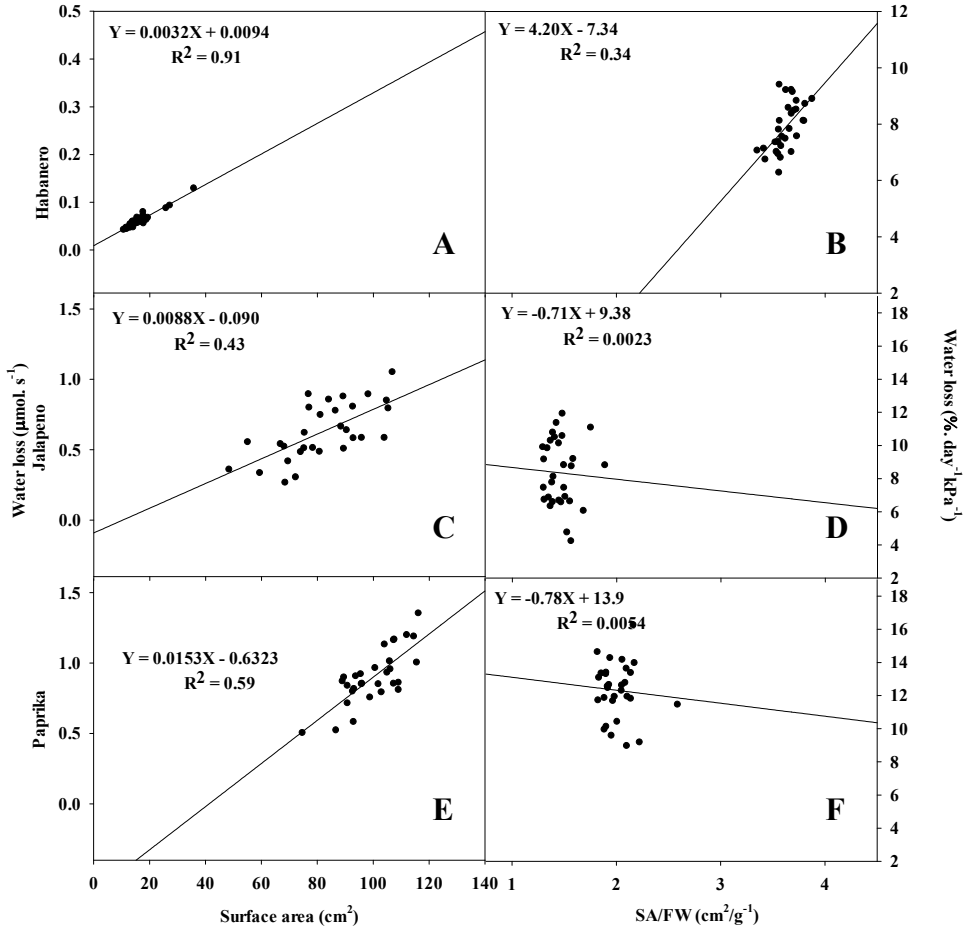


Figure 4.3 The correlation between water loss (µmol.s^{-1}) at 20 °C and predicted surface area (A, C and E) and the correlation between water loss (%. day^{-1} kPa^{-1}) at 20 °C and the predicted surface area and fresh weight ratio (SA/FW) (B, D and F) of Habanero (A and B), Jalapeño (C and D), and Paprika (E and F). The surface area was predicted from an initial fresh weight. Each data point represents an individual fruit.

4.3.4 Comparison of water vapour permeance between cracked and non-cracked Jalapeño fruit

Cracking is a physical defect that affects the quality of some fresh produce such as apple, cherry, tomato, chilli and pepper. The presence of cracks on the surface of skin allows high moisture loss from the fruit and hence significantly influences shrivel and wilting of fresh produce. Cracking in Jalapeño is a result of splitting of the pericarp along the fruit which can occur in small or large fruit and green or red

fruit (Fig. 4.4), however mild cracking are still marketable and not regarded as consumer defect.

Figure 4.4 Examples of cracking in Jalapeño

Water vapour permeance (P'_{H_2O}) characterises the fruit skin as a barrier to water vapour diffusion. Water vapour permeance differences between cracked and non-cracked fruit including consideration of the water loss through the skin, pedicel and calyx or picking scar were studied.

Water vapour permeance P'_{H_2O} (µmol·s^{-1}m^{-2}Pa^{-1}) of the whole cracked and non-cracked Jalapeño was compared. An approximately 3 times higher P'_{H_2O} ($P < 0.05$) was observed in cracked fruit in comparison to non-cracked fruit at 20 °C (Fig. 4.5) indicating that cracking on fruit skin substantially accelerates water loss in chilli. This was similar to Maguire et al. (1999b) who showed a correlation between water vapour permeance and proportional of cracking area in apple.

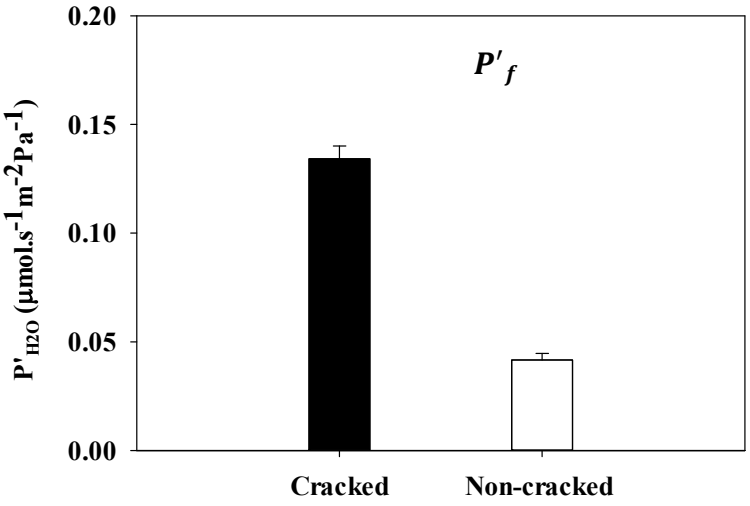


Figure 4.5 Water vapour permeance (µmol.s^{-1}m^{-2}Pa^{-1}) of the whole cracked and non-cracked Jalapeño fruit including pedicel and calyx at 20 °C. Each bar represents mean ± S.E. (n = 10 fruit).

To investigate the route of water loss in Jalapeño P'_{H_2O} was also determined for each fruit structure of Jalapeño. Both cracked and non-cracked fruit were waxed on calyx and pedicel or alternatively the fruit skin to determine the P'_{H_2O} from each structure. Pedicel and calyx showed higher P'_{H_2O} than fruit skin in both cracked and non-cracked fruit. In cracked fruit, water vapour permeance of pedicel and calyx was only 2 times higher ($P < 0.05$) than fruit skin whereas it was 10 times higher ($P < 0.05$) in non-cracked fruit (Fig. 4.6). For pedicel and calyx, there was no difference on P'_{H_2O} between cracked and non-cracked fruit due to cracking occurring only on fruit skin. Previously, calyx P'_{H_2O} has been reported as 14, 18 and 1000 times higher than fruit skin in bell pepper (Diaz-Perez et al., 2007), eggplant (Diaz-Perez, 1998) and tomato (Cameron & Yang, 1982; Diaz-Perez, 1998) respectively.

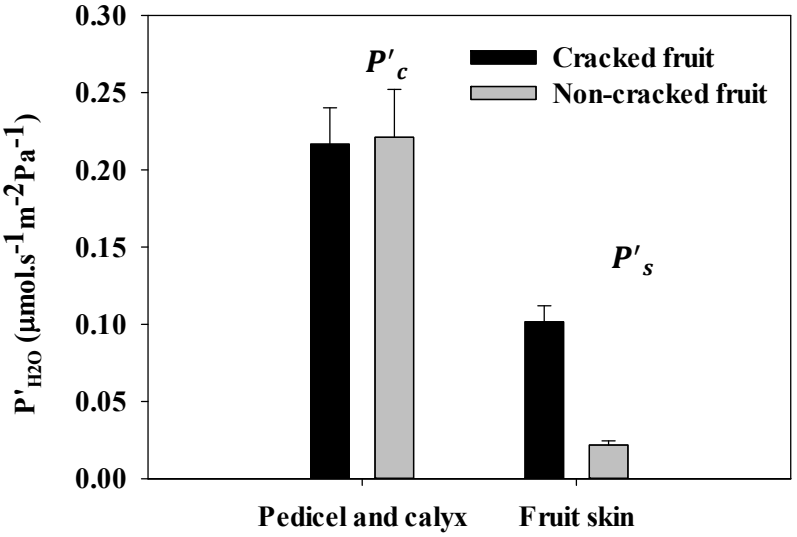


Figure 4.6 Water vapour permeance (µmol.s^{-1}m^{-2}Pa^{-1}) of individual parts of cracked and non-cracked Jalapeño fruit during storage at 20 °C. Each bar represents mean ± S.E. (n = 10 fruit).

Water vapour permeance is reported per unit area. Therefore surface area of each structure is to be taken into account in order to calculate the exact proportion of water which is lost via each fruit structure. Cracking had a major influence on the total water loss in Jalapeño (Fig. 4.7). In cracked fruit, the proportion of water which was lost via fruit skin was higher ($P < 0.05$) than that lost from pedicel and calyx (Fig. 4.7). Meanwhile, in non-cracked fruit approximately equal proportions of water

migrated via the skin or pedicel and calyx (Fig. 4.7). While water loss occurred from both fruit skin and stem areas of Jalapeño, with the stem and calyx being far more permeable to water vapour transfer, cracking on fruit skin still resulted in significant increases in total fruit water loss. This was contrast to the results of Maalekuu et al. (2005) who reported that most water loss in some peppers occurred through the fruit surface, with small amount occurring from the calyx and stem.

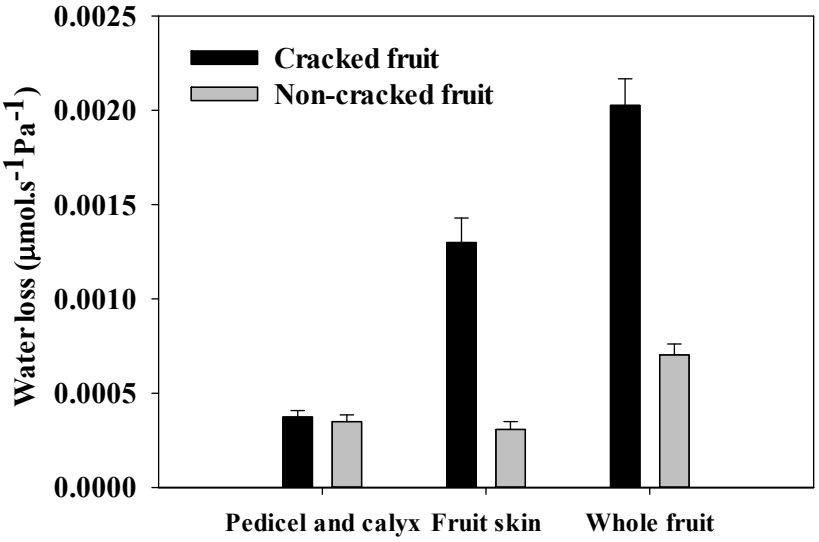


Figure 4.7 Water loss proportions (µmol.s^{-1}Pa^{-1}) of individual parts in cracked and non-cracked Jalapeño fruit during storage at 20 °C. Each bar represents mean ± S.E. (n = 10 fruit).

4.3.5 Effect of temperature on water vapour permeance of Jalapeño fruit

In general, temperature directly affects water loss largely by altering driving force (Δp_{H_2O}). This research investigated the effect of temperature on the water vapour permeance of Jalapeño. Previous research showed that P'_{H_2O} of citrus leaf cuticle increased rapidly with temperatures because of structural changes to the cuticular membrane (Schönherr et al., 1979). High temperature increased water loss, which was reported to affect P'_{H_2O} by recrystallisation of cuticular lipids and developed the hydrophilic holes (Eckl & Gruler, 1980), while Schreiber & Schönherr (1990) found a disorder of the interface between the matrix of polymer and the cuticular lipids.

Water vapour permeance (P'_{H_2O}) of Jalapeño fruit increased (P < 0.05) with temperature (Fig. 4.8). In this work, only cracked fruit were studied as cracking showed a major impact on water loss in Jalapeño with an application of wax on pedicel and calyx to understand whether waxing only these areas can delay the overall water loss. Fruit with wax applied to the calyx and pedicel area had significantly lower (P < 0.05) P'_s than control fruit (P'_f). This result indicated that waxing of the calyx could reduce the rate water loss (approx 10 %) in Jalapeño fruit even at high storage temperature.

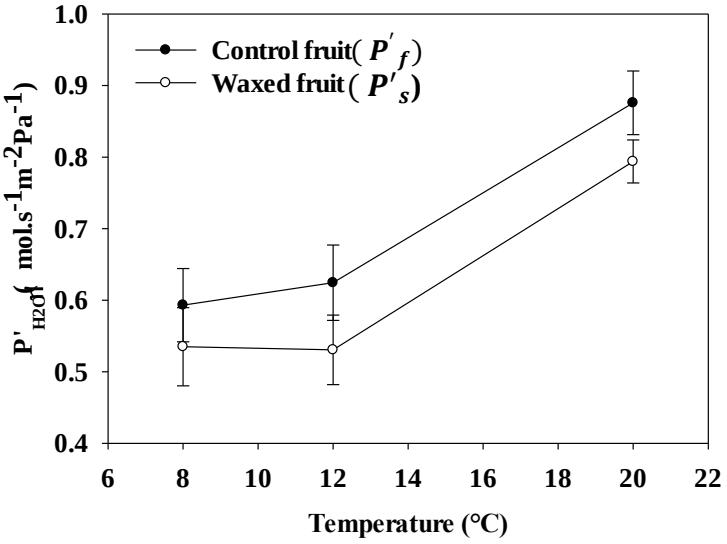


Figure 4.8 Water vapour permeance (µmol.s^{-1}m^{-2}Pa^{-1}) of Jalapeño with and without wax on calyx and stem area and stored at 8, 12, and 20 °C, 85 - 90 %RH. Data represent means ± S.E. (n = 30 fruit).

4.3.6 Effect of maturity at harvest on water vapour permeance of Jalapeño fruit

Maturity at harvest can also affect rate of water loss from chillies and peppers (Sastry et al., 1978; Ben-Yehoshua, 1987; Diaz-Perez et al., 2007). Previous data of Jalapeño stored at 8, 12, and 20 °C (Fig. 4.8) were rearranged by maturity at harvest from 5 - 7 WAFS (Fig. 4.9). P'_{H_2O} of chilli fruit harvested at 5 WAFS was higher (P < 0.05) than fruit harvested at 7 WAFS (Fig. 4.9). These results are similar to Diaz-Perez et al. (2007) who found higher P'_{H_2O} in immature bell pepper indicating incompletely develop of the skin structure of immature fruit. Immature fruit have also been found to have a higher weight loss (%) than ripen fruit of avocado (Cutting

& Wolstenholme, 1991) and banana (Adeniji & Barimalaa, 2008). For European plum the mass of cuticular membrane (mg.fruit^{-1}) increased with fruit maturation by increasing cutin and wax deposit on fruit skin (Knoche & Peschel, 2007). In contrast, weight loss (%) in red bell pepper has been reported to be higher than the loss in green fruit which was related to higher membrane permeability and membrane leakage (Lurie & Ben-Yehoshua, 1986; Lurie et al., 1986). In this research, there was no significant difference ($P > 0.05$) of P'_{H_2O} between fruit harvested at 6 and 7 WAFS during storage at different temperatures in waxed fruit, while for control fruit, there was no significant difference between fruit harvested at 5 and 6 WAFS during storage (Fig. 4.9).

In this research, Jalapeño fruit which were waxed only on the calyx and pedicel (the non-consumed portion) showed lower P'_{H_2O} by approximately 10 % than control fruit at storage conditions (8 and 12 °C, Fig. 4.8). Waxing mainly influenced P'_{H_2O} when wax was applied to Jalapeño fruit harvested at 6 WAFS, but not for fruit harvested at 5 or 7 WAFS which showed no difference of P'_{H_2O} between waxed and control fruit (Fig. 4.9).

In general, wax or an edible impermeable coating could be applied to the whole fruit to reduce water loss. There are a number of studies on waxing in many fruit such as lemon, cucumber, and eggplant (Anon., 2004; Thirupathi et al., 2006), pepper (Lerdthanangkul & Krochta, 1996; Conforti & Ball, 2002; Conforti & Zinck, 2002) and apricot (Ayranci & Tunc, 2004). In pepper, milk protein (Lerdthanangkul & Krochta, 1996), hydrocolloid-lipid (Conforti & Ball, 2002 and Conforti & Zinck, 2002) and methyl cellulose-polyethylene glycol-stearic acid (MC-PEG-SA) (Ayranci & Tunc, 2004) were used to coat to the whole fruit to delay water loss and maintain postharvest quality. The rate of water loss can be reduced in all waxed fruit by approximately 10 - 30 % in comparison to control fruit depending on coating types and pepper cultivars (Lerdthanangkul & Krochta, 1996; Conforti & Ball, 2002; Conforti & Zinck, 2002).

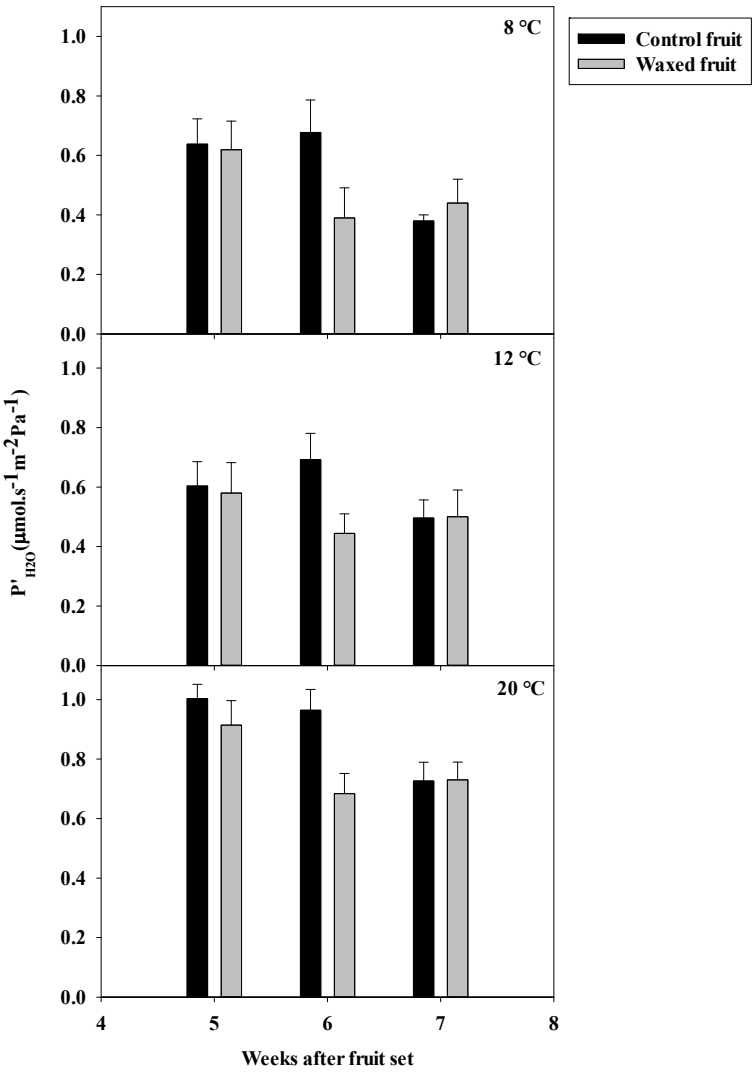


Figure 4.9 Water vapour permeance (µmol·s^{-1}m^{-2}Pa^{-1}) of Jalapeño fruit harvested at different maturity stages with and without applying wax on calyx and stem area and stored at 8, 12, and 20°C, 85-90 %RH. Each bar represents mean ± S.E. (n = 4 - 8 fruit).

However, some considerations relating to food intolerances, for example wheat gluten or milk protein, religious beliefs, and antimicrobial additives, which are on occasions constituents of or additions to edible coating, should be made aware to some consumers (Bourtoom, 2008; Valencia-Chamorro et al., 2011). Application of these coatings to non-consumed fruit structures (i.e. peel, calyx and pedicel) can be an alternative way to reduce these problems. However, for cracked Jalapeño fruit, waxing only the calyx and pedicel only had an approximately 10 % reduction of the

rate of water loss during storage at 8 °C, which can be explained as water loss in cracked fruit mainly occurring from fruit skin (Fig. 4.7).

P'_{H_2O} in this experiment (Fig. 4.8 - 4.9) were approximately 8 times higher than when compared to P'_{H_2O} from the previous experiment (Fig. 4.5). These different results were collected at dramatically different RH. Data collected in Fig. 4.5 were from experiments conducted in a cabinet with fan operation. Dry and wet bulb temperatures were measured at 19 and 16.3 °C, respectively, which equates to 78 % RH. Meanwhile trials resulting in Fig. 4.8 and 4.9 were conducted in a controlled temperature room (RH set point = 95 %) with the measured RH fluctuating between 90 - 98 %, which resulted that dry and wet bulb temperatures being very similar. The driving force (Δp_{H_2O}) of fruit at higher RH was approximate 7 - 8 times lower than fruit at lower RH which directly affected water loss and the subsequent calculation of P'_{H_2O}. These errors in wet bulb and fruit temperature measurement severely influence P'_{H_2O} quantification. Utto (2001) also found small errors in wet bulb and skin temperature can lead to large errors in P'_{H_2O} estimation at different temperatures. Despite these differences between these two experiments, it was a challenge in the calculation of converting water loss to water vapour permeance data. An error occurred was from calculation error rather data collecting error so the relative differences between the treatments still remained no matter what number of the magnitude.

4.3.7 Microscopy images of Jalapeño skin

To investigate the nature of cracking on fruit skin, microscopy work was conducted. Microscopy images of cracked and non-cracked fruit were captured by fluorescence microscopy. The images from normal and fluorescent (blue and green) light showed that the surface of cracked fruit was torn; producing an open hole which created a path of low resistant for water loss (Fig. 4.10B-D). This tear is not present on non-cracked fruit skin (Fig. 4.10A). The size of tear depended on the severity of cracking symptom. Fruit with a small cracking area showed a small hole on the surface (Fig. 4.10B) while fruit with severe cracking was more dramatic (Fig. 4.10D). These images clearly showed that cracked chilli fruit were more prone to water loss than

non-cracked fruit because of their skin surface. Aloni et al. (1999) also observed cracking in pepper fruit initiated by mini-cracks on the cuticle, which expanded to crack and split the epidermal walls. Further study was conducted to determine the components on the fruit skin surface. Berberine-Aniline blue fluorescence staining was selected to detect lignified walls. Generally, the staining colour of suberin lamellae is fluorescence blue-white or blue colour and fluorescence yellowish-green for cuticle (Brundrett et al., 1988; Ma et al., 2004). However the fluorescence images in this work were not clearly observed. Photobleaching may have caused this problem as Brundrett et al. (1988) mentioned that light intensity and wavelength can fade Berberine-Aniline staining. Previously, Ma et al. (2004), following Brundrett et al. (1988), found a weak staining in some cultivars of soybean seeds. These authors attributed the poor staining of some cultivars to the surface deposit compositions (e.g. cutin, lignin and callose), which influence permeability to water.

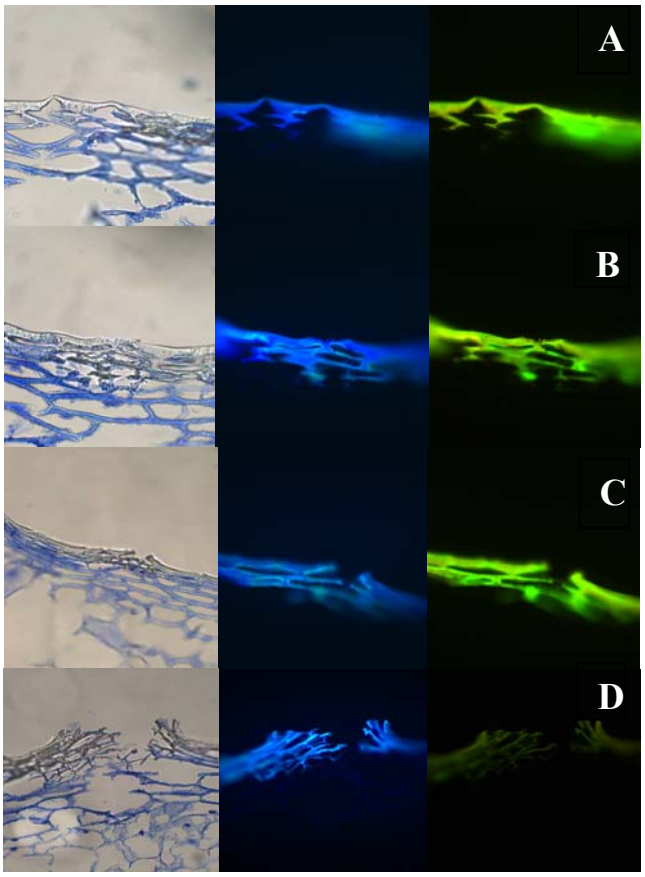


Figure 4.10 Sections of Berberine-Aniline blue staining of Jalapeño fruit skin developed from non-cracked (A) to severe cracked fruit (D) by using light microscope with visible light (left) and UV fluorescent illuminators (blue (middle) and green (right) fluorescence).

4.3.8 Modelling the rate of water loss of Jalapeño during storage at different storage conditions

A model was developed to predict the rate of water loss of Jalapeño during storage under different environmental and fruit conditions. Development of such a model allows estimation of the time to reach a critical water loss (that may cause shrivel, for example) as influenced by manageable factors. With this information, strategies to inhibit water loss can be assessed on the basis of the potential to extend shelf life.

Modelling the rate of water loss (r'_{H_2O}) of Jalapeño was predicted by rearrangement of equation 4.1;

$$r'_{H_2O} = P'_{H_2O} A(\Delta p_{H_2O}) \qquad \textbf{Eq. 4.7}$$

Given a known water vapour permeance (P'_{H_2O}) and surface area (A), the rate of water loss is a direct function of the storage condition (temperature and humidity). Assuming that fruit temperature is equal to environmental temperature, RH becomes a major influence of the driving force (Δp_{H_2O}). Equation 4.7 can be subsequently modified to the following:

$$r'_{H_2O} = P'_{H_2O} A[\, p^{sat}_{H2O}(T)\left(1 - \frac{RH}{100}\right)] \qquad \textbf{Eq. 4.8}$$

The model of % water loss of Jalapeño during storage at different conditions was developed to predict an approximate storage life of cracked and non-cracked Jalapeño. A scenario of storage at 8 °C (optimum temperature, from chapter 3) and 80 % RH was used as a baseline scenario in which manipulation of conditions or fruit characteristics were compared. P'_{H_2O} of cracked and non-cracked Jalapeño at 20 °C at RH 80 % was 0.134 and 0.042 µmol.s^{-1}m^{-2}Pa^{-1}, respectively (Fig. 4.5). It was shown in Fig. 4.8 that P'_{H_2O} (at RH 95 %) at 8 °C was 67 % of P'_{H_2O} at 20 °C therefore P'_{H_2O} (at RH 80 %) at 8 °C was estimated at 0.089 and 0.028 µmol.s^{-1} m^{-2} Pa^{-1} for cracked and non-cracked fruit, respectively. This paragraph above basically outlined the base line conditions

Given this base line condition, the model was used to manipulate some conditions and study the effect of the time to reach 5 % water loss which is assumed to be the time of shrivel developed (Wills et al., 2007). Factors studied include variation in fruit weight, permeance, wax, temperature (affecting both permeance and driving

force) and RH (affecting driving force). Input values of each factor of interest for the model simulation are shown in Table 4.2.

Table 4.2 Values of each parameter for each factor of interest using in the developed model

Factor of interest	P'_{H_2O} ($\mu mol.s^{-1}m^{-2}Pa^{-1}$)	A (m^2)	T (°C)	Fruit weight (g)	RH (%)
Base-line	(Fig. 4.5 and 4.8)	(Fig.4.1C)			
Non-cracked fruit	0.028	0.0070	8	40	80
Cracked fruit	0.089	0.0070	8	40	80
Fruit weight	(Fig. 4.5 and 4.8)				
Non-cracked fruit	0.028	0.0037	8	20	80
Non-cracked fruit	0.028	0.0120	8	70	80
Cracked fruit	0.089	0.0037	8	20	80
Cracked fruit	0.089	0.0121	8	70	80
$\boldsymbol{P'_{H_2O}}$	(Fig. 4.5 and 4.8)				
Non-cracked fruit	0.020	0.0070	8	40	80
Non-cracked fruit	0.040	0.0070	8	40	80
Cracked fruit	0.067	0.0070	8	40	80
Cracked fruit	0.107	0.0070	8	40	80
Waxing	(Fig. 4.5 and 4.8)				
Cracked fruit	0.079	0.0070	8	40	80
Temperature	(Fig. 4.5 and 4.8)				
Non-cracked fruit	0.0300	0.0070	12	40	80
Non-cracked fruit	0.0416	0.0070	20	40	80
Cracked fruit	0.0960	0.0070	12	40	80
Cracked fruit	0.134	0.0070	20	40	80
RH					
Cracked fruit	0.028	0.0070	8	40	70
Cracked fruit	0.028	0.0070	8	40	90
Non-cracked fruit	0.089	0.0070	8	40	70
Non-cracked fruit	0.089	0.0070	8	40	90

To test the sensitivity of water loss to possible variances in fruit weight, small to large fruit (20, 40 and 70 g) were studied. Cracked fruit were predicted to reach 5 % water loss (where shrivel can be observed) within 10 days while non-cracked fruit could be stored for 30 days before shrivel develops (Fig. 4.11A). Fruit weight did not show a high impact on rate of water loss, resulting in a minimal impact on time to develop shrivel symptoms.

When investigating the range of P'_{H_2O}, previously observed in the population high P'_{H_2O} cracked fruit (0.107 μmol.s^{-1}m^{-2}Pa^{-1}) were predicted to reach 5 % water loss in less than 10 days, while storage life would be reduced to 20 days in high P'_{H_2O} non-cracked fruit (0.04 μmol.s^{-1} m^{-2} Pa^{-1}) (Fig. 4.11B).

Temperature (8 - 20 °C) (with 80 % RH) and RH (70 - 90 %) (at 8 °C) were studied in cracked and non-cracked Jalapeño. P'_{H_2O} at different temperatures was taken from Fig. 4.5 and followed the trend from Fig. 4.8. Cracked fruit were predicted to lose 5 % water within 3 days at 20 °C while the occurrence of shrivel can be delayed for 4 to 7 days when fruit were stored at 12 and 8 °C respectively in comparison to fruit stored at high temperatures (12 and 20 °C) respectively (Fig. 4.12A). For non-cracked fruit, shrivel can be observed within 8, 22 and 30 days of storage at 20, 12 and 8 °C, respectively (Fig. 4.12A). However, the actual water loss at 8 and 12 °C from the experiment was slower than the predicted water loss of cracked fruit. This may be explained as fruit used in the experiment were in LDPE bags and stored in the temperature cabinet resulting in high RH. In addition, a significant boundary layer of stagnant air can be created on the fruit skin due to negligible air flow within the packaging. Both of these effects would be expected to result in slower water loss rate from the experiment than predicted in which the permeability data is deliberated collected from a high velocity air environment to eliminate the boundary layer effect (Fig. 4.12A). However, water loss of Jalapeño at 20 °C from the experiment was faster than that predicted, which may be explained as fruit in the experiment were stored at room temperature (with no RH control), so RH might be low and induce higher water loss at 20 °C. This was found in the prediction showing that RH at 70 % (at 8 °C) was predicted to lose 5 % water within 6 days for cracked fruit while the

storage life can be extended to 21 days when fruit were stored at 90 % RH (at 8 °C) (Fig. 4.12B).

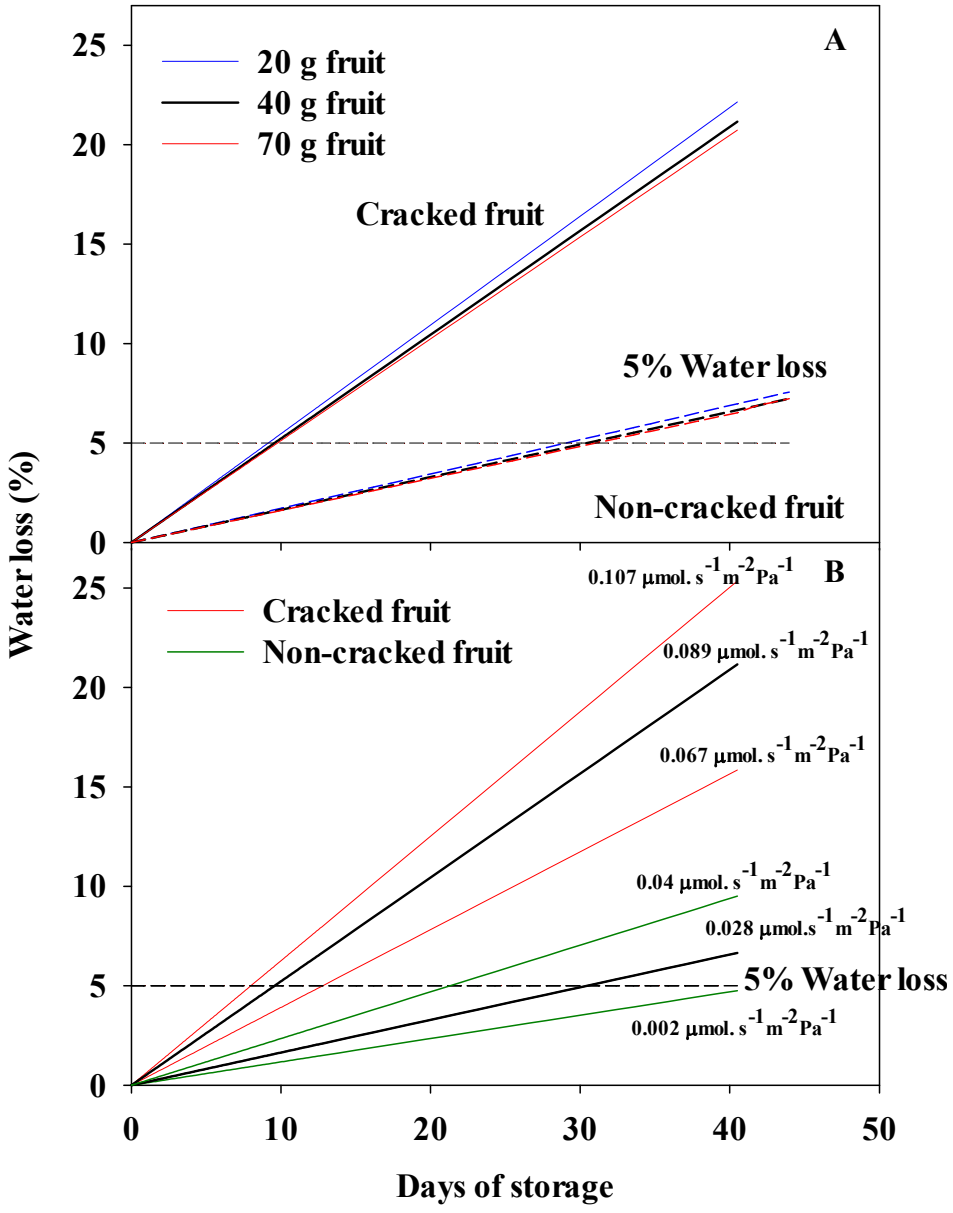


Figure 4.11 Predicted % water loss of cracked and non-cracked Jalapeño (A) weighing from 40 - 70 g and (B) different P'_{H_2O} from 0.067 - 0.107 µmol.s^{-1}m^{-2} Pa^{-1} in cracked Jalapeño and from 0.002 - 0.04 µmol.s^{-1}m^{-2}Pa^{-1} in non-cracked Jalapeño during storage at 8°C and 80 % RH. The black line represents a base Fline scenario of 40 g fruit and average P'_{H_2O} during storage at 8 °C and 80 % RH. All predictions were made using the model described in Eq. 4.8.

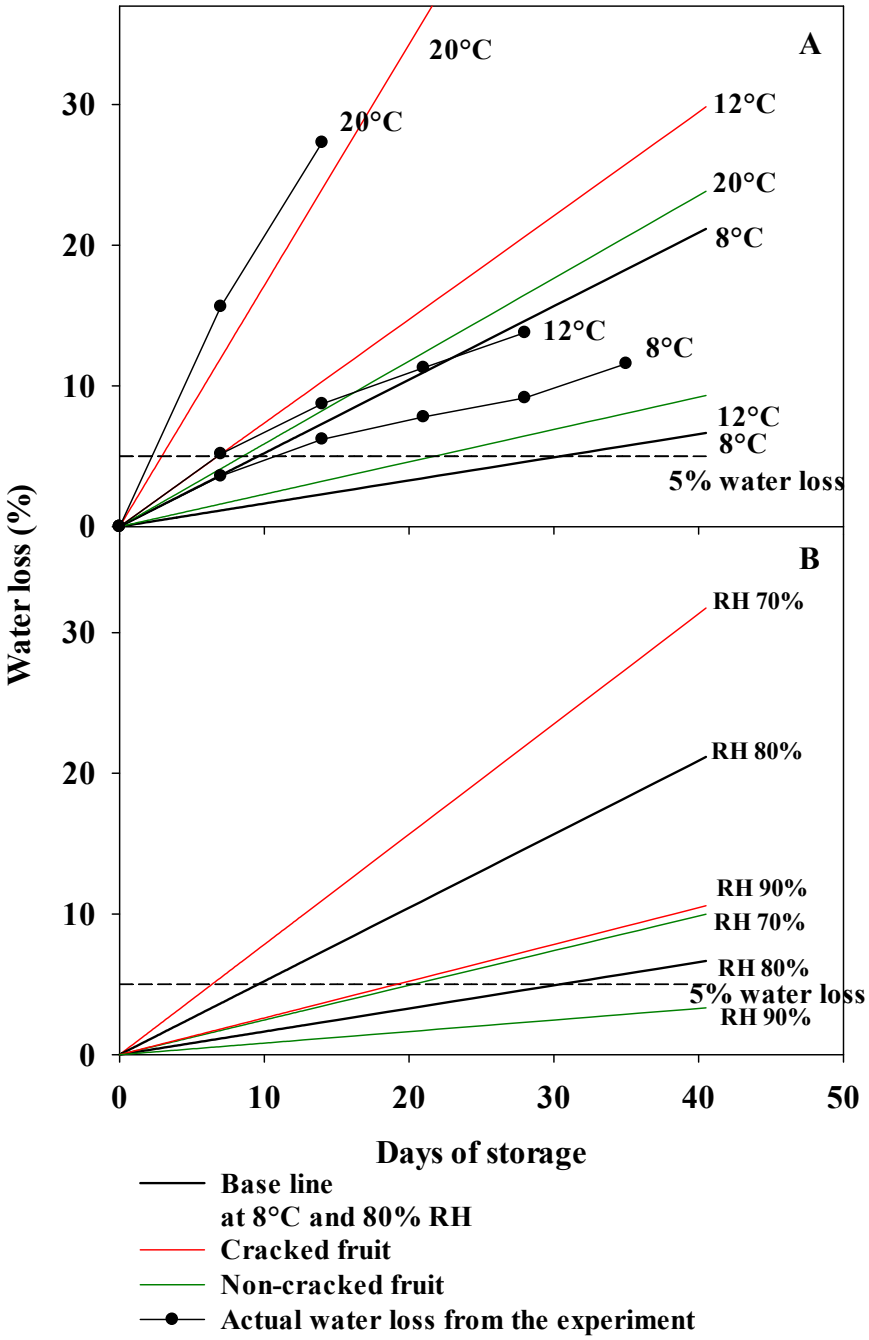


Figure 4.12 Predicted % water loss of cracked and non-cracked Jalapeño during storage at different temperatures from 8 - 20 °C and 80 % RH (A) and at different RH from 70 - 90 % at 8 °C (B) by using the model described in Eq. 4.8 and compared with water loss from Jalapeño during storage at 8 - 20 °C and 75 - 80 % RH in the experiment.

Application of wax on calyx and pedicel was studied as a method to reduce water loss. In this simulation, P'_{H_2O} of cracked fruit at 8 °C was fixed at 0.089 and 0.079 μmol.s^{-1}m^{-2}Pa^{-1} for non-waxed and waxed fruit, respectively (from Fig. 4.5 and 4.8). This simulation demonstrated that waxing only calyx and pedicel results in the loss of 5 % water within 12 days, which is 1 to 2 days later than non-waxed fruit (Fig. 4.13). As water is mainly lost from fruit skin, particularly in cracked fruit, waxing only calyx and pedicel did not highly influence water loss. An alternative strategy to reduce water loss may be to completely coat fruit with edible wax. As stated previously, the rate of water loss can be reduced in all waxed fruit by approximately 10 - 30 % in comparison to control fruit, depending on coating types and pepper cultivar (Lerdthanangkul & Krochta, 1996; Conforti & Ball, 2002; Conforti & Zinck, 2002). Application of edible coating or wax particularly in cracked fruit should be investigated as a potential way to delay water loss.

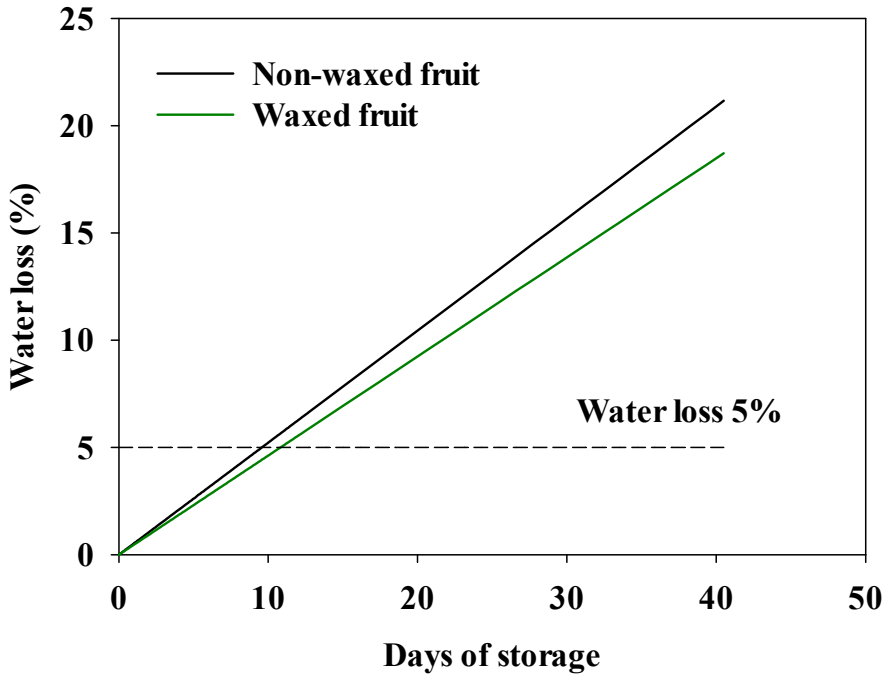


Figure 4.13 Predicted % water loss from cracked Jalapeño comparing between applying wax on calyx and pedicel and non-waxed fruit during storage at 8°C and 80 % RH by using the model described in Eq. 4.8.

4.4 Conclusions

Water loss is a major problem for chilli quality; three chilli varieties showed higher water loss at high temperature (20 °C). A correlation was found between surface area and water loss, which indicated that fruit with high surface areas tended to lose more water. For Jalapeño fruit, water loss in cracked fruit was approximate 3 times higher than in non-cracked fruit. Cracking on fruit skin of Jalapeño strongly affected water loss meaning that water loss mainly occurred through the cracks on fruit skin. Cracking was observed as open holes on microscopy images. Water loss via calyx and pedicel was significant in non-cracked Jalapeño as water loss occurred through both stem area and fruit skin at a similar rate.

A model was developed to predict the shelf life (using 5 % water loss as time to shrivel development) of Jalapeño during storage by conducting a sensitive analysis on the potential factors (such as fruit weight, water vapour permeance (P'_{H_2O}), temperature and RH) that influence time to 5 % water loss. Compared to the base line case of 40 g fruit stored at 8 °C and 80 % RH, it took 10 and 30 days to reach 5 % water loss in cracked and non-cracked Jalapeño respectively. Increasing temperature from 8 to 12 or 20 °C reduced time to reach 5 % water loss to only 7 or 3 days in cracked fruit and 22 or 8 days in non-cracked fruit. Storage at high RH (90 %) delayed water loss by doubling the time to reach 5 % water loss (from 10 to 20 days in cracked fruit and from 30 to 60 days in non-cracked fruit), but low RH (70 %) reduced the time to reach 5 % water loss in both cracked and non-cracked fruit (Fig. 4.14). Changes in fruit weight and P'_{H_2O} showed less effect in the time to 5 % water loss. Overall, time to 5 % water loss is most sensitive to temperature or RH, indicating that water loss in Jalapeño is most easily controlled with good storage condition management.

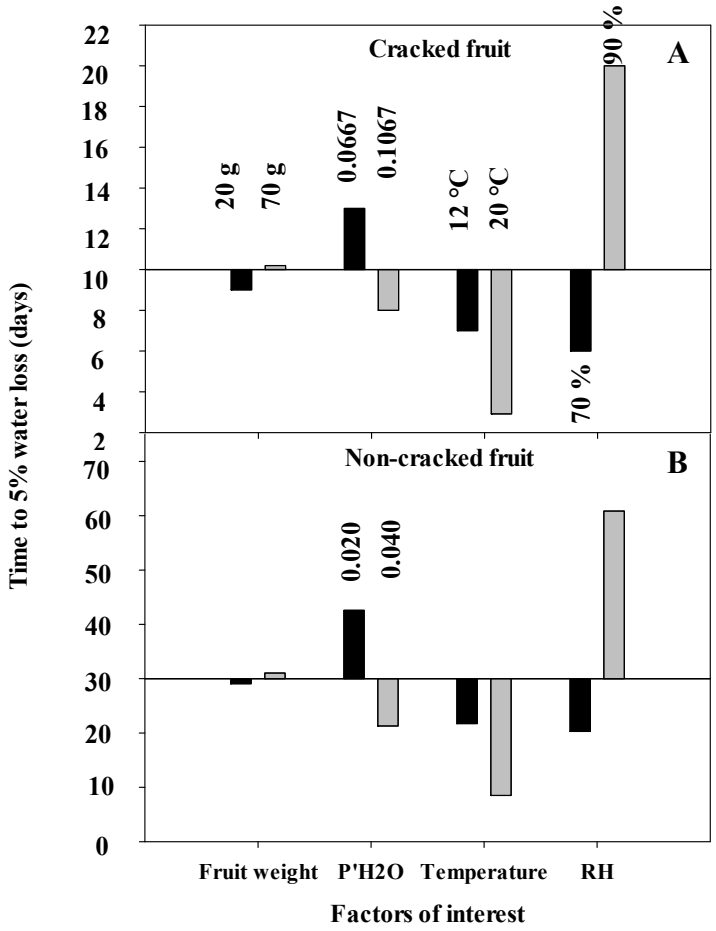


Figure 4.14 Percentage changes in time to 5 % water loss for (A) cracked fruit and (B) non-cracked fruit after applying the parameter measured. Each factor of interest was described in Table 4.2 and compared to the same base line case (40 g fruit stored at 8 °C and 80 % RH, P'_{H_2O} = 0.089 and 0.028 µmol.s^{-1}m^{-2}Pa^{-1} for cracked and non-cracked fruit, respectively).

Application of wax on calyx and pedicel of Jalapeño may delay water loss within 1 - 2 days. Therefore, waxing fruit skin or even the whole fruit should be investigated to prolong the shelf-life of Jalapeño particularly cracked fruit. This model should be developed further to predict water loss of chillies during storage at different conditions.

CHAPTER 5

Pre-harvest factors affect Jalapeño weight, shape and colour

5.1 Introduction

Fruit size, shape and colour are the most important visual consumer qualities of chillies and peppers (Berke et al., 2005). Size and shape vary depending on variety (Biles et al., 1993; Barrera et al., 2005; Barrera et al., 2008). Fruit yield is contributed by the number of marketable fruit per plant and the weight of individual fruit (Russo, 2008; Sermenli & Mavi, 2010). Most chilli and pepper fruit are sold by weight. Therefore, physical properties such as size and shape are important attributes influencing the marketing of chilli. Colour is another important attribute which differs for each cultivar (Davies et al., 1970; de Guevara et al., 1996; Gómez et al., 1998; Hornero-Mendez & Minguez-Mosquera, 2000, 2002). Generally, colour changes of chillies and peppers occur as a result of chlorophyll degradation with a significant increase of carotenoid content (de Guevara et al., 1996; Hornero-Mendez & Minguez-Mosquera, 2002). The demand for coloured chilli and pepper varies depending on the intended use. The aim for growers is to produce a high yield of product with consistent physical quality for consumers.

In chapter 3, postharvest quality of chilli was found to have variability for some attributes such as colour and phytochemical compounds. Variable Paprika colour has previously been reported even when fruit were harvested at similar times after anthesis (Worku et al., 1975). These variations may be influenced by several factors including genetic variation, flowering order, position on plant and growing conditions (Worku et al., 1975; Wien, 1997; Thang, 2007). In this chapter, effects of pre-harvest factors such as time of planting, position on plant, maturity stage and crop load were determined on physical properties (weight, shape and colour) of Jalapeño during fruit growth, maturation and ripening. Data were collected over two seasons from commercial and PGU glasshouses by monitoring individual fruit development from the point of flowering. Understanding more about these variations can aid growers to manipulate the crop in order to produce consistently high quality chillies and peppers.

5.2 Materials and methods

Jalapeño fruit were planted in a commercial glasshouse (Orcona Chillis 'N Peppers) located at Napier, New Zealand. Individual flowers were tagged at full bloom allowing weeks after flowering to be used as the maturity measurement. Fruit were harvested weekly from 1 to 9 weeks after flowering (WAF) (section 2.2 and 2.3.2). Data collection was hampered by approximately 50 % of tagged flowers abortion resulting in reduced fruit numbers in the sample population from what was initially planned.

The further season, Jalapeño fruit were planted in a glasshouse at the Plant Growth Unit, Massey University, Palmerston North from monthly sequential plantings (August - October). On this occasion, maturity was redefined as weeks after fruit set (WAFS) to reduce the amount of fruit lost due to flower abortion (section 2.2 and 2.3.3).

Fruit were harvested fortnightly from 6 to 10 WAFS (section 2.3.3) while also noting the nodal location of growth on the plant. Plants were also manipulated to have either high (fruit on every node) or low (fruit on every 4^{th} node) crop load. The impact of different time of planting, position on plant and crop load on fruit weight, shape and colour was assessed. Temperature and humidity were recorded by Tinytag Ultra (Gemini) data loggers located within a box with fan to prevent influence of direct sun exposure). These experiments involved periodic fruit removal, which may affect maturation and competition of remaining fruit. Another additional set of plants in each sequential planting were strip picked i.e. all fruit present on the plants were harvested once fruit from the first node reached 6, 8 or 10 WAFS. Therefore each plant consisted of fruit with a range of maturity stages.

Jalapeño fruit weight was measured once fruit had equilibrated to room temperature (2 hours after harvest). Fruit length was measured from calyx (excluding pedicel) to the tip of fruit with vernier callipers. Circumference was measured from the widest point of fruit by using string and ruler. Jalapeño fruit were assumed to be a cylindrical shape so fruit volume was calculated from volume $= \pi r^2 h$ (h = fruit

length) and fruit density was calculated from the equation 2.4 (section 2.5.1). Colour of Jalapeño was measured by spectrophotometer (section 2.4.4).

5.3 Results

5.3.1 Fruit weight and shape

5.3.1.1 Weight and shape of Jalapeño fruit planted in a commercial and a PGU glasshouse during maturation

In the commercial glasshouse, fruit weight of Jalapeño increased rapidly from 3 to 30 g during the first 4 weeks after flowering, plateauing for the next two weeks before increasing again from 6 to 9 WAF (Fig. 5.1A). Fruit length and circumference were dependent on maturity at harvest with the majority of growth occurring in the first 2 WAF (Fig. 5.1B).

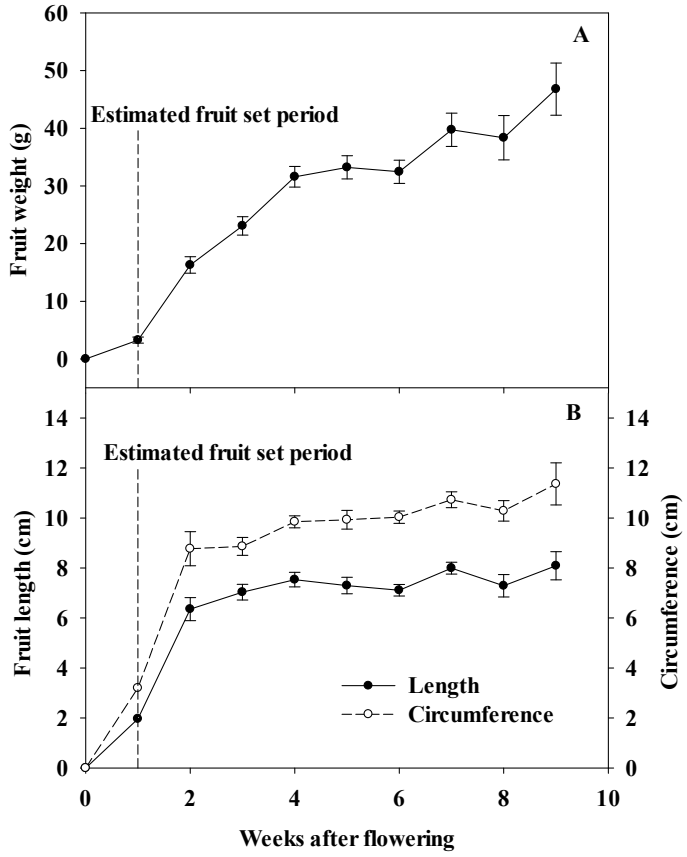


Figure 5.1 Fruit weight (A), length and circumference (B) of Jalapeño planted in a commercial glasshouse. Fruit were harvested during development defined by weeks after flowering (n = 3 – 44 fruit).

For Jalapeño planted in the glasshouse at Plant Growth unit, fruit weight and circumference increased ($P < 0.05$) during development particularly in the first 6 weeks (Fig. 5.2 A and C). However, Jalapeño fruit of 7 WAFS (from plant when all fruit presented on the plant were harvested when fruit from the first node reached 10 WAFS) were unusually small (Fig. 5.2). The length of Jalapeño fruit at different maturities were not different ($P > 0.05$) after 2 WAFS (Fig. 5.2B). Volume of Jalapeño fruit was determined by assuming fruit as cylindrical in shape. Fruit volume tended to increase as fruit weight increased during fruit development (Fig. 5.2A and 5.3A). When fruit density was calculated from fruit weight and volume, it varied with fruit weight and volume of Jalapeño during maturation (Fig 5.3B).

Overall, Jalapeño grew rapidly in weight, volume and circumference through 6 WAFS but fruit length reached maximum about 2 WAFS (Fig. 5.2. and 5.3). Thus fruit elongated fully during an initial stage of fruit development, and then fruit expansion began to occur (Fig. 5.2B and C). Density of chilli tended to increase during initial fruit development and then remained reasonably stable at more mature stages (Fig. 5.3B). When fruit weight at all stages was plotted against fruit density, fruit density is observed to increase with fruit weight (Fig. 5.4) which may relate to both pericarp and placenta development. However the weak correlation may depend on the different proportion of seeds and placenta which are variable in each individual Jalapeño (Fig. 5.5).

In the commercial glasshouse, maturity was gauged from time of flowering while in the PGU glasshouse maturity was measured from fruit set. In order to compare between experiments, fruit set was assumed to occur approximately about 1 WAF by observation (black dashed line in Fig. 5.1A and B). At the same maturity stage, Jalapeño fruit weight from the PGU glasshouse was slightly higher than fruit from the commercial glasshouse. However, fruit from the PGU glasshouse seemed to gain no weight after 6 WAFS while fruit from the commercial glasshouse continued to develop even after 8 WAF (about 7 WAFS) (Fig. 5.1A and 5.2A). Length (6 - 8 cm) and circumference (8 - 12 cm) of fully mature Jalapeño were similar in both the commercial and PGU glasshouses (Fig. 5.1B and 5.2B and C).

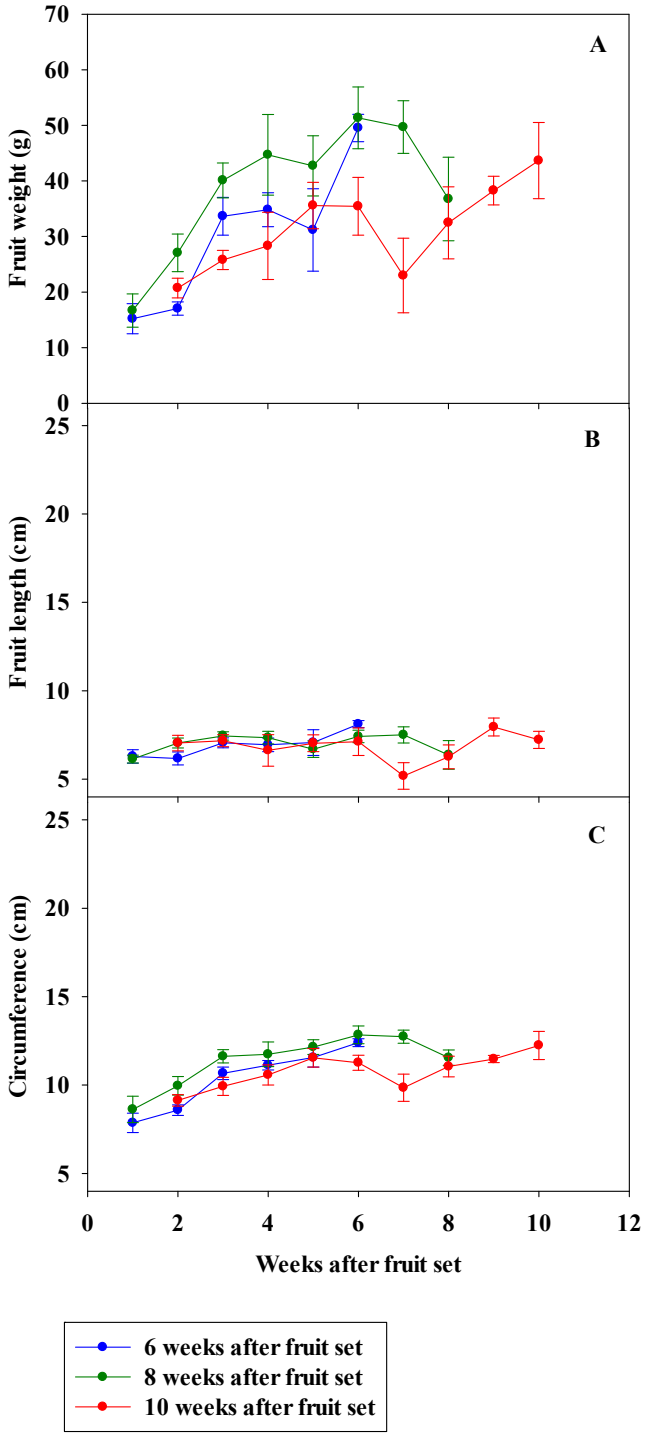


Figure 5.2 Fruit weight (A), length (B) and circumference (C) of Jalapeño planted in a PGU glasshouse. Fruit were harvested during development defined by weeks after fruit set. All fruit presented on the plant were harvested when fruit from the first node reached 6 (Blue), 8 (Green), and 10 (Red) weeks after fruit set. Data represent means ± S.E. (n = 2 - 10 fruit).

Jalapeño plants came from F1 seeds which were expected to produce uniform fruit. However, the observed results displayed some variability. In the same plant, fruit from the one leader (43 g) was approximate 2 times larger than fruit from another leader (20 g). In addition, the variation was also found between fruit from different plants that were treated similarly and were expected to be similar. For example, some plants produce very small (approx 15 - 22 g) fruit while other plants treated similarly produced fruit of more than 30 g. This observed large variation adds complexity to the explanation of the findings in this research.

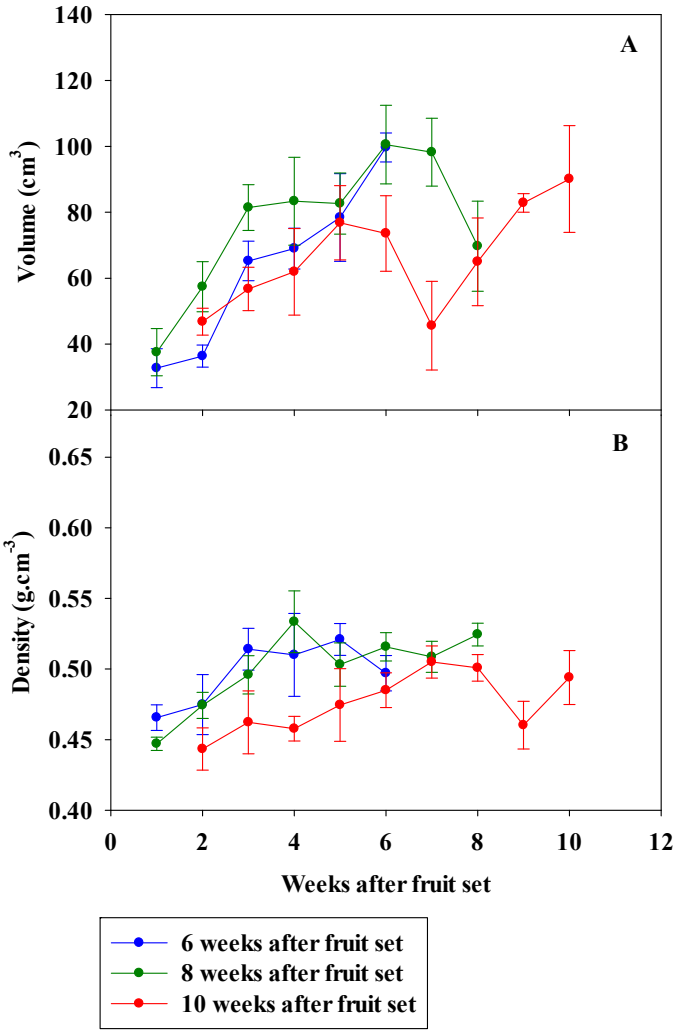


Figure 5.3 Fruit volume (A) and density (B) of Jalapeño planted in a PGU glasshouse. Fruit were harvested during development defined by weeks after fruit set. All fruit presented on the plant were harvested when fruit from the first node reached 6 (Blue), 8 (Green), and 10 (Red) WAFS. Data represent means ± S.E. (n = 2 - 10 fruit).

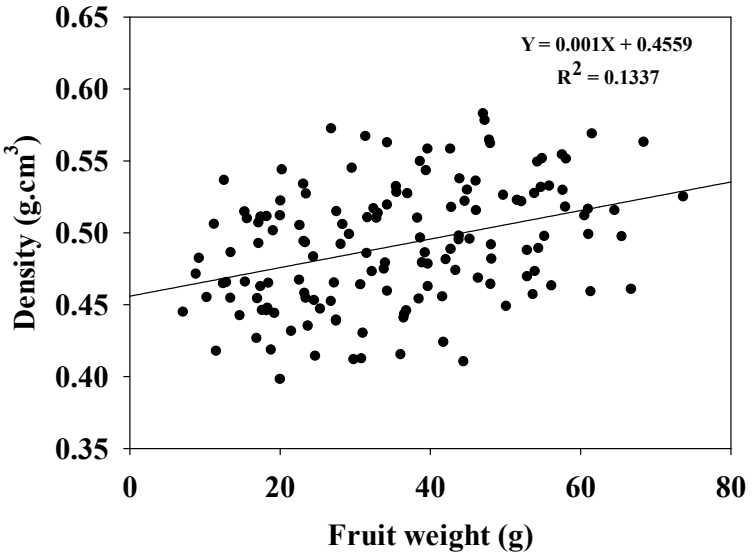


Figure 5.4 The correlation between fruit weight and density.

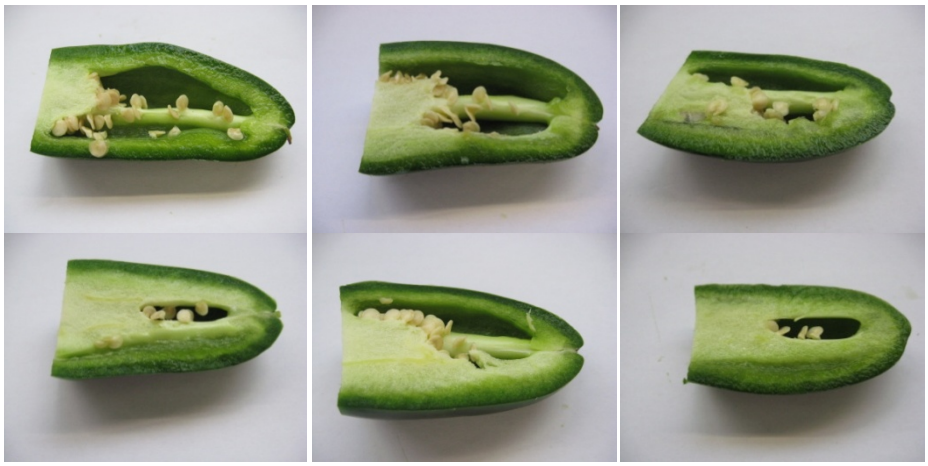


Figure 5.5 Variation of pericarp thickness and placenta proportion in Jalapeño. (Note that fruit were cut in half equally).

5.3.1.2 Effects of time of planting, position on plant, maturity at harvest and crop load on fruit weight and shape

In this experiment, weight and shape of Jalapeño from plants planted at sequential plantings (August - October) were measured in fruit harvested from different positions on the plant at 6, 8 and 10 WAFS and these fruit were harvested from both high and low crop load plants.

Final fruit weight of Jalapeño was influenced by position on the plant ($P < 0.05$), which peaked at nodes 5 to 8 depending on maturity at harvest and time of planting (Fig. 5.6). Fruit weight was lower at both higher and lower nodes. This trend was clearly observed in Jalapeño fruit planted in August (Fig. 5.6A - C). Jalapeño fruit planted in August and harvested at 8 and 10 WAFS were larger ($P < 0.05$) than fruit harvested at 6 WAFS (Fig. 5.6A - C) while fruit harvested at 10 WAFS seemed to be smaller when planted later in the year (Fig. 5.6F and I). Comparison between high and low crop load showed that Jalapeño fruit from low crop load plants were larger than fruit from high crop load plants only when fruit were planted in August and harvested at 6 and 10 WAFS (Fig. 5.6A and C) and fruit planted in October and harvested at 8 WAFS (Fig. 5.6H). However, overall crop load did not affect fruit weight ($P > 0.05$) of Jalapeño irrespective of timing of planting or fruit maturity at harvest. When date of fruit set is considered with respect to final fruit weight, fruit weight of Jalapeño planted in August tended to increase in fruit which were set from October to November and peaked ($P < 0.05$) in fruit which were set in December (Fig. 5.7A - C), after which fruit weight began to decrease in fruit which were set later (Fig. 5.7A - C). In other plantings, there was no difference ($P > 0.05$) of fruit weight in fruit which were set at different times (Fig. 5.7D - I).

Overall, there was no significant difference ($P > 0.05$) on fruit weight of Jalapeño planted at different times except Jalapeño fruit planted in August and harvested at 10 WAFS, which were larger than fruit planted other times. However the number of fruit harvested from Jalapeño plants planted in August (315) was higher than from those planted in September (216) and October (134) due to a blossom end rot outbreak. Approximately 40 - 50 % of chilli fruit from plants planted in September and October were affected by this disorder. Calcium deficiency is a major factor on blossom end rot (BER) occurrence in peppers, in addition to environmental factors (high temperature, high light intensity, water deficit and high salinity) which also stimulate BER occurrence (Aktas et al., 2003). In Jalapeño, the rot began to appear as a brown area at the blossom end of the fruit, with this spot elongating and darkening (Fig. 5.8). Application of calcium was treated to Jalapeño plants once the BER was observed but the BER symptoms remained. The contaminated fruit were removed from the plants to reduce development of blossom end rot in the

glasshouse. Therefore only fruit shapes from the August planting were presented (Fig. 5.9). Jalapeño fruit size varied from 3.6 - 8.5 cm in length, 2.5 - 5.0 cm in width and 7.7 - 13 cm in circumference. Overall, fruit from nodes 4 to 10 were longer and larger ($P < 0.05$) than fruit from other nodes (Fig. 5.9) while fruit harvested at 6 WAFS were shorter and smaller than fruit harvested at other ages (Fig. 5.9A and D). Crop load did not affect ($P > 0.05$) fruit length but the circumference of fruit from low crop load plants was larger ($P < 0.05$) than fruit from high crop load plant at some maturity stages and positions on plant (Fig. 5.9).

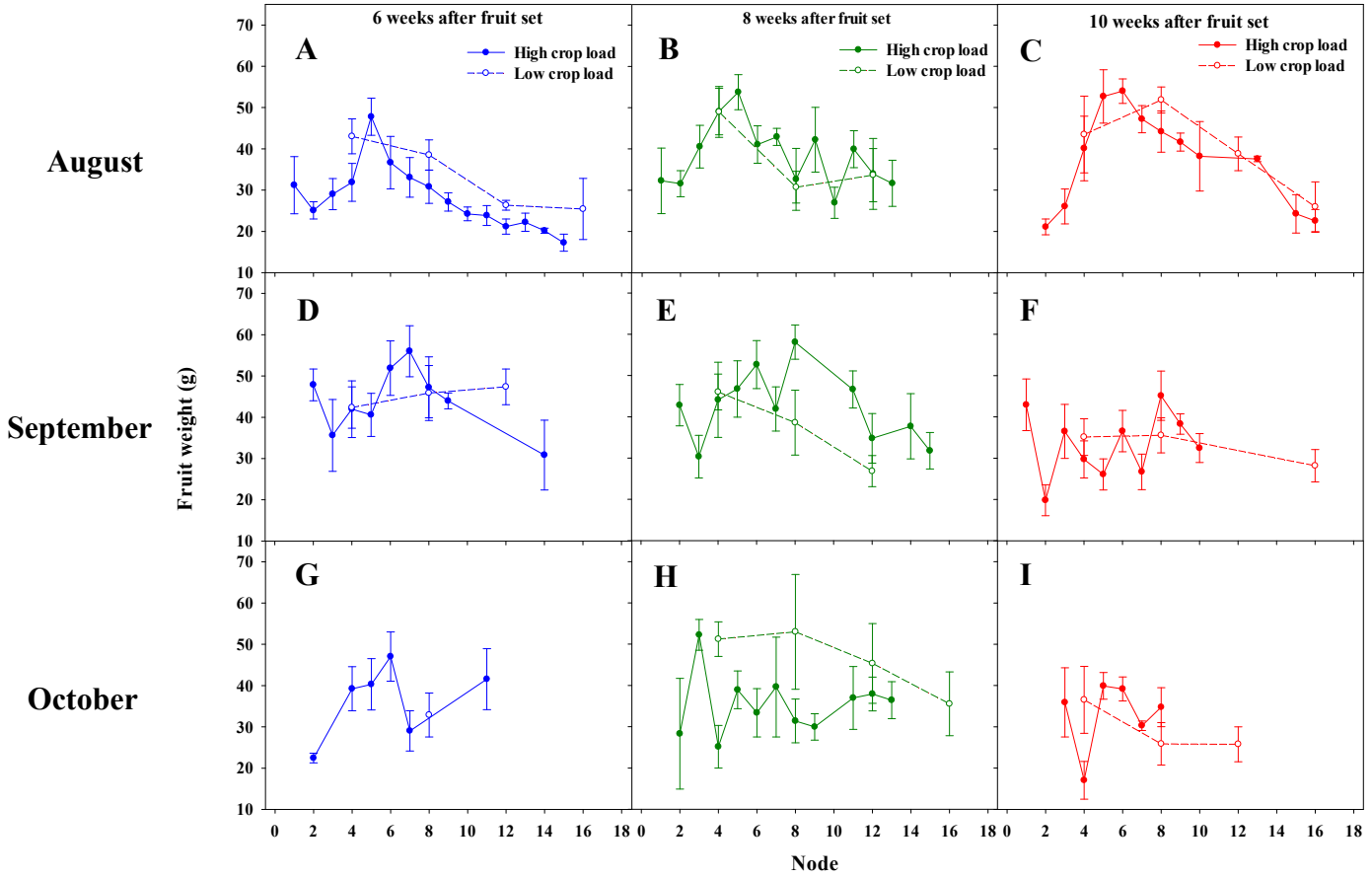


Figure 5.6 Weight of Jalapeño fruit from sequential plantings; August (A - C), September (D - F), and October (G - I) and harvested at 6, 8, or 10 weeks after fruit set from different nodes on the plant. Fruit were from high crop load (closed symbol and solid line) and low crop load (opened symbol and dash line) which was achieved by leaving fruit on the plant at nodes 4, 8, 12 and 16. Data represent means ± S.E. (n≥3).

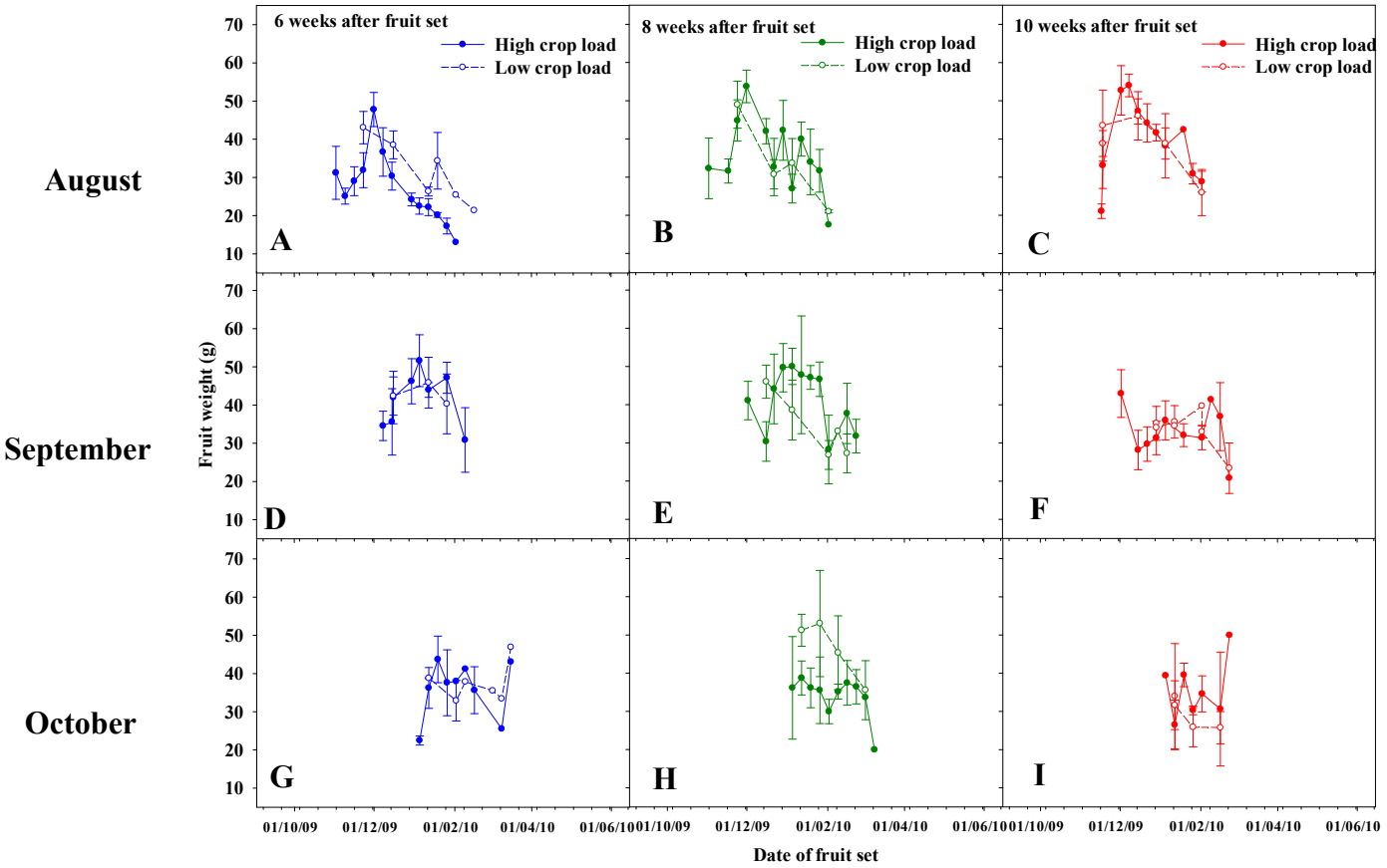


Figure 5.7 Weight of Jalapeño fruit from sequential plantings; August (A - C), September (D - F), and October (G - I) and harvested at 6, 8, or 10 weeks after fruit set as a function of date of fruit set. Fruit were from high crop load (closed symbol and solid line) and low crop load (opened symbol and dash line) which was achieved by leaving fruit on the plant at nodes 4, 8, 12 and 16. Data represent means ± S.E. (n≥3).

Figure 5.8 Blossom end rot in Jalapeño

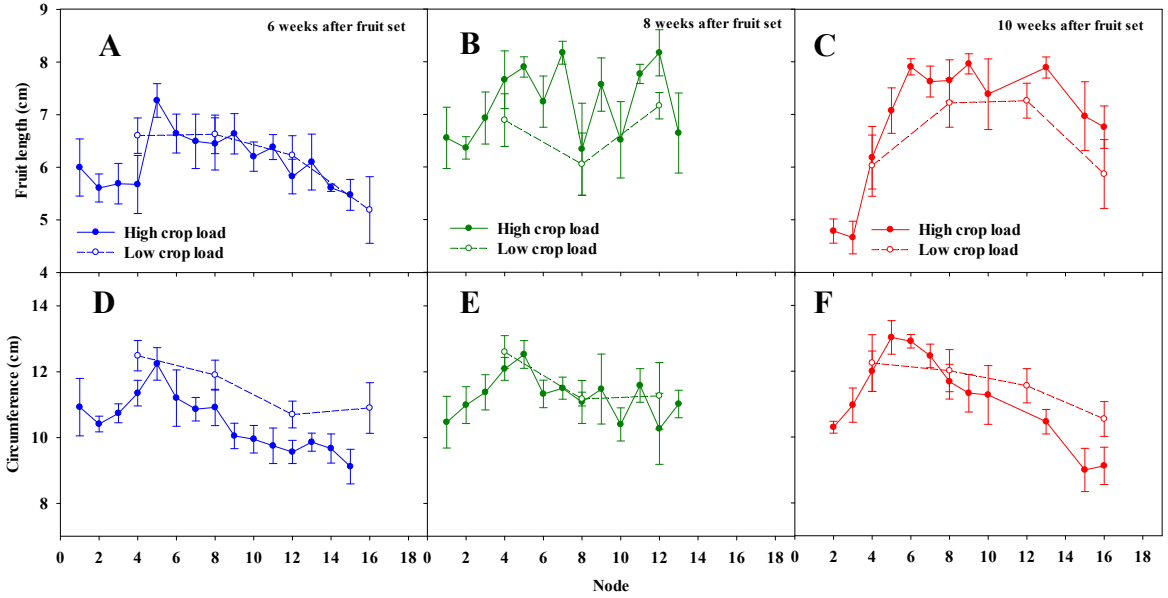


Figure 5.9 Fruit length (A - C) and circumference (D - F) of Jalapeño fruit from plants germinated in August and harvested at 6, 8, or 10 weeks after fruit set from different nodes on the plant. Fruit were from high crop load (closed symbol and solid line) and low crop load (opened symbol and dash line) which was achieved by leaving fruit on the plant at nodes 4, 8, 12 and 16 of the plant only. Data represent means ± S.E. (n≥3).

Temperature in the glasshouse was controlled between 16 - 25 °C with a heater or fan ventilation operated when required. Despite this temperature followed the expected seasonal patterns (Fig. 5.10) increasing from October to February over the summer period and then decreasing until June as winter approached. Day temperature rose to maximum at 25 °C in summer and minimum at 18 °C in winter while night temperature was controlled to not drop below than 16 °C. As relative humidity (RH) is a function of temperature, absolute humidity was used to present the actual water vapour in the air. Absolute humidity peaked during February and day time showed higher absolute humidity than night time (Fig. 5.10). Although Jalapeño planted in August were produced larger fruit when fruit were set in December and developed through January and February (Fig. 5.7A - C), which may be a preferable growing condition, this peak was not found in fruit planted later (Fig. 5.7D - I). Therefore, position on plant may have a higher impact on fruit size than temperature and absolute humidity during fruit development.

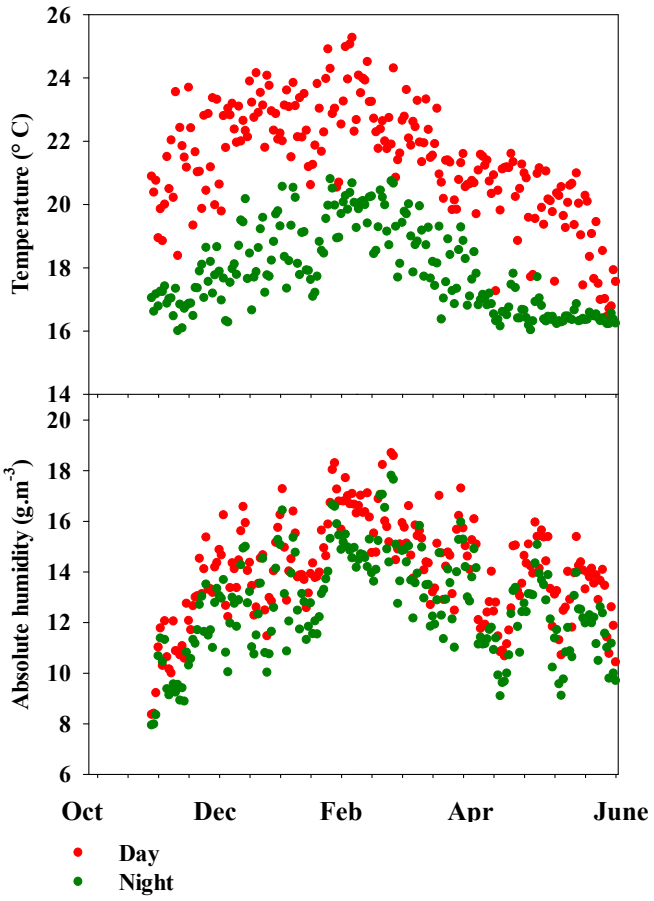


Figure 5.10 Average day (6 am - 6pm) and night (6pm - 6am) temperature and absolute humidity in a PGU glasshouse during fruit development.

5.3.2 Colour

5.3.2.1 Colour changes of Jalapeño planted in a commercial and a PGU glasshouse during fruit development

L* values of Jalapeño from the commercial glasshouse increased ($P < 0.05$) during development (Fig. 5.11A). Meanwhile a* value tended to decrease in fruit harvested from 2 to 6 WAF and then increased once fruit were mature. However there was no significant difference ($P > 0.05$) among a* values during fruit maturation (Fig. 5.11B) due to high variation of a* values observed in chilli harvested after 7 WAF, which were from a combination of green and red fruit (Fig. 5.11A and B). The b* values varied ($P < 0.05$) with maturity stage (Fig. 5.11C). Jalapeño fruit began to change colour from dark green to red after 6 - 9 WAF (approximately 5 - 8 WAFS).

For Jalapeño planted in the PGU glasshouse and harvested when fruit from the first node reached 6, 8 and 10 WAFS, three colour parameters (L*, a* and b* values) tended to increase ($P < 0.05$) during fruit maturation (Fig. 5.12A - C). Colour began to change from green to red after 6 WAFS. Colour change of Jalapeño was delayed when more fruit were left on the plant (Fig. 5.12A - C; the red line).

Overall, colour changes from green to red in Jalapeño both grown in the commercial and PGU glasshouse began to occur after 6 WAFS, therefore harvesting fruit at breaker stage (i.e. from 6 - 8 WAFS) seem to be a good maturity index for red Jalapeño demand because fruit at this maturity stage can later change to red colour during handling and storage.

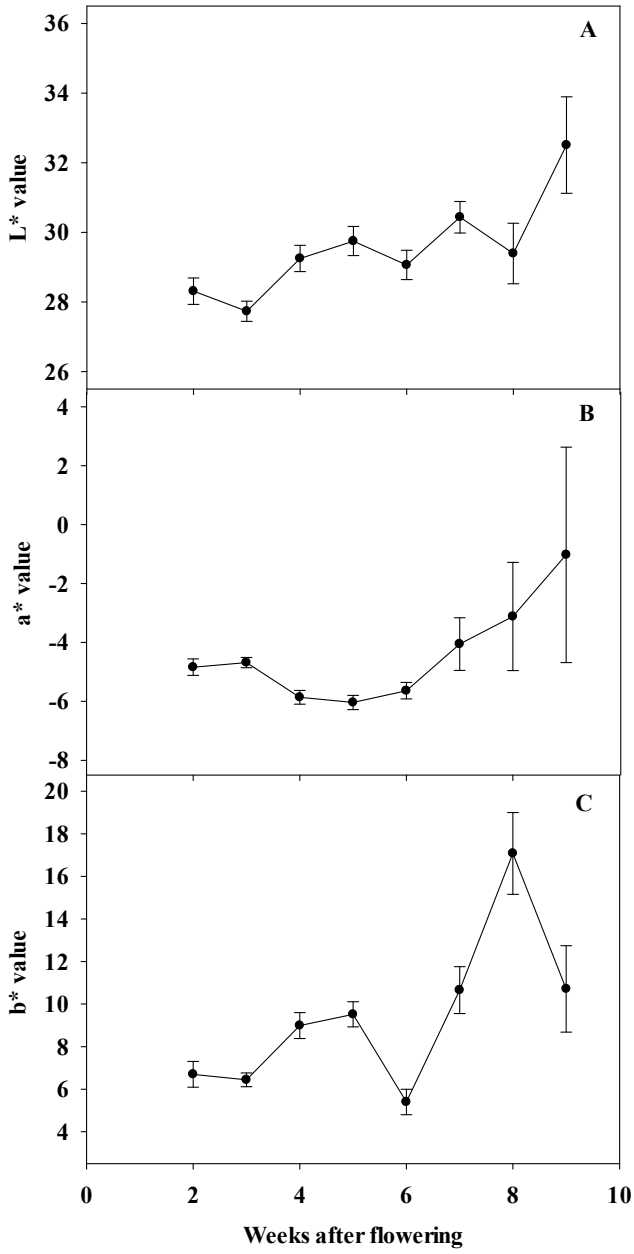


Figure 5.11 Fruit lightness (L* value) (A), redness (a* value) (B) and yellowness (b* value) (C) of Jalapeño planted in a commercial glasshouse. Fruit were harvested at different maturity stages defined as weeks after flowering. Data represent means ± S.E. (n = 6 - 50 fruit)

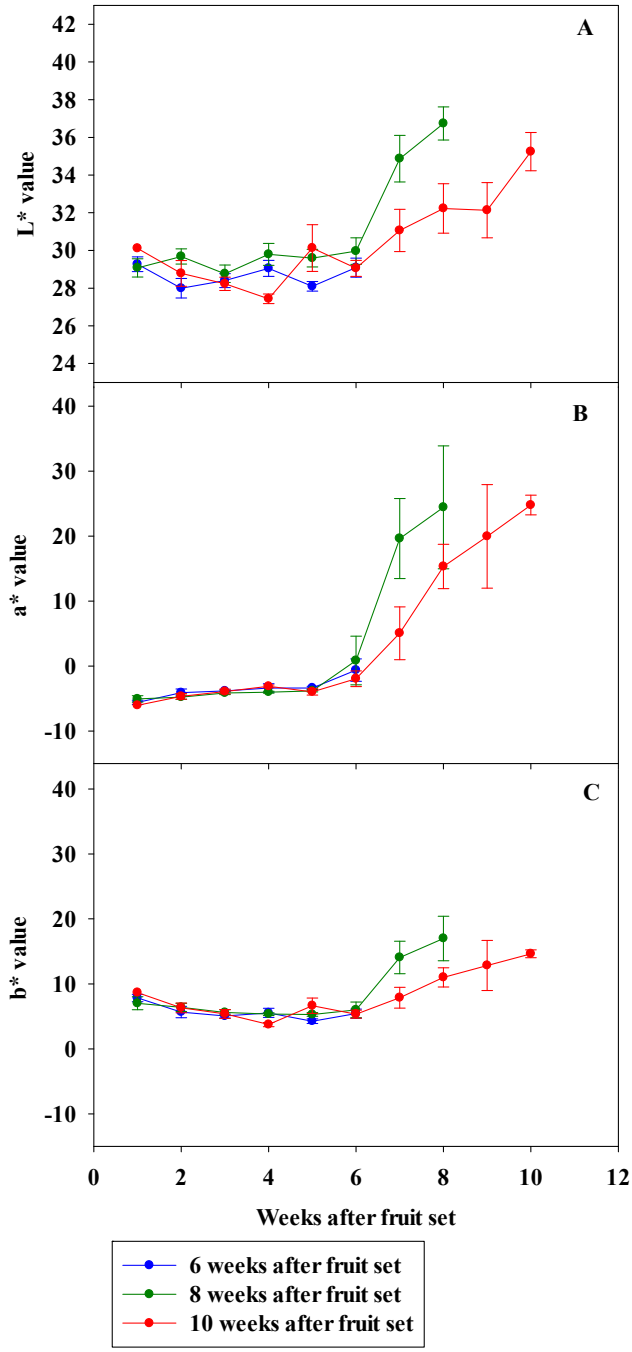


Figure 5.12 Fruit lightness (L*) (A), redness (a* value) (B) and yellowness (b* value) (C) of Jalapeño planted in a PGU glasshouse. All fruit presented on the plants were harvested when fruit from the first node reached 6 (Blue), 8 (Green), and 10 (Red) WAFS. Data represent means ± S.E. (n = 2 - 10 fruit).

5.3.2.2 Effects of time of planting, position on plant, maturity at harvest and crop load on colour of Jalapeño fruit

Effects of pre-harvest factors were studied on colour changes of Jalapeño. Fruit planted in the PGU glasshouse were harvested at 6, 8 and 10 WAFS. Fruit harvested at 6 WAFS were mostly green (70 %) while 95 % of fruit harvested at 8 WAFS were red and all fruit harvested at 10 WAFS were red (Table 5.1). L*, a* and b* values all increased ($P < 0.05$) with fruit age (Fig. 5.13 - 5.15) and fruit harvested at 10 WAFS showed fully red colour with high a* and b* values.

Table 5.1 Percentage of red Jalapeño fruit planted from sequential plantings: (August, September and October) and harvested at 6, 8, and 10 WAFS

Time of planting	WAFS		
	6	8	10
August	34 (137)	88 (92)	100 (98)
September	27 (66)	100 (86)	100 (73)
October	18 (38)	97 (64)	100 (27)
Total	30 (241)	95 (242)	100 (198)

* Numbers in bracket represent number of total fruit in each lot and red fruit were defined based on visual skin colour and a positive a* value.

Time of planting did not affect ($P > 0.05$), L* and b* value but there was a significant difference ($P < 0.05$) on a* value as fruit from plants planted in September and October showed higher a* value than fruit from plants planted in August (Fig. 5.14).

Position on plant also affected colour of Jalapeño. L* value of fruit harvested at 6 WAFS at higher nodes tended to be higher than L* values of fruit at lower nodes (5.13A, D and G) and fruit from plants planted in August and harvested at 8 WAFS also showed the same trend (Fig. 5.13B) while L* values of fruit harvested at other times were constant along the plant (Fig. 5.13C, E, F, H, and I). Jalapeño fruit harvested at 6 WAFS remained green at lower nodes until node 6 and then fruit tended to change to red colour at higher nodes (node 6 onward), particularly fruit from plants planted in August (Fig. 5.14A, D and G). Meanwhile fruit harvested at

other maturity stages were red along the plant (Fig. 5.14C, E, F, H, I) with the exception of fruit planted in August and harvested at 8 WAFS in which a* values tended to increase with node as fruit at lower nodes were at breaker stage at harvest (Fig. 5.14B). For b* value, fruit harvested at 6 WAFS and at higher nodes showed higher b* value than fruit at lower nodes particularly fruit planted in August (Fig. 5.15A, D and G), but b* value remained stable in fruit harvested at other maturity stages (Fig. 5.15C, E - F, H - I) except fruit planted in August and harvested at 8 WAFS, for which b* value tended to increase with node (Fig. 5.15B). Crop load did not affect colour ($P > 0.05$) in terms of L*, a* or b* values irrespective of nodes or maturity (Fig. 5.13 - 5.15).

Overall, fruit harvested at 10 WAFS were fully red mature at harvest so there were no significant differences ($P > 0.05$) in the colour parameter. For fruit harvested at 8 WAFS, fruit tended to change to red colour at higher nodes only in fruit planted in August, while fruit planted later were fully red at harvest. Colour changes in fruit harvested at 6 WAFS began to occur at higher nodes, which were clearly shown in fruit planted in August.

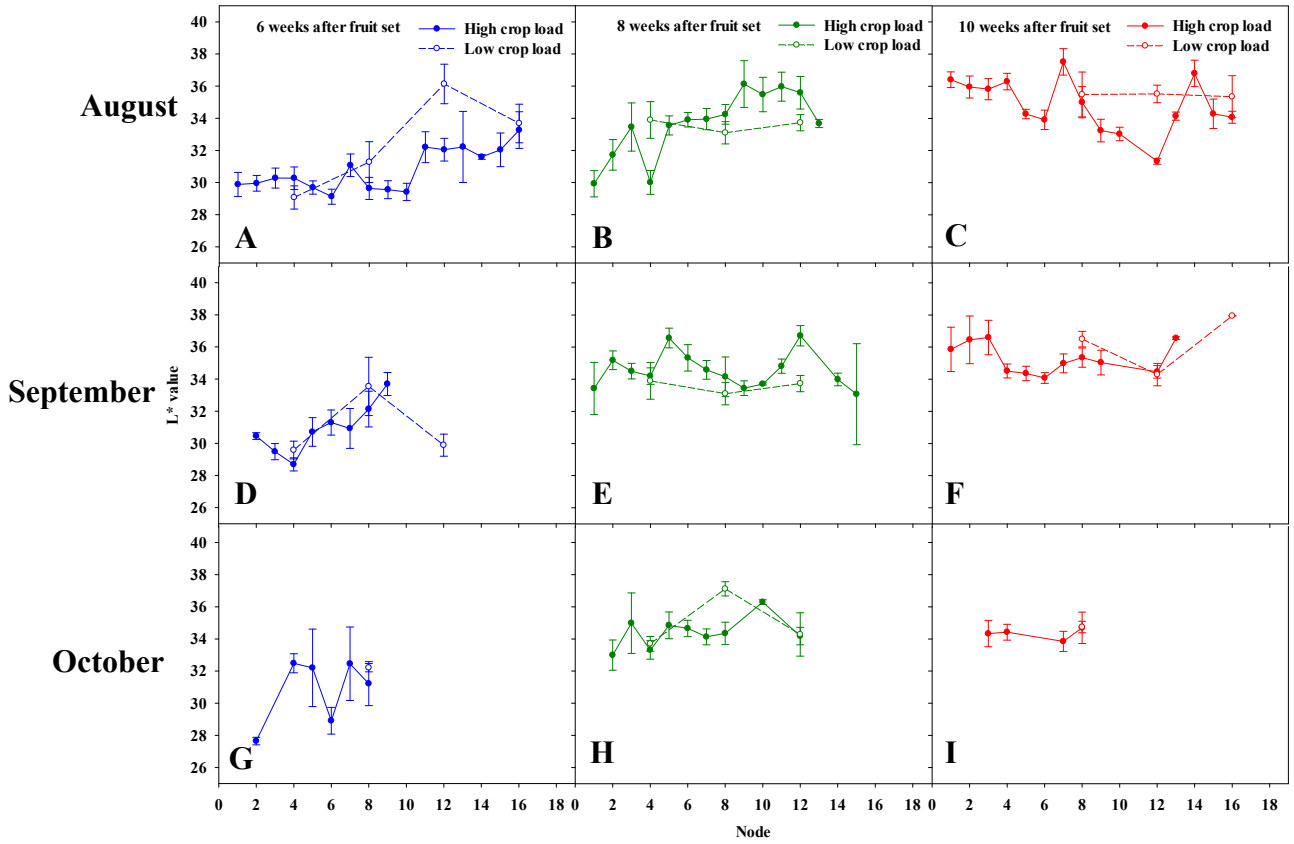


Figure 5.13 Fruit lightness (L*) of Jalapeño from sequential plantings; August (A - C), September (D - F) and October (G - I) and harvested at 6 (Blue), 8 (Green), and 10 (Red) weeks after fruit set from different nodes on the plant. Fruit were from high crop load (closed symbol and solid line) and low crop load (opened symbol and dash line) which was achieved by leaving fruit on the plant at nodes 4, 8, 12 and 16. Data represent means ± S.E. (n ≥ 3).

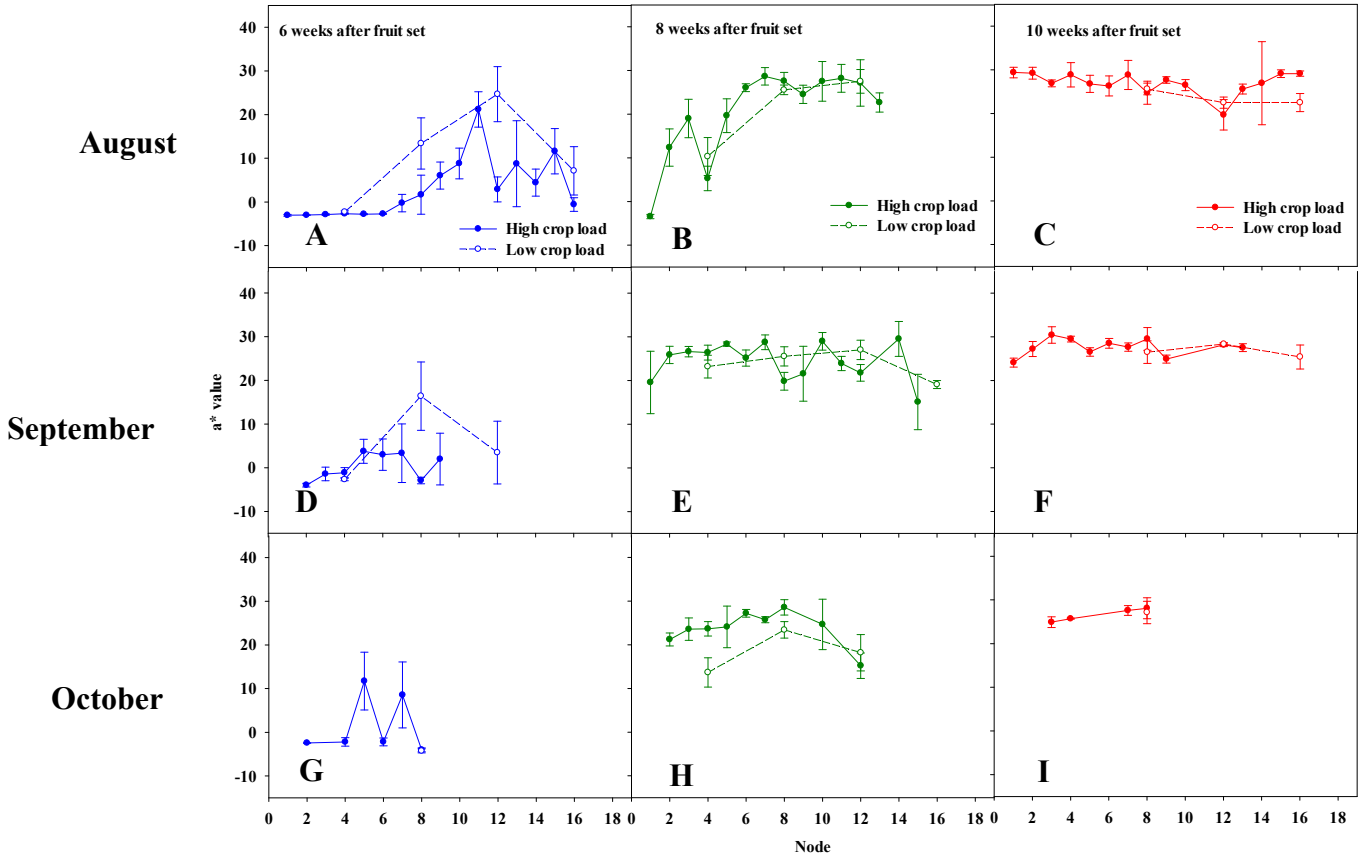


Figure 5.14 Fruit redness (a*) of Jalapeño from sequential plantings; August (A - C), September (D - F) and October (G - I) and harvested at 6 (Blue), 8 (Green), and 10 (Red) weeks after fruit set from different nodes on the plant. Fruit were from high crop load (closed symbol and solid line) and low crop load (opened symbol and dash line) which was achieved by leaving fruit on the plant at nodes 4, 8, 12 and 16. Data represent means ± S.E. (n ≥ 3).

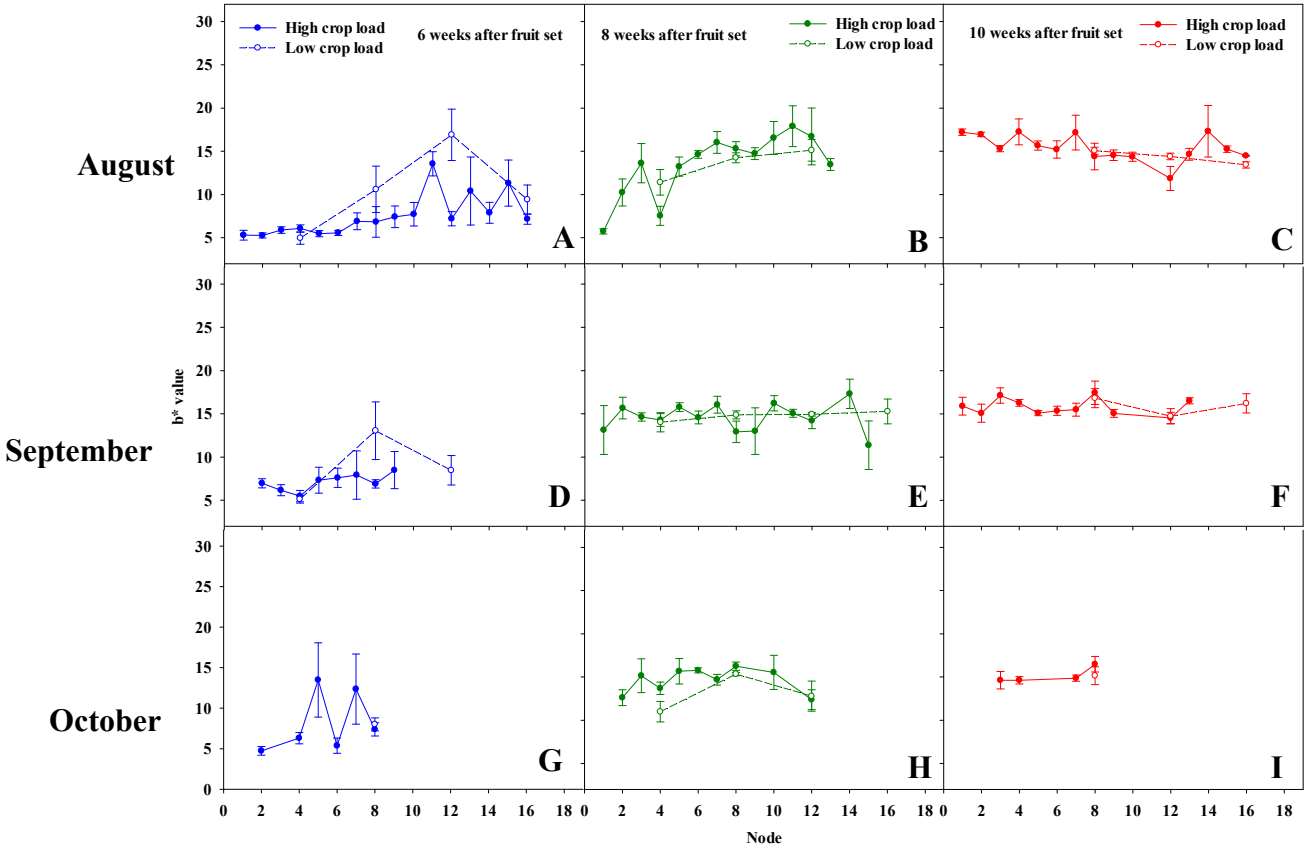


Figure 5.15 Fruit yellowness (b*) of Jalapeño from sequential plantings; August (A - C), September (D - F) and October (G - I) and harvested at 6 (Blue), 8 (Green), and 10 (Red) weeks after fruit set from different nodes on the plant. Fruit were from high crop load (closed symbol and solid line) and low crop load (opened symbol and dash line) which was achieved by leaving fruit on the plant at nodes 4, 8, 12 and 20. Data represent means ± S.E. (n ≥ 3).

5.3.2.3 The relationship between colour, total chlorophyll, and total carotenoids

Jalapeño fruit were harvested from dark green to red colour (Fig. 5.16). L* and b* values slightly increased while a* value increased rapidly when fruit turned red (Fig. 5.17). During colour change of Jalapeño from green to red, an increase of a* value correlated to a decrease of total chlorophyll (Fig. 5.18A) and an increase of total carotenoid concentration (Fig. 5.18B). Total chlorophyll gradually decreased exponentially (R^2 = 0.86) with a* value until zero when fruit were red (Fig. 5.18A), while a polynomial correlation for total carotenoids and a* value was found (R^2 = 0.72) (Fig. 5.18B). However, high variation was found when these correlations were used to predict total chlorophyll and carotenoid in Jalapeño fruit by using a* value. A large difference of total chlorophyll and carotenoid was found when compared between predicted values and measured values. Overall, colour change from green to red of Jalapeño is due to a combined contribution of chlorophyll degradation and production of total carotenoids.

Figure 5.16 Jalapeño fruit harvested from green to red colour.

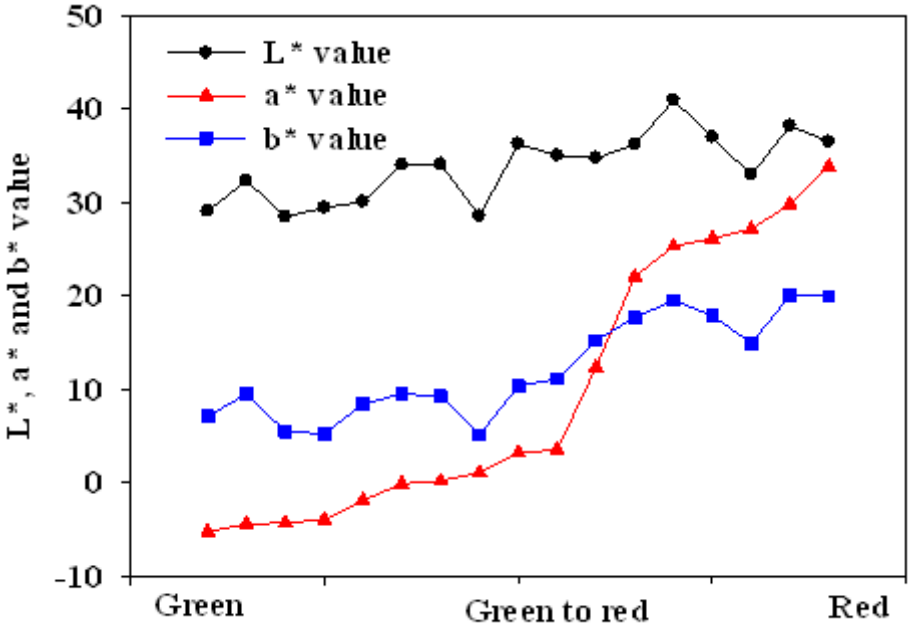


Figure 5.17 L*, a* and b* values of Jalapeño fruit harvested from green to red (Fig. 5.16). Data were arranged by ranked a* value.

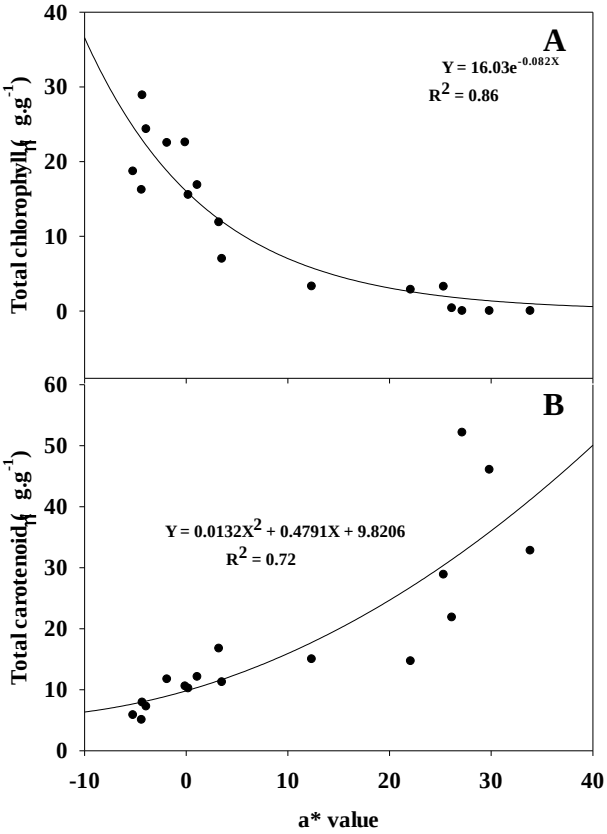


Figure 5.18 Correlation between total chlorophyll (A) and carotenoid (B) of Jalapeño with a* value.

5.3.3 Cracking

Cracking symptoms were observed overall in 53 % of Jalapeño fruit planted in the PGU glasshouse. Understanding the factors relating to the occurrence of cracking in Jalapeño may help to reduce this symptom or avoid growing Jalapeño in improper conditions. The incidence of cracked fruit at each month was studied in order to determine conditions this triggered cracking (Fig. 5.19).

Jalapeño fruit harvested at 6 WAFS showed less than 20 % cracked fruit from all three sequential plantings (Fig. 5.19A, D and G) while fruit harvested at 8 and 10 WAFS developed more than 20 % cracked fruit when fruit were set during December – February, particularly in Jalapeño planted in September and October (Fig. 5.19E, F, H and I).

High incidence of cracked fruit was found when fruit were set during December and January (Fig. 5.18E, F, H and I) and later developed for 6 - 10 weeks. These results may relate to rapid fruit development during this period, which was shown in Fig. 5.7. During this period the range of day and night temperatures were 21.2 - 22.9°C and 18.0 - 19.5°C respectively (Table 5.2). The high absolute humidity between December to February may cause a reduction in fruit transpiration resulting in an increase in turgor pressure in the fruit, making them more prone to crack (Aloni et al., 1998). In addition, the shrinkage and expansion ratio which is influenced by growing conditions such as sunlight may relate to cracking of Jalapeño (Aloni et al., 1999; Moreshet et al., 1999). No clear effect of crop load on number of cracked fruit was observed (Fig. 5.19).

However, unlike Jalapeño plants planted in September and October, Jalapeño plants planted in August did not produce highly cracked fruit although fruit were set during December - February (Fig. 5.19B and C). This suggests that older plants are less susceptible to cracking. Therefore time of fruit set may not be a major impact on fruit cracking with plant age and fruit maturity potential having a higher impact on fruit cracking incidence.

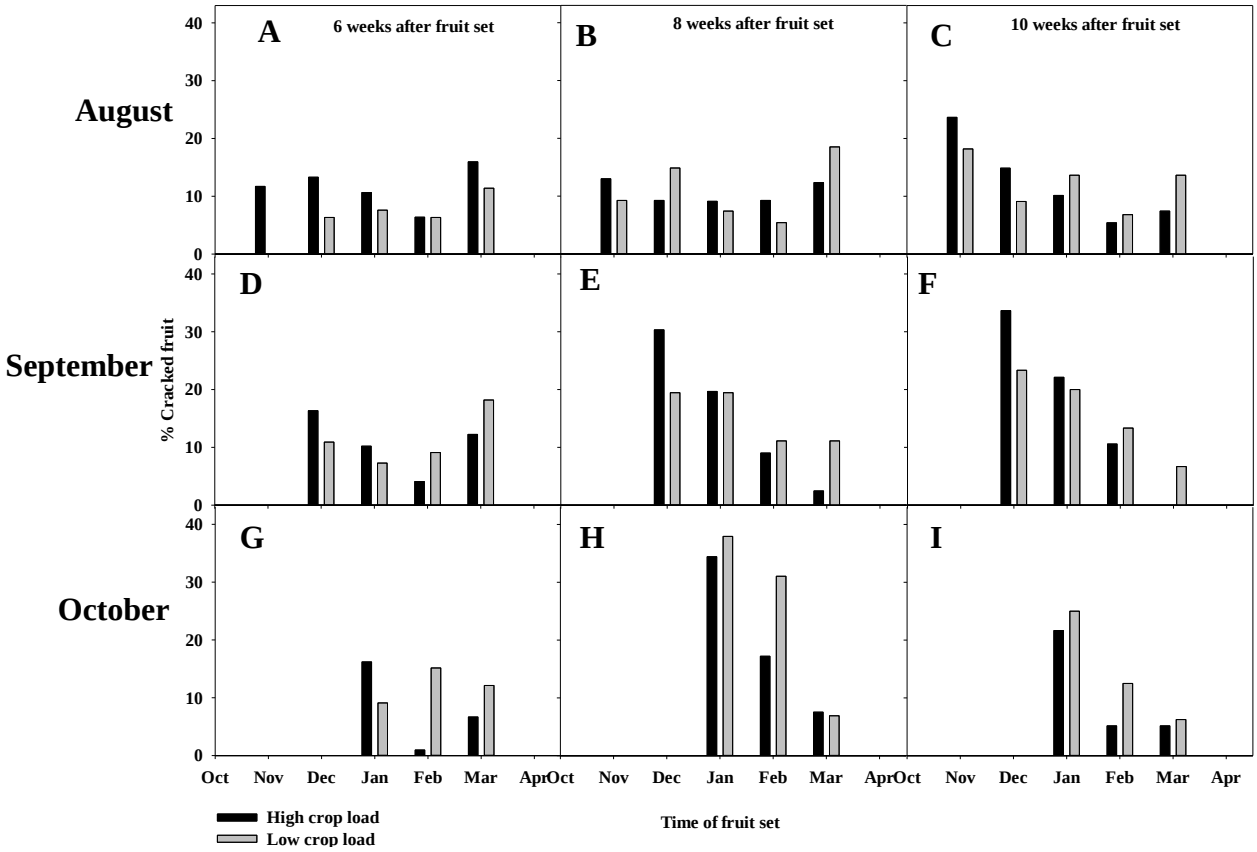


Figure 5.19 Percentage of cracked Jalapeño fruit from sequential plantings: August, September, and October and harvested at 6, 8, 10 weeks after fruit set. Fruit were from high and low crop load, which was achieved by leaving fruit on the plant at nodes 4, 8, 12 and 16.

Table 5.2 Average day and night temperature, absolute humidity and the differences between day and night temperature and absolute humidity in the PGU glasshouse from November to May

	Temperature			Absolute humidity ($g.m^3$)		
	Day	Night	Diff	Day	Night	Diff
November	21.1	17.2	3.9	12.0	10.7	1.3
December	22.8	18.3	4.4	14.2	12.7	1.4
January	22.9	19.1	3.7	15.4	13.8	1.6
February	22.7	19.5	3.2	16.1	14.8	1.3
March	21.2	18.0	3.3	14.5	13.3	1.1
April	20.3	16.7	3.6	13.5	12.1	1.2
May	18.9	16.4	2.5	13.0	11.4	1.6

5.4 Discussions

5.4.1 Fruit size and shape

Fruit growth of *Capsicum annuum* varies between varieties. Generally, fruit grow by 2 - 9 times (from 2 - 4 g to 4 - 35 g) to the developed green fruit stage (Hornero-Mendez & Minguez-Mosquera, 2002). In this work, weight of Jalapeño increased rapidly during the first 3 WAFS (Fig. 5.1A), similar to New Mexican peppers, in which fruit weight increased 4 times (from 20 to 80 g) between 3 - 5 WAF (Biles et al., 1993). Fruit weight of Amazonic hot pepper accessions showed a gradual increase in weight from 0.5 to 3.5 g at full maturity, which took approximately 5 - 6 weeks (Barrera et al., 2005; Barrera et al., 2008) although fruit were small. However the growth rate of these fruit was not high when compared with other larger chillies or pepper varieties. Fruit weight of sweet peppers increased in a linear manner from 1 - 8 weeks after anthesis (Tadesse et al., 2002), but in this work fruit weight of Jalapeño increased linearly only during initial fruit development (up to 3 - 4 WAFS) (Fig. 5.1A and 5.2A). After 6 WAFS, Jalapeño fruit weight tended to be stable (Fig. 5.2). This result is similar to New Mexican and other peppers, which show a decrease of fruit weight during final ripening that might be related to water loss (Biles et al., 1993; Hornero-Mendez & Minguez-Mosquera, 2002). Meanwhile Tadesse et al. (2002) found that sweet pepper continued to grow until 8 weeks after anthesis, which was a suggested period to harvest sweet pepper as fruit were fully mature.

In addition, fruit weight of Jalapeño planted in the PGU glasshouse was higher than those planted in the commercial glasshouse when compared at the same age (Fig. 5.1A and 5.2A). However fruit from the commercial glasshouse continued to grow up to 8 WAF (Fig. 5.1) while fruit from the PGU glasshouse seemed to be fully developed at 6 WAFS (Fig 5.2). Chillies planted commercially were harvested from different plants whereas a range of fruit of different ages was harvested from a single plant at one time in a PGU glasshouse. Therefore, one possible explanation for the lack of growth in fruit over 6 WAFS in the controlled environmental experiment may be the continued competition for metabolites from all the fruit on the plant. Other potential competing fruit may have been removed in the commercial glasshouse, resulting in reduced competition and hence allowing mature fruit (> 6 WAFS) to continue to grow.

Similar to previous research, fruit growth pattern of Jalapeño in this research followed a single sigmoid curve with maturity (Biles et al., 1993; Tadesse et al., 2002; Barrera et al., 2005; Barrera et al., 2008). Barrera et al. (2008) concluded that three stages of chilli fruit development existed, which were cell division, cell expansion with some peaks of respiration, then a stable stage with a non-climacteric pattern as fruit were fully mature.

Specific fruit characteristics of different chilli and pepper varieties related to cell elongation and expansion. For pepper fruit with a tubular shape, the length increases faster than diameter, while for fruit with ball shape the diameter increases faster than the length (Tadesse et al., 2002; Barrera et al., 2005; Barrera et al., 2008). In this research fruit length of Jalapeño developed early during the initial growth (by 2 WAF), while fruit circumference increased later during fruit development (Fig. 5.1B, 5.2B and C). Therefore, the tubular form of Jalapeño is likely to be a result of initial cell division followed by cell expansion until full maturity.

Effects of pre-harvest factors were also studied with respect to time of planting, position on plant, maturity at harvest and crop load. Fruit weight of Jalapeño harvested at 6 WAFS was less than Jalapeño fruit harvested at 8 and 10 WAFS (Fig. 5.6). Fruit weight of Jalapeño varied with the position on the plant ($P < 0.05$) and peaked at nodes 5-8 (Fig. 5.6), with this trend most clearly demonstrated in fruit planted in the August planting (Fig. 5.6A - C). Differences in fruit development caused by position on plant have previously been studied in pear (Wang et al., 2010), kiwifruit (Lawes et al., 1990; Tombesi et al., 1993; McPherson et al., 2001; Remorini et al., 2007), custard apple (George & Nissen, 1988), cherimoya (González & Cuevas, 2008), and strawberry (Sachs & Izsak, 1972). Fruit weight of kiwifruit at the top of the canopy was higher than fruit from the bottom (Remorini et al., 2007). In contrast cherimoya and strawberry fruit from basal positions develop larger fruit than fruit from apical positions (Sachs & Izsak, 1972; González & Cuevas, 2008), while in some pear cultivars fruit, the lower order ($2^{nd} – 4^{th}$) position in inflorescence showed the best quality (Wang et al., 2010). The observed results from this research may indicate that the proximity of fruit from lower nodes to sources of nutrients and water closer than fruit from higher nodes may result in larger fruit at the base of the

plant. In addition, fruit at lower nodes had less competition of assimilate from previously formed fruit than fruit at higher nodes. However, position on plant in these previous works referred to fruit position within the canopy or inflorescence which differed in production quite substantially from the *Solanaceae* family. Due to pepper plants grow rapidly simultaneous with fruit growth and production, both competitions by neighbouring fruit and also growing plant should be considered.

Crop load has been reported to impact fruit size and yield. For example, plants with higher fruit number produced smaller fruit than plants with lower fruit number (Dorland & Went, 1947); larger fruit were found in low crop load treatments but the fruit yield is reduced (Atkins, 1990; Stopar et al., 2002; González & Cuevas, 2008). However in this work, crop load treatments did not affect fruit weight of Jalapeño (Fig. 5.6 and 5.7), which was similarly reported for cherry tomato (Gautier et al., 2005), cherimoya (González & Cuevas, 2008) and strawberry (Sachs & Izsak, 1972). In strawberry, Sachs & Izsak (1972) showed that pruning flowers which formed later (inferior blossoms) did not increase the fruit size of strawberry on the earlier nodes, however pruning flowers induced uniformity of strawberry fruiting.

Effects of crop load and position on plant on fruit growth was influenced by assimilate movement (Lawes et al., 1990; González & Cuevas, 2008) as defined by source and sink relationships. The lack of response of fruit size to crop load manipulation may be a result of fruit demand not being limited by the availability of a source in the high crop load treatment (González & Cuevas, 2008). New cultivars should be developed to produce more uniform fruit with high yield or special conditions can be established such as applying fertilizer to create sufficient source strength for fruit development.

Other such as growing season might have a larger impact than crop load, for example relationships between crop load and fruit weight in kiwifruit varied year by year (McPherson et al., 2001). In this research, Jalapeño plants only allowed one fruit to develop per node for high crop load treatment. For future work, there is potential for more than 1 fruit to grow on each node simultaneously and study on a second flush of production after the first flush is harvested.

Growing conditions and time of planting are considered as important factors influencing flower and fruit development. Cochran (1936) concluded that growing temperature influenced time of flowering and fruit set of peppers. High yield was found when pepper plants were grown at 27 °C - day temperature and between 12 - 22 °C - night temperature, depending on plant age, as the optimum night temperature decreased with increased plant age (Dorland & Went, 1947). In this work, the temperature in the PGU glasshouse was maintained between 15 - 25°C by heater or fan (Fig. 5.10). Large Jalapeño fruit were found at high growing temperature (23 °C day temperature and 19 °C night temperature) (Fig. 5.7).

5.4.2 Fruit colour

It has been well established that during fruit ripening, colour changes of chillies and peppers from green to red or yellow or orange depending on variety and cultivar (Davies et al., 1970; Minguez-Mosquera & Hornero-Mendez, 1993, 1994b; de Guevara et al., 1996; Deli et al., 1996; Gómez et al., 1998; Hornero-Mendez et al., 2000; Hornero-Mendez & Minguez-Mosquera, 2000; Hornero-Mendez et al., 2002; Hornero-Mendez & Minguez-Mosquera, 2002; Perez-Lopez et al., 2007; Pino et al., 2007). Generally, colour changes of chillies and peppers are initiated in the range of 3 - 8 weeks after full bloom (Biles et al., 1993; Tadesse et al., 2002; Barrera et al., 2005; Barrera et al., 2008). This work found that colour changes in Jalapeño occurred after 6 WAFS (approximately 7 WAF) (Fig. 5.11 and 5.12 and Table 5.1).

During fruit ripening, green colour disappears due to chlorophyll degradation. Previous research has reported that chlorophyll degradation in *Capsicum annuum* fruit begins from 20 - 33 DAF with the rate of degradation varying between cultivars. Red pepper cultivars with high growth rates such as ‘Belrubi’, ‘NuMex’, ‘Bola’ and ‘Delfin’, lost 40 - 60 % of chlorophyll at the beginning of the colour change stage, while chlorophyll concentration in the ‘Mana’ cultivar, which had a lower growth rate decreased at a later stage (Hornero-Mendez & Minguez-Mosquera, 2002). Chlorophyll concentration in Jalapeño decreased with an increase of a* value, which was related to the colour change of fruit skin from green to red (Fig. 5.16 and 5.17).

Total carotenoids increased from 4 - 100 times during colour changes of chillies and peppers (Hornero-Mendez et al., 2000; Hornero-Mendez & Minguez-Mosquera, 2000; Hornero-Mendez et al., 2002), while carotenoid concentration in Jalapeño increased from 8 - 10 times (Fig. 5.18). Carotenoids in red pepper consist of capsanthin and capsorubin, which increase during ripening while lutein disappears when fruit are fully red (Davies et al., 1970; Marín et al., 2004; Menichini et al., 2009). Carotenoid levels in chillies and peppers are affected by cultivar, maturity at harvest, and harvest time (Minguez-Mosquera & Hornero-Mendez, 1993, 1994a, b; Deli et al., 1996; Markus et al., 1999; Hornero-Mendez et al., 2000; Hornero-Mendez & Minguez-Mosquera, 2000; Hornero-Mendez et al., 2002).

Overall colour change was a result of chlorophyll degradation which coincided with an increase of total carotenoid concentration (Fig. 5.17). Exponential correlation of total chlorophyll (R^2 = 0.87) and polynomial correlations of carotenoid (R^2 = 0.72) were observed for the a* value of Jalapeño (Fig. 5.18). A high linear correlation (R^2= 0.96) between total carotenoid and L* value has previously been reported in sweet pepper (Perez-Lopez et al., 2007). However, in this work a weak correlation (R^2 = 0.27) was found between total carotenoid and L* value of Jalapeño (data not shown).

Colour change of Jalapeño from green to red colour in terms of L*, a* and b* values showed a gradual increase of L* and b* values while a* value rapidly increased when fruit changed colour green to red (Fig. 5.11 and 5.12). Previously a decrease of L* value and increase of a* and b* values were found in sweet pepper during ripening (Perez-Lopez et al., 2007), while L* values of paprika cultivars were highest in the reddish-green stage not in the fully red stage and, the highest of b* values were found in the green stage (de Guevara et al., 1996). As the patterns in L*, a* and b* differ substantially between each chilli variety, not only does it suggest that each variety has its own set of colour tones, but it also suggests that the coordination of the loss of chlorophyll and accumulation of carotenoid compound that drive the colour changes, differs between each chilli variety.

Maturity at harvest is also related to colour changes of chillies and peppers after harvest. Approximately 30 % of Jalapeño fruit harvested at 6 WAFS were red, while

fruit harvested at 8 and 10 WAFS were almost all red (Table 5.1). Jalapeño is consumed as both green and red fruit depending on consumer preference. Managing harvest maturity is an obvious tool in this case to satisfy requirements of consumers.

In this research, position on the plant affected ($P < 0.05$) colour of Jalapeño. Colour of fruit harvested at 6 WAFS, and from lower nodes in particular tended to be darker and remained green longer than fruit from higher nodes (Fig. 5.13A, D and G; Fig. 5.14A, D and G and Fig. 5.15A, D and G). This result is similar for apple (Nawar et al., 1996; Nilsson & Gustavsson, 2007), kiwifruit (Tombesi et al., 1993) and star fruit (Zabedah et al., 2009) in which internal or shaded fruit showed darker colour than those exposed to the sun light. Paprika pepper grown in the greenhouse showed lower lightness (L*) and redness (a*) values or darker colour because these plants received less sunlight (Gómez et al., 1998) which indicated that colour changes of pepper is influenced by temperature and light (de Guevara et al., 1996). In addition hue angle of apple and peach located on the outside of the tree was higher than those of fruit located inside of the tree (Lewallen, 2000; Nilsson & Gustavsson, 2007). Although light and temperature influenced colour change in ripening chillies (Gómez et al., 1998; Montefiori et al., 2005), by 8 - 10 WAFS all fruit were fully red on the plant. Therefore effect of position on plant can be studied when fruit are not fully red at harvest. This can be observed in fruit harvested at 6 or 8 WAFS particularly from August planting, the delayed maturation of fruit at lower nodes may be explained as fruit were developing in the cooler conditions which showed slower ripening.

5.4.3 Cracking

Cracking, a physiological disorder in chillies and peppers, has previously been reported to have a tendency to occur in full size fruit (Aloni et al., 1998; Aloni et al., 1999). In tomato, cracking begins to occur in fruit at 6 - 7 WAFS (Bakker, 1988). This was similar to Jalapeño in this work which showed higher % cracked fruit at the 8 and 10 WAFS harvests than fruit harvested at 6 WAFS (Fig. 5.19). This may be a result of weakening cuticle during fruit expansion at fully mature stages which may reduce elasticity of the fruit skin (Bakker, 1988; Aloni et al., 1999; Opara et al., 2010). Jalapeño plants planted earlier tended to produce fruit with a lower cracking occurrence than plants planted later, particularly during the rapid growth period (Fig.

5.19). In addition, Jalapeño plants planted in September and October suffered from blossom end rot and resulted to low fruit number. Based on this work, plant age and fruit maturity at harvest showed a higher impact on cracking than growing conditions so Jalapeño should be germinated early (by August) to avoid cracking appearance in fruit developed during summer.

In addition, high day and night temperature difference has been reported to cause shrinkage-expansion of fruit skin and cracking development (Aloni et al., 1999; Opara et al., 2010). High night RH reduces transpiration in fruit, therefore excess water may accumulate in the fruit causing fruit swelling and finally crack (Peet, 1992; Aloni et al., 1998; Moreshet et al., 1999). Previous research found that fruit removal also increased cracking as these few fruit left on the plants received more assimilates and rapidly developed (Peet, 1992), however no clear result between crop loads and cracking was found in this work.

5.5 Conclusion

Fruit weight of Jalapeño increased during fruit development by fruit elongation in early stages and continued with fruit expansion until mature. Pre-harvest factors influenced fruit size and colour of Jalapeño. Location of fruit on the plant showed a major impact on fruit size as fruit tended to be larger at nodes 5 - 8 describing by the competition for assimilates and the distance from nutrients and water. Meanwhile maturity at harvest was the major factor influencing colour of Jalapeño as colour changes from green to red in Jalapeño began to occur after 6 WAFS. Time of planting also affected fruit weight and colour. For example, fruit harvested at 10 WAFS seemed to be smaller when planted later in the year and fruit planting and harvesting at 6 WAFS resulted in slower change of colour at lower nodes.

A high incidence of cracking was found in fruit harvested at 8 and 10 WAFS, particularly when fruit were set and developed on younger plants, at high day and night temperature and high absolute humidity. However, this evidence was not found in Jalapeño plants of older age, even in fruit which were set during summer period which indicated that occurrence of cracking in Jalapeño was mainly influenced by plant ages and maturity at harvest.

Overall, crop load seemed not to show an impact on fruit size and colour. Thinning leaders during production is essential for decreasing the risk from plant collapse due to weight. Later, fruit thinning is not needed as there is no benefit to generating a low crop load (with the limitation of two leaders per plant with a single fruit per node and only on the first flush of fruit production). Therefore, effects of pre-harvest factors (such as time of planting, position on plants and maturity at harvest) should be considered together to produce uniform Jalapeño fruit with uniform weight and colour.

CHAPTER 6

Pre-harvest factors affect phytochemical compounds in Jalapeño

6.1 Introduction

Fruit and vegetables are good sources of vitamins and phytochemicals. In addition they are regarded as being beneficial for human health by protecting against diseases such as cancer, cardiovascular disease, and diabetes (Kaur & Kapoor, 2001). Chilli has a high vitamin content and antioxidant activity (Howard et al., 1994; Contreras-Padilla & Yahia, 1998; Estrada et al., 2000; Materska & Perucka, 2005; Conforti et al., 2007; Deepa et al., 2007; Serrano et al., 2010; Alvarez-Parrilla et al., 2011). Concentration of these phytochemical compounds has been reported to vary with genotype, maturity stage, growing season, edible part, growing conditions, and postharvest handling (Lee et al., 1995; Harvell & Bosland, 1997; Contreras-Padilla & Yahia, 1998; Estrada et al., 1999a; Sun et al., 2007; Monforte-Gonzalez et al., 2010). Improved understanding of pre-harvest factors (i.e. time of planting, position on plant, maturity at harvest and crop load) on phytochemical compounds in chillies could help to deliver more consistent products. For example it could assist in selecting an optimal time for harvest when chilli fruit produce the highest amount of beneficial compounds.

The objectives of this research were:

1. To study effects of maturity at harvest on phytochemical composition in Jalapeño planted in a commercial glasshouse;
2. To improve extraction methods for selected phytochemical compounds in Jalapeño;
3. To study effects of pre-harvest factors (i.e. time of planting, position on plant, maturity at harvest and crop load) on phytochemical composition in Jalapeño planted in a PGU glasshouse.

6.2 Materials and methods

Jalapeño fruit planted in a commercial glasshouse located in Napier and in a glasshouse at Plant Growth Unit, Massey University (PGU) were studied. Growing condition and plant management were explained in section 2.2, 2.3.2 and 2.3.3. In

the commercial glasshouse, Jalapeño fruit were harvested at different maturities; ascorbic acid, total capsaicinoids, antioxidant activity (AOX) and total phenolic concentration (TPC) were measured.

The effects of pre-harvest factors (i.e. time of planting, position on plant, maturity at harvest and crop load) on phytochemical compounds were studied in Jalapeño fruit planted in a PGU glasshouse. A set of Jalapeño plants was planted sequentially from August to October. Fruit from each planting were harvested from different nodes at 6, 8, or 10 weeks after fruit set (WAFS). The chilli plants were treated to deliver a high crop load (one fruit per node) and low crop load (leaving fruit only at nodes 4, 8, 12 and 16) (section 2.2 and 2.3). Only fruit from nodes 4, 8, 12 and 16 were measured in this experiment. As fruit were progressively removed from the plants in this approach, a separate experiment was carried out in which all fruit present on the plants were harvested once fruit from the first node reached 6, 8 or 10 WAFS in the PGU glasshouse. This strip-pick experiment gave suggestions of how fruit phytochemical composition varied between nodes with the oldest fruit present at the lowest nodes.

In this chapter, ascorbic acid and total capsaicinoid concentration (including capsaicin and dihydrocapsaicin) were measured by HPLC. The details of extraction method and evaluation of ascorbic acid and capsaicinoids are described in section 2.6.2 and 2.6.4 respectively. Antioxidant activity (AOX) of Jalapeño was assessed by ferric reducing antioxidant power (FRAP) assay (section 2.6.1) and total phenolic concentration (TPC) was measured by Folin-Ciocalteu assay (section 2.6.3).

6.3 Results

6.3.1 Ascorbic acid

6.3.1.1 Ascorbic acid in Jalapeño from a commercial glasshouse during fruit maturation

Ascorbic acid concentrations of these fruit were sampled at varying maturities. Ascorbic acid concentration of Jalapeño harvested at different maturities showed high variability during fruit maturation (Fig. 6.1) with no statistically significant

differences (P > 0.05). Comparing between fruit size, ascorbic acid concentration was higher (P < 0.05) in larger fruit (> 40 g) than in small fruit (≤40 g) (Table 6.1).

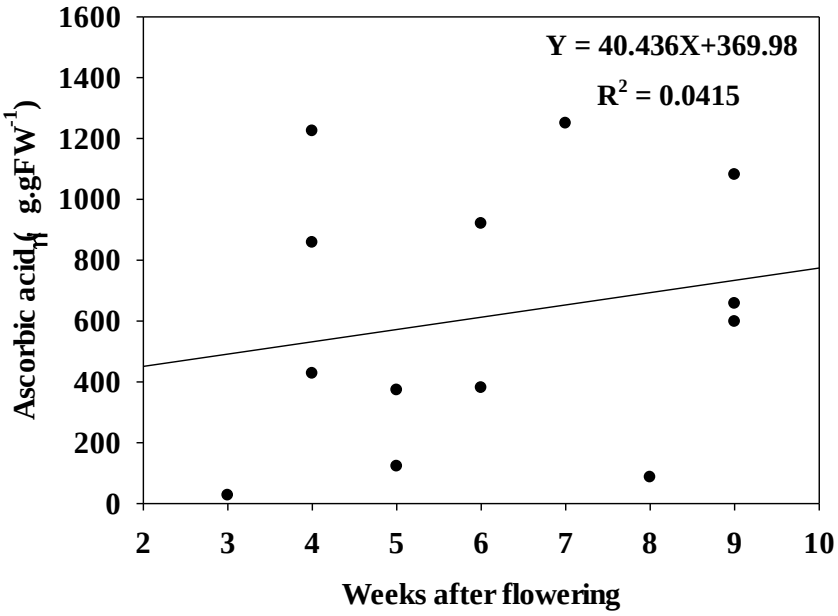


Figure 6.1 Ascorbic acid concentration (µg.gFW^{-1}) of Jalapeño planted in a commercial glasshouse during fruit maturation. Each point represents an individual chilli fruit.

Table 6.1 Ascorbic acid concentration at different fruit sizes

Size	Ascorbic acid concentration (µg.gFW^{-1})
Small (≤ 40 g)	458 ± 134 b
Large (> 40 g)	862 ± 116 a

Data represent mean ± S.E. (n = 10 fruit). Numbers followed by different letters differ significantly (P < 0.05) following analysis of variance and least significant difference (LSD) mean separation procedures.

6.3.1.2 Variation in ascorbic acid in Jalapeño fruit of different ages harvested at a single time

In a strip pick experiment, all Jalapeño fruit present on the plant were harvested when fruit from the first node reached 6, 8 or 10 WAFS. A range of maturities at harvest was found in these fruit from mature to young fruit (low to high nodes).

Ascorbic acid concentration in Jalapeño tended to decrease ($P < 0.05$) in younger fruit at higher nodes (Fig. 6.2A). However, high variation still remained in fruit from the same node which may be explained by fruit from the same node sometimes having different maturities; these data were an average of different individual fruit from different plants. When these same data were rearranged by the actual maturity of each fruit (defined as WAFS), ascorbic acid concentration of Jalapeño increased significantly ($P < 0.05$) with maturity at harvest and peaked at 6 and 7 WAFS, and then ascorbic acid concentration tended to decrease after 7 WAFS (Fig. 6.2B). Ascorbic acid concentration during fruit development ranged from 195 - 1400 $\mu g.gFW^{-1}$.

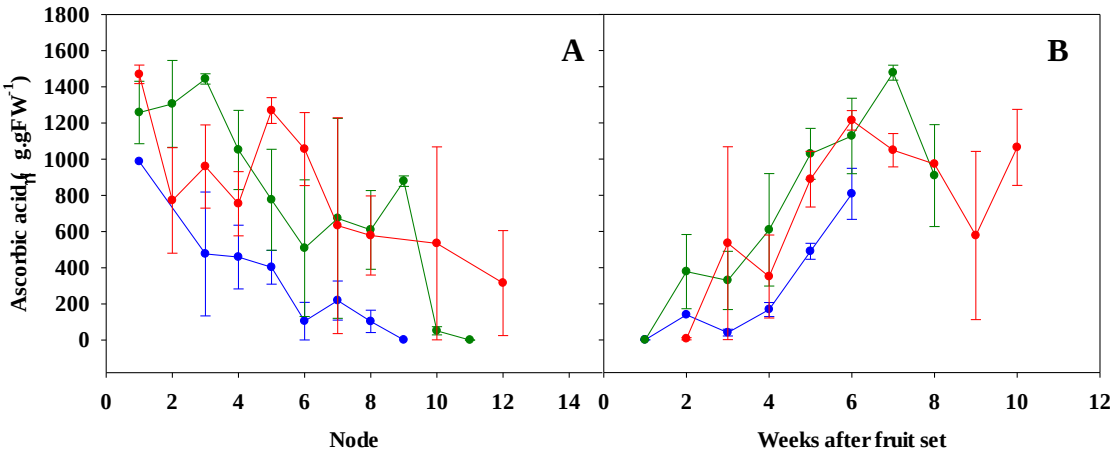


Figure 6.2 Ascorbic acid concentration of Jalapeño planted in a PGU glasshouse. Fruit were harvested from different nodes (A) and the same data were represented by actual fruit maturity (B). All fruit presented on the plant were harvested at one time when fruit from the first node reached 6 (Blue), 8 (Green), and 10 (Red) WAFS. Data represent means ± S.E. (n = 2 - 24 fruit).

6.3.1.3 Effects of time of planting, position on plant, maturity at harvest and crop load on ascorbic acid in Jalapeño planted in a PGU glasshouse

Ascorbic acid concentration in Jalapeño from both high and low crop load plants which were planted at different times (August - October) and sequentially harvested when fruit at each node reached 6, 8 or 10 WAFS was measured using only fruit from the first flush of production on nodes 4, 8, 12, and 16.

Due to the location of the fan in the glasshouse (Fig. 2.1), there was a possibility of variation in ascorbic acid concentration along the row which may from temperature fluctuation. To test this hypothesis, plants were grouped by location in a glasshouse (3 plants located close to the fan = C; 4 plants located in the middle of the glasshouse = M; 3 plants located far from the fan = F). There was no difference ($P > 0.05$) in ascorbic acid concentration among these groups (data not shown). Therefore, plants could be safely used as a replication.

Ascorbic acid concentration in Jalapeño planted in August and September were higher ($P < 0.05$) than in fruit planted in October, where some data were missing due to blossom end rot, resulting in low fruit number (Fig. 6.3G - I). Ascorbic acid concentration of Jalapeño from different nodes and crop loads which were harvested when each fruit reached 6, 8 or 10 WAFS ranged from 18 - 1400 $\mu g.gFW^{-1}$. Mostly, ascorbic acid concentration tended to decrease ($P < 0.05$) at higher nodes (Fig. 6.3). Fruit at nodes 4 and 8 had the highest ascorbic acid concentration and it was maintained to 10 WAFS, particularly for the August planting (Fig. 6.3A - C). Fruit from nodes 12 or 16 did not show high ascorbic acid concentration except in the August and harvested at 6 WAFS, for which ascorbic acid concentration remained high at node 12 (Fig. 6.3A). Comparing maturities at harvest, ascorbic acid concentration in Jalapeño harvested at 8 and 10 WAFS tended to be higher ($P < 0.05$) than in fruit harvested at 6 WAFS, particularly in fruit from node 4 and the August and September planting dates (Fig. 6.3A - F). Jalapeño fruit from high crop load plants showed higher ($P < 0.05$) ascorbic acid than fruit from low crop load plants (Fig. 6.3). Overall, fruit from high crop load plants contained 979 $\mu g.gFW^{-1}$ of ascorbic acid which was approximately 20 % higher than in fruit from low crop load plants.

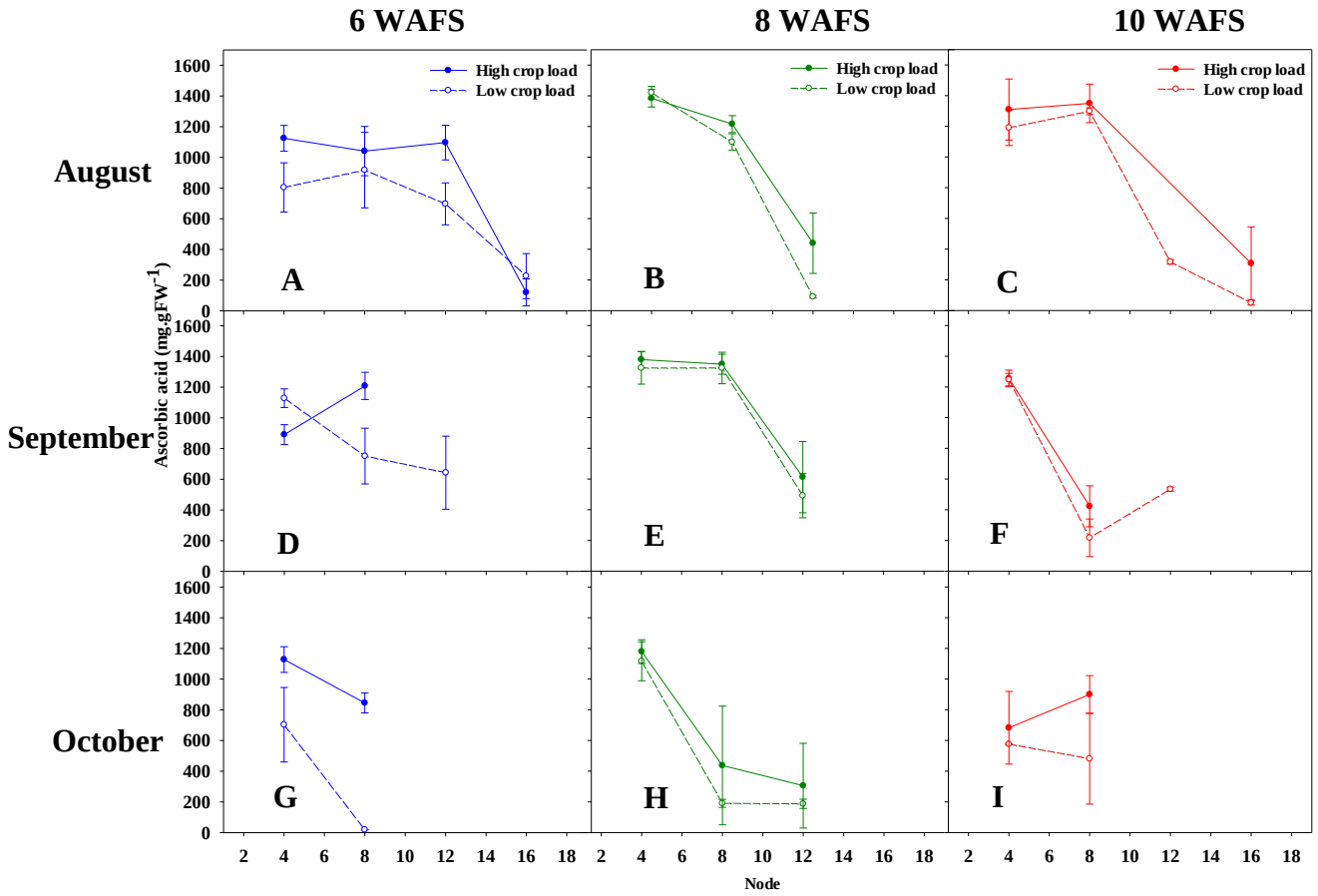


Figure 6.3 Ascorbic acid concentration (µg·gFW^{-1}) of Jalapeño from sequential plantings; August (A - C), September (D - F), and October (G - I) and harvested at 6 (Blue), 8 (Green), and 10 (Red) weeks after fruit set from different nodal positions on the plant. Fruit were from (closed symbol and solid line) high crop load and (opened symbol and line) low crop load which achieved by leaving fruit on the plant at nodes 4, 8, 12, and 16. Data represent means ± S.E. (n = 2 - 5 fruit).

6.3.2 Total capsaicinoids

6.3.2.1 Capsaicin in Jalapeño planted in a commercial glasshouse

Capsaicin concentration in Jalapeño planted in the commercial glasshouse during fruit development was determined. Fruit weight did not show a strong effect on capsaicin concentration (Fig. 6.4). Capsaicin concentration in fruit with similar weight ranged from 0 - 1000 μg.g DW^{-1} and no capsaicin was found in several fruit of various weights (Fig. 6.4).

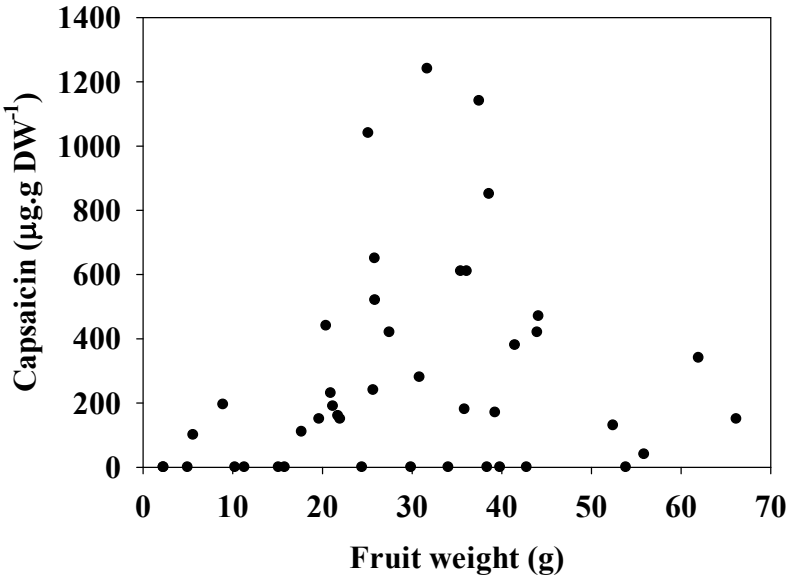


Figure 6.4 Capsaicin concentration in Jalapeño as a function of fruit weight. Each point represents an individual chilli fruit.

When the same data were reanalysed by fruit maturity, capsaicin concentration was fairly consistent during fruit development although fruit harvested at 3 and 6 WAFS showed lower capsaicin concentration ($P < 0.05$) than fruit harvested at other maturities (Fig. 6.5). However, high variability of capsaicin concentration was found in Jalapeño at some maturities, which may be explained by fruit to fruit and plant to plant variation and the low number of fruit at each maturity stage. Capsaicin concentration in fruit from some plants was not consistently detectable even when harvested at different maturities (Table 6.2). Among fruit harvested at the same maturity, capsaicin concentrations were variable in both fruit from different plants and even fruit from the same plant (Table 6.2).

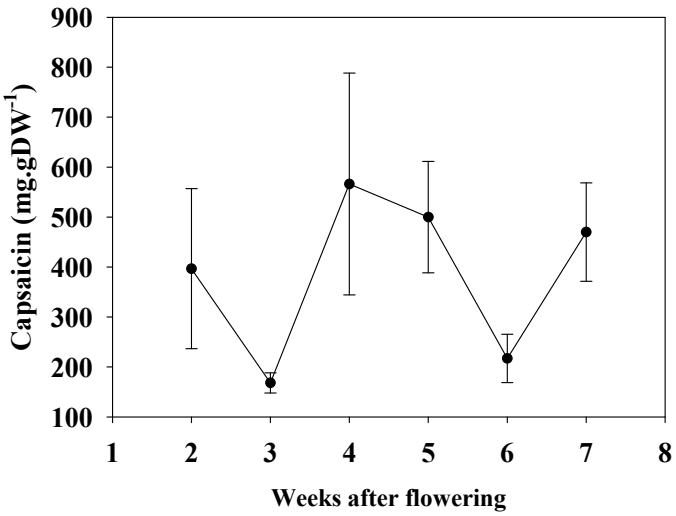


Figure 6.5 Capsaicin concentration (µg·gDW^{-1}) in Jalapeño planted in a commercial glasshouse during fruit development. Data represent means ± S.E. (n = 3 - 11 fruit).

Table 6.2 Capsaicin concentration in Jalapeño fruit harvested from the same or from different plants (ND = not detectable).

Plant	Maturity stage (Weeks after fruit set)	Capsaicin (µg.gDW^{-1})
A	3	ND
	4	ND
B	3	95
	3	ND
	5	610
	5	420
	7	280
	7	610
C	2	ND
	4	ND
	6	ND
	8	ND
D	2	100
	3	150
	6	160

6.3.2.2 Method verification for total capsaicinoid extraction

Due to the observed high variation in capsaicinoid concentration from fruit to fruit, it was decided to test the effect of extraction process. The ratio of sample (g) and extraction solvent (acetronitrile) (mL) was modified from 1:10, 1:16 and 1:20. Previously, Collins et al. (1995) recommended that samples should be > 1 g of powder to produce consistently reliable capsaicinoid measurement. Due to a limit of sample size in this work, 0.5 g of chilli powder was tested for reliability and used for further analysis. Then the extraction process at the optimum ratio was tested at varying durations (1 - 4 hours) of heating at 80 °C.

Overall, the ratio of sample to extraction solvent of 1:20 and heating for 4 hours were the best conditions for total capsaicinoid extraction (Table 6.3 and 6.4). A second extraction was conducted to ascertain whether total capsaicinoids still remained in the residue. Less than 20 % of total capsaicinoids were found in the second extraction (data not shown); therefore only one extraction was used for further measurements.

Table 6.3 Total capsaicinoid concentration at different ratios of sample: extraction solvent.

Sample: extraction solvent ratio	Total capsaicinoids ($\mu g.gDW^{-1}$)
1:10	436 ± 92 b
1:16	534 ± 153 a
1:20	592 ± 124 a

Data represent means ± S.E. (n = 3). Numbers followed by different letters differ significantly ($P < 0.05$) following analysis of variance and least significant difference (LSD) mean separation procedures.

Table 6.4 Total capsaicinoid concentration after different heating periods.

Heating period (hour)	Total capsaicinoids ($\mu g.gDW^{-1}$)
1	412 ± 125 b
2	438 ± 110 b
4	502 ± 149 a

Data represent means ± S.E. (n = 3). Numbers followed by different letters differ significantly ($P < 0.05$) following analysis of variance and least significant difference (LSD) mean separation procedures.

6.3.2.3 Variation in capsaicinoid concentration in Jalapeño fruit of different ages harvested at a single time

Total capsaicinoid concentrations were studied in Jalapeño planted in the PGU glasshouse when all fruit present on the plant were harvested once fruit from the first node reached 6, 8 or 10 WAFS. Total capsaicinoid concentrations ranged from 44 - 1147 µg.gDW^{-1} during fruit maturation (Fig. 6.6). Total capsaicinoids increased at the early stage of fruit development and then capsaicinoid concentration seemed reasonably stable in fruit from week 3 onwards, (Fig. 6.6). This stage of fruit maturity may coincide with placenta and seed production, while Maga (1975) reported that no pungency was detected in chilli fruit before 4 WAFS. The capsaicinoid production may differ in each chilli variety. However, high variation of capsaicinoids was found even in fruit from the same plant so fruit to fruit variation had a major impact on variation of total capsaicinoids in Jalapeño.

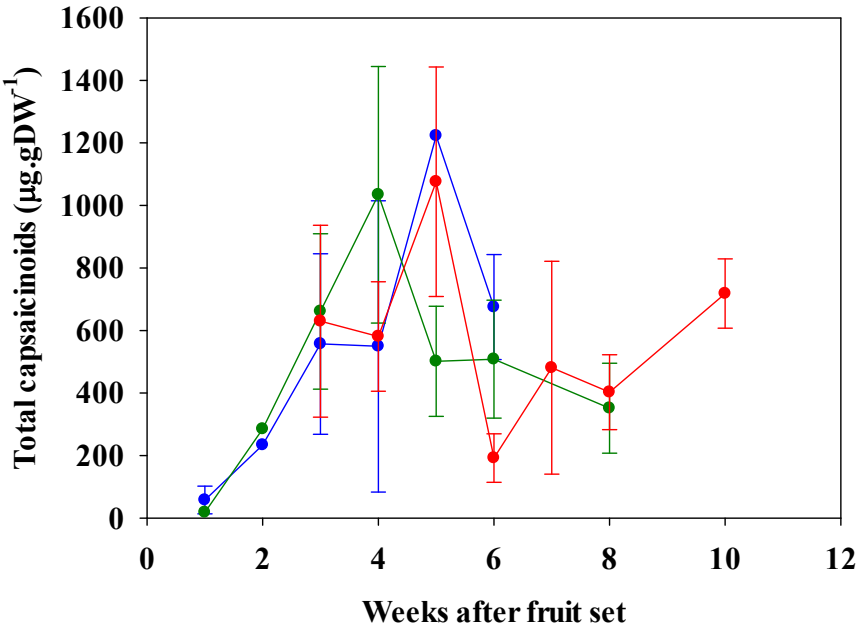


Figure 6.6 Total capsaicinoids (µg.gDW^{-1}) of Jalapeño planted in a PGU glasshouse. Fruit were harvested during fruit development defined by weeks after fruit set. All fruit presented on the plant were harvested at one time when fruit from the first node reached 6 (Blue), 8 (Green), or 10 (Red) weeks after fruit set. Data represent means ± S.E. (n = 3 - 14 fruit).

6.3.2.4 Effects of time of planting, position on plant, maturity at harvest and crop load on total capsaicinoids in Jalapeño planted in a PGU glasshouse

Total capsaicinoids were studied in Jalapeño planted from sequential plantings and fruit were harvested from different nodes when each fruit reached 6, 8 or 10 WAFS. Location in a PGU glasshouse (as previously mention in ascorbic acid) and time of planting did not affect ($P > 0.05$) total capsaicinoid concentration so location and time of planting were used as replications (Fig. 6.7).

Total capsaicinoid concentrations were similar among fruit harvested at different maturity stages (Fig. 6.7), and there were no significant differences ($P > 0.05$) in total capsaicinoid concentration by node, or maturity stage. Total capsaicinoid concentrations ranged from 680 - 982 $\mu g.gDW^{-1}$. Fruit from low crop load plants contained 893 $\mu g.gDW^{-1}$ of total capsaicinoids which was higher ($P < 0.05$) than fruit from high crop load plants which contained 693 $\mu g.gDW^{-1}$ of total capsaicinoids (Fig. 6.7).

Capsaicinoids are mainly produced in the placenta (Iwai et al., 1979; Rowland et al., 1983; Zamski et al., 1987; Thiele et al., 2008; Broderick & Cooke, 2009). To validate this statement total capsaicinoids of Jalapeño were measured in pericarp, placenta, and seed (Table 6.5). More than 70 % of total capsaicinoids were found in placenta with a concentration approximately 50 times higher ($P < 0.05$) than in the pericarp. In addition, when total capsaicinoid concentration was measured from different sections of Jalapeño fruit, the top section contained the highest total capsaicinoids ($P < 0.05$) than middle and bottom sections (Table 6.6) which can be related to the varying proportion of placenta and seed in each part of fruit. This confirmed that total capsaicinoids were not consistently distributed across the whole fruit. The observed results may explain the high variation of total capsaicinoid concentration in previous section. The differences between samples could be conceded by variability coming from different proportions of seed, placenta and pericarp being found in each fruit.

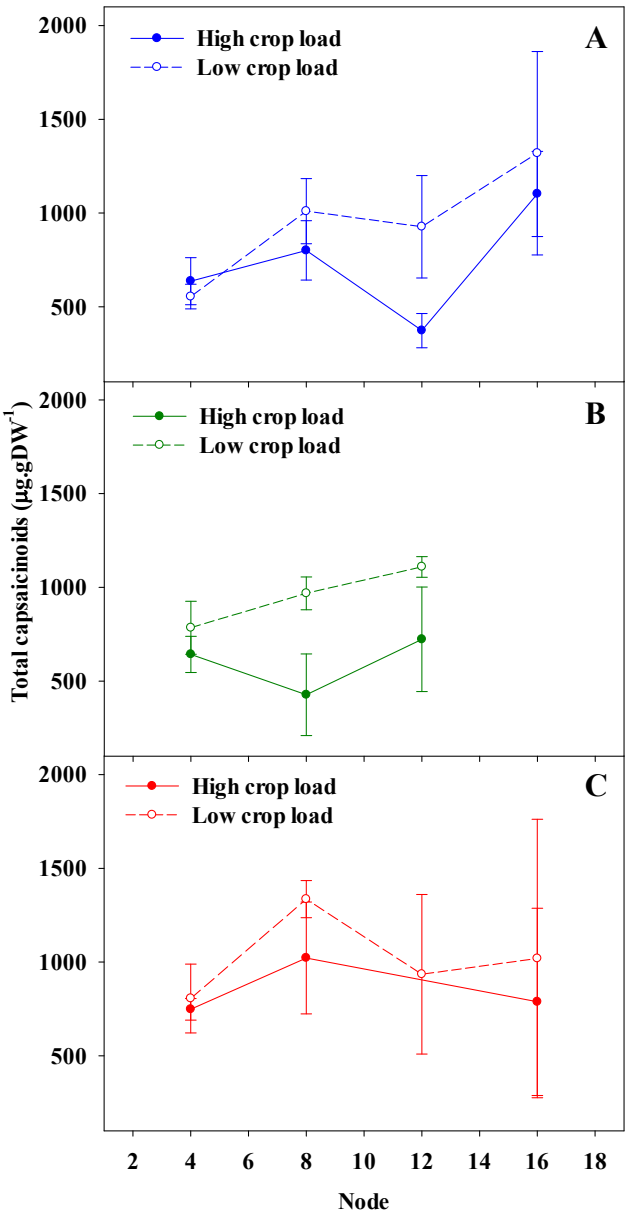


Figure 6.7 Averages of total capsaicinoid concentration (A - C) of Jalapeño fruit planted in a PGU glasshouse and harvested at 6 (Blue), 8 (Green), and 10 (Red) weeks after fruit set from different nodal positions on the plant. Fruit were from (closed symbol and solid line) high crop load and (open symbol and dashed line) low crop load, which was achieved by leaving fruit on the plant at nodes 4, 8, 12 and 16. Data represent means of total capsaicinoids from three sequential plantings ± S.E. (n = 2 - 12 fruit).

Table 6.5 Total capsaicinoid concentration (μg.gDW^{-1}) and content (μg) in pericarp, placenta, and seed of Jalapeño fruit (40 g) harvested at 7 WAFS

Plant parts	Total capsaicinoids (μg.gDW^{-1})	Dried weight (g)	Average total capsaicinoids (μg)
Pericarp	189 ± 44 c	2.9	543
Placenta	10576 ± 2197 a	0.6	5787
Seed	1726 ± 645 b	0.9	1587

Data represent means ± S.E. (n = 3). Numbers followed by different letters differ significantly (P < 0.05) following analysis of variance and least significant difference (LSD) mean separation procedures.

Table 6.6 Total capsaicinoid concentration (μg.gDW^{-1}) of Jalapeño from different sections

Plant section	Total capsaicinoids (μg.gDW^{-1})
Top	1178 ± 430 a
Middle	414 ± 245 b
Bottom	121 ± 46 b

Data represent means ± S.E. (n = 3). Numbers followed by different letters differ significantly (P < 0.05) following analysis of variance and least significant difference (LSD) mean separation procedures.

Since total capsaicinoid concentrations were measured in halves of Jalapeño fruit containing variable proportion of pericarp, placenta, and seed, a small experiment was set up to determine the influence of different proportions of pericarp, placenta, and seed particles on total capsaicinoid concentration of the samples. Jalapeño fruit was dissected in halves and proportions of pericarp, placenta and seed were weighed and recorded. These samples were oven dried and ground into powder form. The particle size of ground tissue was measured by a Mastersizer 2000 (Hydro MU, Malvern Instruments Inc., Worcestershire, UK). A Monte-Carlo simulation was developed by Dr. Andrew East to predict the effect of proportions of different tissue in each sample on the measured total capsaicinoids (Appendix I). Simulations showed an enormous potential range of total capsaicinoid concentration due to sub-sampling the standard ground mixture. This careful analysis has confirmed that a

high variation of the measured total capsaicinoid concentration can be explained simply by the inclusion of different proportions of each part (placenta, seed, and pericarp) in the 0.5 g sample used for extraction.

6.3.3 Antioxidant activity and total phenolic concentration

6.3.3.1 Antioxidant activity (AOX) and total phenolic concentration (TPC) in Jalapeño planted in a commercial glasshouse

The antioxidant activity (AOX) expressed as FRAP values of water extracts averaged from 438 - 813 $\mu mol.L^{-1}$ and significantly increased ($P < 0.05$) during fruit development (Fig 6.8A) but this trend was not seen in ethanol extracts. Maturity at harvest did not show any effects ($P > 0.05$) on TPC in either solvent (Fig 6.8B). Both FRAP and TPC values were significantly lower ($P < 0.05$) in 50 % ethanol extracts than in water extracts. Jalapeño harvested at 6 WAF showed the highest antioxidant activity measured by FRAP assay while TPC remained stable during fruit development (Fig. 6.8). High AOX assessed by FRAP assay in fruit harvested at 6 WAFS was not proportional to the TPC (R^2 = 0.24 and 0.07 in water and 50 % ethanol extracts respectively) (data not shown). These results indicated that phenolic compounds might not be a major contributor to the antioxidant activity in Jalapeño chilli.

6.3.3.2 Method verification

In second season, the extraction method was improved by testing the efficiency of different extraction solvents; ethanol (80 %) + 1 % HCl (acidified EtOH), methanol, and water (Table 6.7). Water and acidified EtOH proved to be a more efficient extraction solvent for AOX than methanol while there was no significant difference ($P > 0.05$) among the extraction solvents on TPC measurement. Therefore, water and acidified ethanol were selected for further method verification.

Previous research stated that to avoid ascorbic acid interference on TPC measurement, the extracts should be heated at 60 °C for 1 hour (Deepa et al., 2007). Therefore heating and non-heating treatments were tested on ethanol and water extracts. In addition, to test whether the period of extraction process showed an

effect on the extraction efficiency; different extraction periods (1 hour or overnight) were studied.

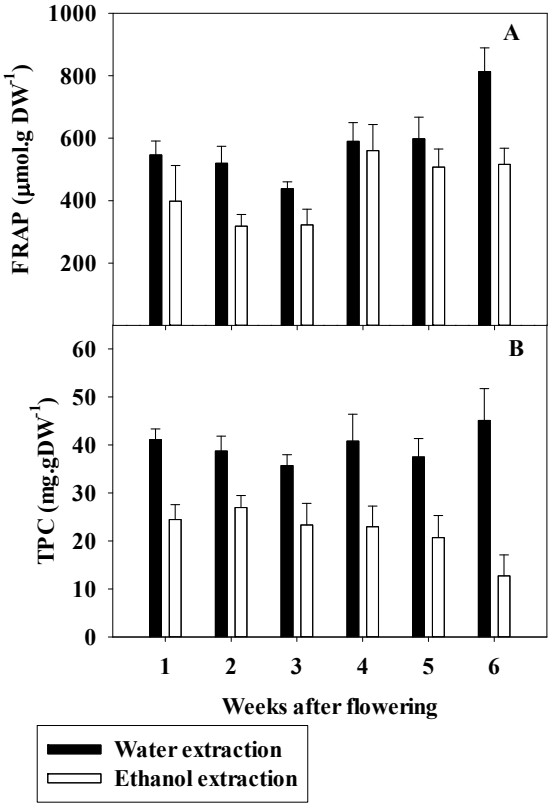


Figure 6.8 Antioxidant activity (AOX) assessed by ferric reducing antioxidant (FRAP) assay (A), and total phenolic concentration (TPC) (B) of Jalapeño during fruit development in both water and 50 % ethanol extracts prepared with 10 mg freeze-dried tissue in 10 ml solvent. Data represent means ± S.E. (n = 3) (***).

Table 6.7 Antioxidant activity (AOX) by FRAP assay and total phenolic concentration (TPC) of Jalapeño extracts from different extraction solvents

Extraction solvents	FRAP (µmol.gDW^{-1})	TPC (mg.gDW^{-1})
80 % Ethanol + 1 % HCl	216 ± 14 a	53 ± 1 a
Methanol	164 ± 12 b	54 ± 1 a
Water	233 ± 9 a	48 ± 2 a

Data represent means ± S.E. (n=3). Numbers followed by same letter did not differ significantly (P > 0.05) following analysis of variance and least significant difference (LSD) mean separation procedures.

(***) Fig. 6.8 from this chapter is included in the poster: Jansasithorn, R., East, A., Hewett, E., Mawson, J., Molan, A., & Heyes, J. 2010. Harvest maturity influences the antioxidant activity in Jalapeño chilli. In 28th International Horticultural Congress 2010: Abstracts (pp. 363 - 363). Lisbon, Portugal.

Overall, acidified ethanol showed a higher extraction efficiency ($P < 0.05$) for AOX measurement than water, while there was no significant difference ($P > 0.05$) between acidified ethanol and water on TPC measurement (Table 6.8). Heating treatment did not affect ($P > 0.05$) AOX and TPC measurement in either solvent, indicating that heating was not necessary in the extraction process for AOX and TPC determination in Jalapeño (Table 6.8). Overall, AOX assessed by FRAP in overnight extraction samples was higher ($P < 0.05$) than 1 hour extraction samples (Table 6.8). Meanwhile, TPC in water extract and heating treatment for 1 hour was lower ($P < 0.05$) than TPC in other treatments. Overall results showed that the best extraction conditions for AOX and TPC measurement were 80 % Ethanol + 1 % HCl without heating treatment and with overnight extraction. This method was selected for the next experiment in this research.

Table 6.8 Antioxidant activity (AOX) by FRAP assay and total phenolic concentration (TPC) of Jalapeño extracts with different conditions.

Solvent	Condition	Extraction period	FRAP ($\mu mol.gDW^{-1}$)	TPC ($mgGAE.gDW^{-1}$)
EtOH (80 % EtOH + 1 % HCl)	Heat	1	123 ± 45	23 ±6
	Heat	Overnight	198 ± 23	25 ±8
	No heat	1	188 ± 89	22 ±7
	No heat	Overnight	189 ± 33	25 ± 6
Water	Heat	1	97 ± 17	15 ±5
	Heat	Overnight	177 ± 96	23 ± 5
	No heat	1	126 ± 26	28 ±8
	No heat	Overnight	182 ± 53	26 ± 4
Solvent			*	NS
Heat			NS	NS
Time			**	NS
Solvent * Heat			NS	**

Data represent means ± S.E. (n = 3).
*, **, NS = Significant at 5 %, 1 % levels following analysis of variance and least significant difference (LSD) mean separation procedures and not significant respectively.
Other interactions were tested but found there was no significantly different.

6.3.3.3 Variation in antioxidant activity (AOX) and total phenolic concentration (TPC) in Jalapeño fruit of different ages harvested at a single time

When all fruit present on the plant were harvested when fruit at the first node reached 6, 8, or 10 WAFS, AOX and TPC proved to be remarkably stable between fruit harvested at different maturities. There were no significant differences ($P > 0.05$) between AOX and TPC as fruit matured (Fig. 6.9). FRAP values ranged from 56 - 218 µmol.gDW^{-1}) (Fig. 6.9A), while TPC remained more stable at 21 - 46 mg.gDW^{-1} (Fig. 6.9B). Fruit harvested when fruit from the first node reached 6 WAFS showed lower AOX, but higher TPC than fruit harvested at more mature stages which indicated that there was no positive correlation between AOX and TPC.

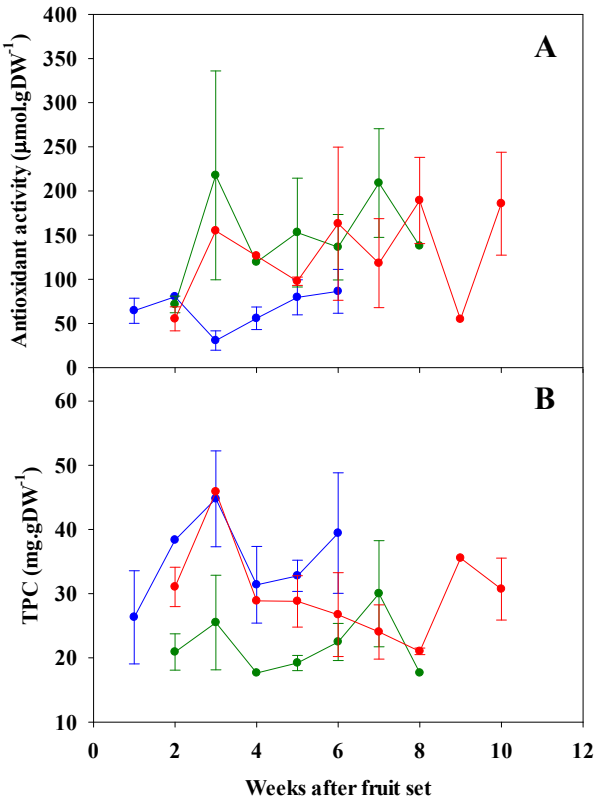


Figure 6.9 Antioxidant activity, AOX (µmol.gDW^{-1}) (A) assessed by ferric reducing antioxidant (FRAP) assay and total phenolic concentration, TPC (mg.gDW^{-1}) (B) of Jalapeño planted in a PGU glasshouse. All fruit presented on the plant were harvested at one time when fruit from the first node reached 6 (Blue), 8 (Green), or 10 (Red) weeks after fruit set. Data represent means ± S.E. (n = 2 - 11 fruit).

6.3.3.4 Effects of time of planting, position on plant, maturity at harvest and crop load on antioxidant activity (AOX) and total phenolic concentration (TPC) of Jalapeño planted in a PGU glasshouse

AOX assessed by ferric reducing antioxidant (FRAP) assay and TPC assessed by Folin-Ciocalteu assay were studied in Jalapeño fruit planted at sequential plantings with different crop loads; fruit were harvested from different nodes when each individual fruit reached 6, 8 or 10 WAFS. Locations in a PGU glasshouse and time of planting did not affect ($P > 0.05$) AOX and TPC concentrations in Jalapeño so average values of AOX and TPC of Jalapeño from different time of plantings were shown in Fig. 6.10.

AOX and TPC concentrations did not vary ($P > 0.05$) among nodes meaning that these data were remarkably consistent in fruit along the plant (Fig. 6.10). Fruit from low crop load plants showed higher AOX and TPC for some maturities, but there was no statistical difference ($P > 0.05$) between crop loads (Fig. 6.10). AOX in Jalapeño fruit harvested at 8 and 10 WAFS was higher ($P < 0.05$) than in fruit harvested at 6 WAFS (Fig. 6.10A - C) while TPC remained stable ($P > 0.05$) with maturity at harvest (Fig. 6.10D - F).

Because of the effect of placenta proportion on total capsaicinoid content, AOX and TPC were also measured in pericarp, placenta and seed to determine whether there were differences in AOX and TPC among the tissues (Table 6.9). Unlike capsaicinoid concentration, there were only small difference of AOX in pericarp, placenta and seed and no significant differences ($P > 0.05$) of TPC were found in different parts of Jalapeño fruit. The 3 times difference of FRAP concentration in placenta and seed was far smaller than the 50 times difference in total capsaicinoid concentration, between placenta and pericarp so there was no strong concern about the impact of tissue variation in the sample on measurement. This analysis implied that the compounds other than total capsaicinoids are probably being responsible for the AOX and TPC.

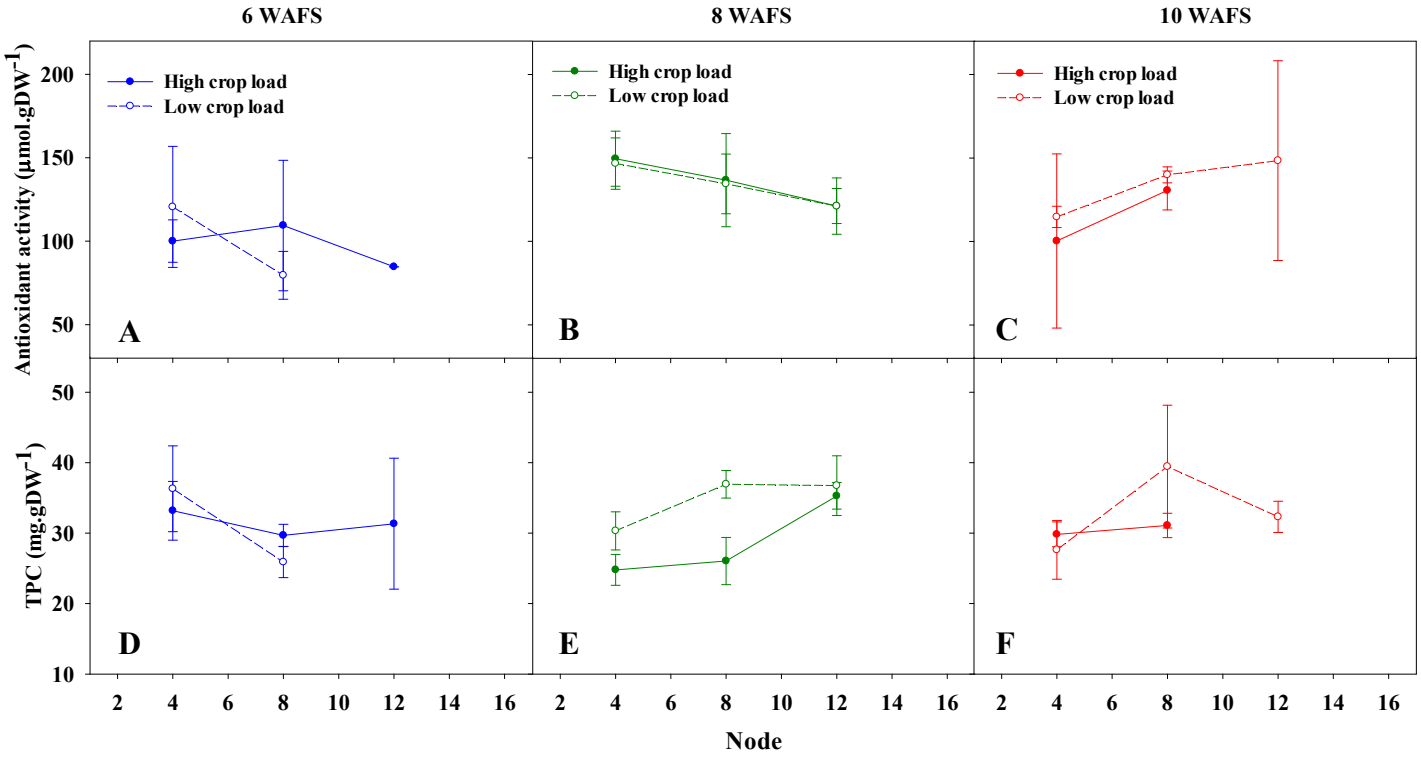


Figure 6.10 Antioxidant activity, AOX (µmol·gDW^{-1}) (A - C) assessed by ferric reducing antioxidant (FRAP) assay and total phenolic concentration, TPC (mg·gDW^{-1}) (D - F) of Jalapeño planted in a PGU glasshouse and harvested at 6 (Blue), 8 (Green), and 10 (Red) weeks after fruit set from different nodal positions on the plant. Fruit were from (closed symbol and solid line) high crop load and (opened symbol and dash line) low crop load which achieved by leaving fruit on the plant at nodes 4, 8 and 12. Data are means of AOX and TPC from three sequential plantings ± S.E. (n = 2 - 13 fruit).

Table 6.9 Antioxidant activity, AOX (μmol·gDW^{-1}) and TPC (mg.gDW^{-1}) in pericarp, placenta, and seed of Jalapeño fruit

Plant parts	FRAP (μmol.gDW^{-1})	TPC (mg.gDW^{-1})
Pericarp	268 ± 7a	44 ± 2a
Placenta	274 ± 1a	52 ± 3a
Seed	104 ± 30b	38 ± 17a

Data represent means ± S.E. (n = 3)
Different letters in the same column represent significant differences ($P < 0.05$) following analysis of variance and least significant difference mean separation procedures.

6.3.4 The correlation between phytochemicals of Jalapeño chilli

The correlation between AOX and TPC were studied to test whether TPC are a major component of the AOX. The weak correlation was found between FRAP and TPC of Jalapeño planted in the commercial glasshouse (Fig. 6.8) which was similar to Jalapeño planted in the PGU glasshouse, where there was no correlation between FRAP and TPC (Fig. 6.11A). Colour, size and maturity data were assessed, but they could not explain the pattern (a group of results that showed high FRAP and low TPC and a group of result which showed low FRAP and high TPC) found in Fig. 6.11A (data not shown). This suggested that phenolic compounds were not a major contributor to the AOX of Jalapeño. No correlation was also found between ascorbic acid and FRAP in Jalapeño planted in the PGU glasshouse (Fig. 6.11B).

To check whether capsaicinoids are antioxidants or contribute to TPC, pure capsaicin in methanol were used instead of the sample in AOX and TPC measurements. Pure capsaicin showed high AOX and contributed to TPC (data not shown), which suggested that placenta of Jalapeño should show high AOX and TPC. However, AOX and TPC did not show a large difference among tissue types, yet the placenta contained 50 times more capsaicinoid concentration than other tissues (Table 6.5). These results indicate that capsaicinoids cannot be well extracted by ethanol which was used as extraction solvent for AOX and TPC measurement. Therefore, the correlation between total capsaicinoids and FRAP or TPC was not studied (Navarro et al., 2006).

Overall, the results indicate that there must be other compounds that can be extracted by ethanol, such as chlorophyll and carotenoids, which are more influential on the AOX and TPC of Jalapeño tissue.

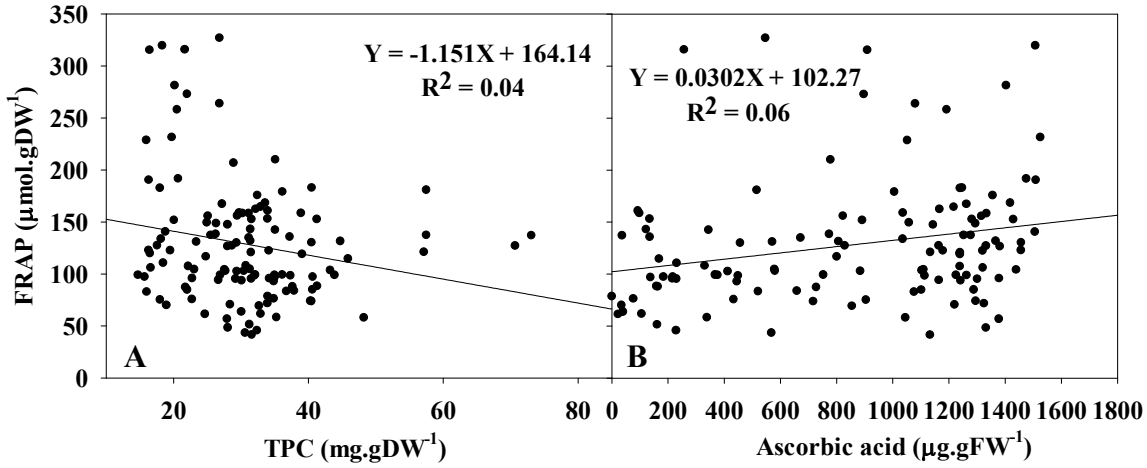


Figure 6.11 Correlations between FRAP and TPC (A), FRAP and ascorbic acid (B) of Jalapeño planted in a PGU glasshouse.

6.4 Discussion

Chillies contain beneficial health compounds such as vitamin C, A (carotenoids) and capsaicinoids (Marín et al., 2004; Materska & Perucka, 2005; Sun et al., 2007; Chuah et al., 2008; Hervert-Hernandez et al., 2010; Alvarez-Parrilla et al., 2011). To measure these compounds accurately, the published methodology should be carefully verified to establish the effect on non-uniform distribution of phytochemical compounds within the fruit.

6.4.1 Phytochemical compounds in each individual part of Jalapeño

A large difference (about 50 times) of capsaicinoid concentration was found between placenta and pericarp of Jalapeño (Table 6.5), with more than 70 % of total capsaicinoids being found in placenta tissue, which was similar to previous research in other peppers (Kozukue et al., 2005; Monforte-Gonzalez et al., 2010). A sampling model also confirmed that variability in the mixture of tissues in the extracted sample used for extraction would lead to high variability of total capsaicinoid concentration. This finding may affect measured total capsaicinoids in previous work. In most research, the whole fruit were ground, combined and weighed from 1 -

10 g of powder used for measuring total capsaicinoid concentration. Either combining fruit to a bulk sample or measuring individual fruit were reported (Estrada et al., 1997; Contreras-Padilla & Yahia, 1998; Estrada et al., 2000, 2002; Kirschbaum-Titze et al., 2002a; Kirschbaum-Titze et al., 2002b; Deepa et al., 2007). Kirschbaum et al. (2002) showed that five fruit combining into one sample was required to give an acceptable precision for capsaicinoid measurement. In this research, total capsaicinoids were measured from half fruit similar to Contreras-Padilla & Yahia (1998) (and the other half was frozen for measuring AOX and TPC). However, this method may lead to differences of total capsaicinoid concentration from different halves. Some research measured capsaicinoids only in the pericarp tissue (Kozukue et al., 2005; Materska & Perucka, 2005) as it is a commonly eaten part. However, many people consume the whole Jalapeño fruit and therefore data are needed from all tissues. Overall, the accuracy of capsaicinoid measurement depends on the difference of capsaicinoid concentrations in each tissue and the proportions of placenta, pericarp and seed in the extracted sample. If there are large differences, the amount of extracted sample would need to be considered more carefully as previous research has been used ranging from 1 - 10 g which may reduce a variability of capsaicinoid measurement. In addition, it also depends on the eaten part of chilli and pepper. If only pericarp is consumed, the general procedure for capsaicinoid measurement can be used to measure capsaicinoids in pericarp.

Ascorbic acid is composed of both ascorbic acid (AA) and dehydroascorbic acid (DHA) but generally, only AA is reported (Rahman et al., 1978; Howard et al., 1994; Howard et al., 2000; Fox et al., 2005; Navarro et al., 2006; Deepa et al., 2007) since less than 1 mg.100g FW^{-1} of DHA has been reported for capsicum fruit (Wimalasiri & Wills, 1983). Therefore, only AA was measured in this work. However, considerable variation in DHA has been reported from undetectable to 26 % of total ascorbic acid in Jalapeño, Serrano, bell pepper and New Mexican chilli (Howard et al., 1994; Osuna-Garcia et al., 1998; Marín et al., 2004). Therefore, DHA of Jalapeño in this research was measured by Tan (2011). Surprisingly, more than 30 % of total ascorbic acid was in the form of DHA. To confirm whether this high proportion might relate to a conversion from AA to DHA during storage (samples had been stored in -70°C freezer for a year), fresh samples were obtained and a similar proportion was

found. Some vegetables such as Swiss chard contain only DHA in fresh fruit as AA is completely and immediately converted to DHA because of a high oxidase activity of enzymes such as ascorbate oxidase, peroxidase and polyphenol oxidase in the tissue (Gil et al., 1998). It seems that ascorbic acid in Jalapeño might transform to DHA easily in both fresh and frozen fruit. Both forms are used as vitamin C when consumed, so future work with chillies should attempt to measure both AA and DHA.

Vitamin C concentrations were also measured from different parts of Jalapeño fruit by Tan (2011) (Table 6.10) who found only 1.5 times difference between pericarp and placenta, but very small amounts of vitamin C were found in seed. Pepkowitz et al. (1944) found only 4 times difference of ascorbic acid among pericarp, placenta and seed in pepper fruit. An average fruit of Jalapeño fruit consists of 72 % pericarp, 16 % pedicel and calyx, 9 % placenta and only 3 % seed, therefore the possibility of seed being in the extraction sample is low. In this case the proportion of individual tissue contained in the extraction sample is unlikely to impact on vitamin C measurement as found for capsaicinoid measurement.

Table 6.10 Total vitamin C in pericarp, placenta and seeds for green and red chillies (Tan, 2011).

	Total vitamin C (mg.g FW^{-1})		
	Placenta	Pericarp	Seed
Red	0.8 ± 0.06 a	1.0 ± 0.04 a	0.05 ± 0.01 b
Green	0.7 ± 0.07 a	1.0 ± 0.05 a	0.05 ± 0.04 b

Numbers followed by same letter did not differ significantly ($P > 0.05$) following analysis of variance and least significant difference mean separation procedures.

Similarly to vitamin C, AOX showed only 3 times differences among pericarp, placenta and seed of Jalapeño, while there were no differences of TPC among different parts of Jalapeño (Table 6.9). This observation can also indicate that total capsaicinoids were not well extracted by ethanol, therefore, the sub-sampling error observed from capsaicinoid measurement may not highly influence ascorbic acid including AOX and TPC measurement.

In conclusion, variation in capsaicinoid concentration among fruit parts does need to be considered in any sampling strategy but ascorbic acid, AOX and TPC do not vary significantly on the fruit and random sampling from half-fruit sampling is adequate.

6.4.2 Effect of pre-harvest factors on phytochemical compounds

6.4.2.1 Time of planting

Jalapeño fruit planted in August and September showed higher ascorbic acid concentration than those planted in October (Fig. 6.3), which may be explained that plants planted earlier having less competition between plant and fruit growth than plants planted during peak season (Adams et al., 2001; Minchin et al., 2010). In addition, fruit and vegetables grown in lower temperatures tended to show higher ascorbic acid, such as mandarin, grapefruit, apple and leafy vegetables (Rosenfeld, 1979; Mozafar, 1994; Lee & Kader, 2000). However total capsaicinoids in Jalapeño were not affected ($P > 0.05$) by time of planting (Fig. 6.7), while a previous study found that Padrón peppers developed during summer showed higher capsaicinoid contents than fruit developed during an autumn period (Estrada et al., 1999a). Like total capsaicinoids, time of planting did not affect ($P > 0.05$) AOX and TPC in Jalapeño (Fig. 6.9).

6.4.2.2 Maturity at harvest

Maturity at harvest is another important factor affecting fruit composition (Lee & Kader, 2000). Ascorbic acid of Jalapeño tended to increase during fruit maturation in both the strip pick experiment and environmental controlled experiment (Fig. 6.2B and 6.3). Similar to previous research, an increase of ascorbic acid concentration in peppers was found during ripening, which peaked at breaker stage (Yahia et al., 2001; Navarro et al., 2006; Deepa et al., 2007) or at fully red stage (Rahman et al., 1978; Howard et al., 1994; Osuna-Garcia et al., 1998; Howard et al., 2000; Marín et al., 2004). Mature fruit contain higher sugar concentration (Mozafar, 1994; Howard, 2006), a precursor of ascorbic acid synthesis, than immature fruit so mature fruit are primed to produce high ascorbic acid. Yahia et al. (2001) also showed a decline of ascorbic acid at fully mature or overripe stages of bell pepper, which correlates to an increase of ascorbic acid oxidase (AAO) at this stage.

Unlike ascorbic acid, capsaicinoid concentration in Jalapeño increased very early at the initial stage of fruit development and then it was consistent until fully mature (Fig. 6.6), while previous research has reported an increase of capsaicinoid concentration from 4 - 7 WAFS (Iwai et al., 1979; Estrada et al., 1997; Contreras-Padilla & Yahia, 1998; Estrada et al., 2000; Gnayfeed et al., 2001; Jha et al., 2001; Deepa et al., 2007; Barrera et al., 2008; Pandey et al., 2010). When Jalapeño fruit were harvested at different fruit ages (6, 8 and 10 WAFS) there was no difference ($P > 0.05$) in total capsaicinoids among these fruit (Fig. 6.7). Although small differences may have been considered variation, it seems unlikely that there is much change in capsaicinoid concentration over fruit reached 6 WAFS.

Overall, AOX in Jalapeño seemed to be consistent through fruit age in the strip pick experiment (Fig. 6.9). For AOX in Jalapeño growing in a PGU glasshouse, fruit harvested at 8 and 10 WAFS showed higher AOX than fruit harvested at 6 WAFS (Fig. 6.10), while TPC remained stable during fruit maturation (Fig. 6.9 - 6.10). An increase of AOX has been reported in most chillies during maturation as biosynthesis of antioxidants occurs during ripening (Howard et al., 2000; Materska & Perucka, 2005; Navarro et al., 2006; Conforti et al., 2007; Deepa et al., 2007; Sun et al., 2007), while TPC showed either a decrease of TPC from green to red fruit (Estrada et al., 2000; Materska & Perucka, 2005; Navarro et al., 2006; Conforti et al., 2007; Deepa et al., 2007; Menichini et al., 2009) or an increase of TPC with fruit maturation (Lee et al., 1995; Howard et al., 2000; Deepa et al., 2007). Genotype, plant age and fruit maturity all influence AOX and TPC (Marín et al., 2004; Conforti et al., 2007; Deepa et al., 2007; Sun et al., 2007; Alvarez-Parrilla et al., 2011). The differences reported between maturity at harvest may be dependent on the method for measuring AOX. For example, red pepper showed potential antioxidant in a β-carotene bleaching test, while green peppers exhibited AOX via lipid peroxidation (Conforti et al., 2007).

6.4.2.3 Position on plant

Phytochemical compounds contained in fruit located at different positions on the plant may vary depending on light and temperature, which are variable along the plant (Mozafar, 1994). Previous research showed that kiwifruit (Remorini et al.,

2007) and starfruit (Zabedah et al., 2009) exposed to the sunlight showed higher ascorbic acid concentration than shaded fruit because a precursor of ascorbic acid is sugar (i.e. mannose or galactose), which can be produced by photosynthesis in the chloroplast of green tissues (i.e. leaf or green fruit). However, this trend was not found in this research; Jalapeño fruit harvested at the same maturity from lower nodes showed higher ascorbic acid concentration than fruit from high nodes (Fig. 6.3). This was similar to Gautier et al. (2005) who found that cherry tomato fruit located close to the plant had higher vitamin C than fruit located far from the plant. Asrey et al. (2007) showed higher vitamin C (ascorbic acid + dehydroascorbic acid) in guava fruit from the middle and lower part of canopies than fruit from the upper canopy, although fruit from upper nodes contained higher sugar content. This suggests no positive correlation between sugar content and vitamin C synthesis. It can indicate that light intensity did not affect the conversion of sugar to vitamin C.

Fruit position on plant did not affect capsaicinoid concentration, AOX or TPC in Jalapeño as they seemed to be consistent along the plant (Fig. 6.7). However, previous research demonstrated either higher pungency in pepper at lower nodes, which was attributed to lesser competition between fruit at lower nodes (Zewdie & Bosland, 2000) or higher capsaicinoid concentration in the apical zone, which was attributed to light exposure stimulating capsaicinoid accumulation (Estrada et al., 2002). However, previous research has reported that pungency and capsaicinoid concentration/ content varied in chilli from the same cultivar grown in the same plot (Harvell & Bosland, 1997), or even fruit harvested from the same plants (Kirschbaum-Titze et al., 2002b; Mueller-Seitz et al., 2008). Little previous information has been reported on comparison of AOX or TPC from fruit at different positions on the plant.

6.4.2.4 Crop load

The lower competition between fruit from low crop load plants did not increase ascorbic acid concentration. In contrast, Jalapeño fruit from high crop load plants showed higher ($P < 0.05$) ascorbic acid concentration than fruit from low crop load (Fig. 6.3). Similarly, peach fruit (particular in peel) from commercial crop load also showed higher ascorbic acid than fruit from low crop (Buendia et al., 2008). The

author suggested that it could be a result of a positive effect on photosynthesis in high crop load treatment which may enhance ascorbic acid synthesis (Buendia et al., 2008). In addition, it has been reported previously that higher ascorbic acid concentration was found in leaves than some fruit (Mozafar, 1994; Asensi-Fabado & Munne-Bosch, 2010) so it may be possible that ascorbic acid is synthesized in leaves and imported to fruit (Osuna-Garcia et al., 1998). Alternatively sugar produced from photosynthesis in leaves is transferred to fruit for ascorbic acid synthesis or chilli fruit may synthesize both sugar and ascorbic acid themselves. The number of fruit on the plant influences assimilates or ascorbic acid synthesis as fruit from high crop load plants showed higher ascorbic acid concentration.

Jalapeño fruit from the low crop load treatment showed higher capsaicinoids than fruit from the high crop load treatment (Fig. 6.7) which may relate to less competition of fruit on the plant for nitrogen, which is known as a precursor for capsaicinoid biosynthesis. However, no previous research has been done on the effect of crop load on total capsaicinoid concentration.

Crop load seemed not to show any effects on AOX and TPC in Jalapeño as they seemed to be consistent along the plant and no difference ($P > 0.05$) was found between high and low crop load treatment (Fig. 6.10). Little previous information has been reported on comparison of AOX or TPC in fruit from different crop load, particularly for chillies and peppers. Stopar et al. (2002) showed an increase of TPC in 'Jonagold' apple from low crop load treatments while crop load did not affect TPC in 'Fuji', 'Gala' and Golden Delicious' (Unuk et al., 2006).

6.4.3 The correlation between phytochemicals of Jalapeño chilli

A correlation between AOX and TPC has been reported in some chillies (Lee et al., 1995; Howard et al., 2000; Deepa et al., 2007; Sun et al., 2007; Serrano et al., 2010). The R^2 values ranged from 0.33 - 0.94, whereas a weak correlation has been found in hot pepper where a low AOX was found in small green fruit which had high TPC (Conforti et al., 2007). A weak correlation between AOX and TPC was also observed in this research as the increase of AOX did not correlate to a consistent trend of TPC during fruit maturation (Fig. 6.8 - 6.10) which may be because TPC

was not the main contributor of AOX in Jalapeño. In addition, total capsaicinoids which contribute to TPC were not well extracted by ethanol in this research as TPC was not high in placenta (Table 6.9). For capsaicinoid extraction, acetronitrile is used for capsaicinoid measurement which indicated that the extracted compounds differ depending on extraction solvents. This was similar to Conforti et al. (2007), who showed high total capsaicinoids but low total phenolic content in red peppers. In addition, different assays for AOX measurement (e.g. FRAP, DPPH, or β-carotene bleaching test) are suitable for specific chemical reactivity and conditions. For example, a correlation between TPC and AOX measured by DPPH assay was found, while no correlations were found when AOX was measured by β-carotene bleaching and bovine brain peroxidation assays in green and red peppers (Conforti et al., 2007). In addition, no correlation was found between AOX and ascorbic acid in this research (Fig. 6.11B), while high correlations (R^2 = 0.89 - 0.97) have been reported previously in sweet and bell peppers (Fox et al., 2005; Serrano et al., 2010). This suggests that ascorbic acid was also not a major contributor of the AOX in Jalapeño. To determine the correlation between AOX and phytochemical compounds in the future work, decisions on the extraction solvent used for each compounds and the specific assays used for assessment should be evaluated.

6.5 Conclusion

Pre-harvest factors such as time of planting, position on plant, maturity at harvest and crop load seemed to showed obvious effects on ascorbic acid, but not on capsaicinoids, AOX or TPC, which mostly showed no significant differences. Jalapeño fruit at lower nodes showed higher ascorbic acid concentration than fruit from higher nodes, while other compounds seemed to be consistent along the plant. Jalapeño fruit from high crop load plants showed higher ascorbic acid concentration than fruit from low crop load plants. This information will be beneficial for chilli growers to produce fruit with higher nutritional value and yield. Ascorbic acid and TPC are not major contributors of the AOX in Jalapeño. Total capsaicinoid measurement can be affected by the differences of capsaicinoid concentration among placenta, pericarp or seed. To measure capsaicinoids accurately, chilli variety in terms of pungency (mild, medium, or hot) and eaten part of chilli should be considered.

CHAPTER 7

Overall discussion and conclusion

7.1 Introduction

The overall goal of this research is to determine an optimum storage temperature for maintaining quality of three chilli varieties (Habanero, Jalapeño and Paprika) and to assess the factors that influence chilli water loss. In order to achieve these goals, chilling sensitivity, water loss and phytochemical changes in chillies were studied during storage at different temperatures. In addition, effects of pre-harvest factors (i.e. time of planting, position on plant, maturity at harvest and crop load) on fruit weight, shape, colour and phytochemical compounds (i.e. capsaicinoids, vitamin C, total phenolic compound and antioxidant activity) were studied to understand the variation on fruit quality which was observed at harvest. This information would be useful to assist production and delivery of uniform chilli fruit that can be stored for long period at optimum temperature.

7.2 Key findings

7.2.1 Optimum storage temperature

It was found that the optimum storage temperature for Jalapeño and Habanero is 8 °C. No chilling injury symptoms were observed during storage for more than 4 weeks (Fig. 3.8 - 3.9). Meanwhile Paprika lost their firmness, particularly tensile strength, at 8 °C although the overall appearance remained acceptable (Fig. 3.6 and 3.10). The loss of firmness at 8 °C indicates that Paprika should be stored at warmer temperatures. Previous research has reported the range of optimum storage temperature for chillies and peppers to be 7 - 13 °C with chilling injury occurring when fruit were stored below 7 °C (Thompson, 1979; Kader, 1996; Gonzalez-Aguilar, 2004). In addition, chilling injury symptoms of Paprika, Jalapeño and Habanero (i.e. severe decay) were visible once fruit were moved from low temperature (0 and 4 °C) to room temperature (Fig. 3.8E and F, Fig. 3.9E and F and Fig. 3.10E and F). This was similar to Ogata et al. (1968) and Lin (2005) who found a rapid deterioration of pepper when fruit were transferred to high temperature. Based on the observed results in this research, it is suggested that loss of firmness

should be considered along with the overall appearance as symptoms of chilling injury in chillies and peppers. Chilling injury symptoms in most chillies become visible when fruit are returned to room temperature.

Storage temperature did not affect total capsaicinoid concentration in Jalapeño harvested at 6 WAFS during storage, while chilli fruit harvested at 8 WAFS showed a decrease ($P < 0.05$) of total capsaicinoids when fruit were moved from low temperature (8 °C) to 20 °C (Fig. 3.11A - B), which was similar to previous research (Kirschbaum-Titze et al., 2002a; Barrera et al., 2005; Gonzalez et al., 2005). Ascorbic acid in Jalapeño decreased ($P < 0.05$) with time of storage at 8 and 20 °C, but no difference ($P > 0.05$) of ascorbic acid concentration was observed between fruit stored at two temperatures (Fig 3.11C and D). Previous research has been reported that fresh-cut red pepper tended to lose greater ascorbic acid concentration during storage for 9 days at 8 °C than 4 °C (Raffo et al., 2008). Ascorbic acid concentration in most chillies and peppers decreased with time during storage at 5 - 10 °C over 8 - 45 days (Gonzalez et al., 2005; Akbudak et al., 2006; Raffo et al., 2008; Avalos Llana & Sgroppo, 2009; Ruiz-Cruz et al., 2010; Sakaldas & Kaynas, 2010).

According to these results, temperature should be controlled to 8 °C during handling and storage to maintain postharvest quality of Habanero and Jalapeño for the maximum time. Paprika requires warmer storage temperature as in practice Paprika fruit are often dried after harvest and processed to powder form. Packaging can be an additional treatment to delay shrivel symptom and protect fruit from external damage.

7.2.2 Water loss in chillies

Water loss is a major postharvest problem in chillies. Water loss of Jalapeño can occur through fruit skin, pedicel, calyx and the picking scar. Cracking on fruit skin accelerates water loss in Jalapeño fruit in comparison to non-cracked fruit. Cracked Jalapeño were found to have a three times higher rate of water loss ($\mu mol.s^{-1}Pa^{-1}$) than non-cracked fruit. Almost 80 % of water loss from cracked fruit occurs via the fruit skin while water loss in non-cracked fruit occurs equally from fruit skin and stem areas

(pedicel, calyx and picking scar) (Fig. 4.7 and Fig 7.1). However, Maalekuu et al. (2005) also showed that most water loss in some peppers occurs through fruit surface with little amount of water loss observing from calyx and stem. Water loss generally occurs through stomata, but stomata are absent on the fruit skin of chillies and peppers therefore water loss occurs through the cuticle of the fruit skin (Blanke & Holthe, 1997).

Wax and cutin are the main lipid components in the pepper culticle (Maalekuu et al., 2005). Lownds et al., (1993) and Parsons et al. (2012) found a correlation between total wax amount and water loss in some peppers. Aliphatic (simple straight chain) cuticle components (e.g. alkanes) create closely packed structures and hence form impermeable crystalline regions of the cuticle while more complex isoprenoid-based compound (e.g. triterpenoid and sterol) may form loose packing and produce a more porous highly permeable cuticle (Casado & Heredia, 1999; Vogg et al., 2004; Parsons et al., 2012). These observations agreed with Lurie & Ben-Yehoshua (1986) and Lurie et al. (1986) who demonstrated higher sterol and phospholipid content in red peppers, which lost more water than green fruit. However, in this research immature Jalapeño fruit showed higher P'_{H_2O} than mature fruit (Fig. 4.9) which may indicate incomplete development of the skin structure in these immature fruit (Diaz-Perez et al., 2007). However, Jalapeño fruit used in this research were harvested from 5 to 7 WAFS where fruit remained green. Therefore fruit in this work were less mature in comparison to red peppers from previous work. To confirm the effect of maturity on water loss in Jalapeño, wider ranges of maturity stages (green - red) and wax constituent composition should be studied in future work.

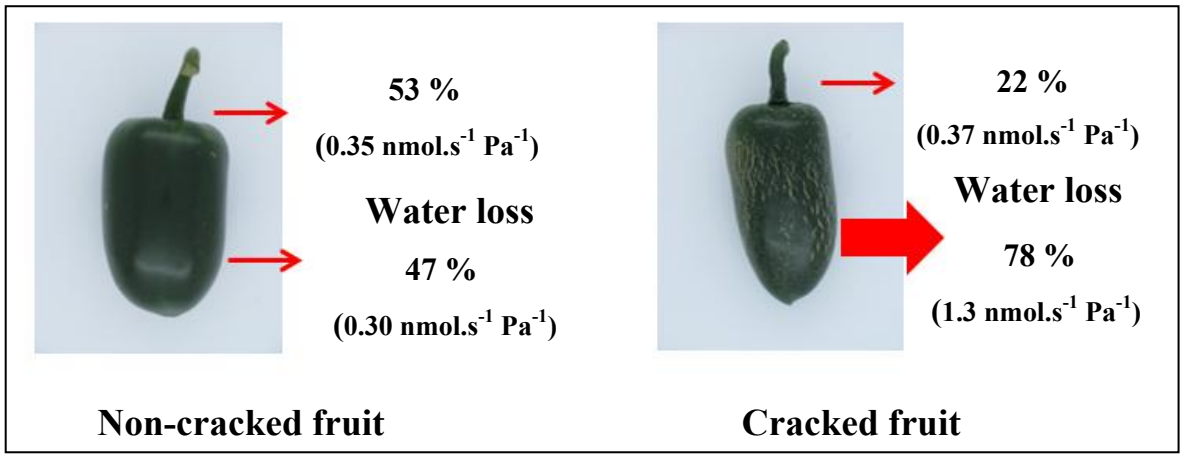


Figure 7.1 Summary of water loss from non-cracked and cracked Jalapeño.

Apart from maturity at harvest which showed that mature Jalapeño (8 and 10 WAFS) fruit showed higher % cracked fruit (> 20 %) than younger fruit (6 WAFS) (Fig. 5.19), growing conditions may relate to cracking incidence in Jalapeño. For example high day temperatures accelerated rapid growth as Jalapeño fruit increased their size rapidly when fruit were set on December - January (Fig. 5.7) and high night absolute humidity (13 - 15 $g.m^3$) on January - March may decrease fruit transpiration which induced fruit to be prone to crack (Table 5.2). Similarly, Aloni et al. (1998) and Moreshet et al. (1999) found higher frequently of cracked fruit from both covered pepper plant and light exposed plant. Therefore monitoring growing conditions (e.g. water supply, temperature and RH) may enable reduction of cracked fruit. Shading the glasshouse during high temperature periods and controlling humidity is suggested to reduce cracking appearance, which has been confirmed in apple (Jackson et al., 1977; Opara et al., 2010).

In addition, chilli or pepper cultivars with a resistance to cracking should be developed. In tomato, thickness of the cuticular membrane in the outer epidermal periclinal walls is higher in cracking resistant cultivars (Matas et al., 2004). Meanwhile Lane et al. (2000) showed that the water uptake (measured by the percentage of fruit weight gained when fruit were immersed in water until they cracked) can be used as a predictor in cherry to classify the susceptibility to cracking as higher water uptake is observed in cracking resistant cultivar. Development of a similar rapid testing method for cracking susceptibility in chillies would be a useful

tool to assist breeders to identify tolerant cultivars. Understanding the factors controlling cracking incidence would also be useful in the breeding programs. In chillies, Johnson & Knavel (1990) confirmed that cracking are controlled by genes which F2 generation (cracking sensitive × cracking resistant cultivars) showed lower cracking score than fruit from sensitive cultivar. Therefore Jalapeño fruit may cross or backcross with selected resistant cultivars to develop desired fruit with lower susceptibility or free from cracking and scarring.

Generally, to delay water loss in fruit and vegetables, wax can be applied to the fruit skin by dipping or spraying (Thirupathi et al., 2006). Previous research demonstrated that coated pepper showed lower weight loss (approx 2 - 22 % depending on coating) than uncoated fruit during storage at 10 °C for 4 weeks (Conforti & Ball, 2002; Conforti & Zinck, 2002). In this research waxing only the calyx and pedicel of cracked Jalapeño reduced the rate of water loss by approximately 10 % during storage at 8 °C. Visible shrivel can be observed when fruit lost more than 5 % water. The waxing only calyx and pedicel of cracked Jalapeño delayed shrivel appearance by only 1 - 2 days (Fig. 4.13), which was not significant difference on storage life of Jalapeño. Waxing the whole fruit should show more impact on delaying water loss in Jalapeño fruit, particularly in cracked fruit in which water is largely lost via the fruit skin (approx 78 %, Fig. 7.1). However, wax should not be applied too thickly to avoid anaerobic respiration which can cause development of off odours (Anon., 2004). In addition, the price of wax and its preparation should be considered as to whether it is worthwhile for value addition in terms of extending the storage life.

Overall, water loss in Jalapeño relates mainly to cracking on the fruit skin, so controlling cracking occurrence on Jalapeño fruit skin is required to reduce water loss during handling and storage. For future work, the effect of day and night temperature and RH should be studied on fruit growth and cracking occurrence in Jalapeño. In addition, developing new chilli cultivars which present less water loss is an alternative way to produce chilli fruit with high quality and long storage life.

7.2.3 Effect of pre-harvest factors on Jalapeño fruit quality

Fruit were harvested at known maturity to determine the effect of fruit maturity on physiological attributes and the interaction of fruit maturity with time of planting, position on plant and crop load.

In the current body of published research there is a number of confounding pre-harvest factors (i.e. position on plant, maturity at harvest and crop load). For example, when fruit are selected at 'mature' and 'immature' stages they may be picked from lower and upper nodes respectively (Osuna-Garcia et al., 1998; Materska & Perucka, 2005; Conforti et al., 2007). To assess these factors independently, in this thesis the studied approach has been carefully applied where either fruit were individually harvested from each node when they reached 6, 8 or 10 WAFS or fruit were strip picked when fruit from the first node reached 6, 8 or 10 WAFS.

7.2.3.1 Time of planting

In this research, Jalapeño were planted sequentially (August, September and October) so that fruit from each planting would represent seasonal replications when harvested at the same maturity stage. Differences between these replications indicated a seasonal effect on chilli growth.

When Jalapeño were harvested at 10 WAFS, fruit planted in August and harvested in December - February weighed 38 g whereas fruit planted later in the year and harvested in February - April weighed 31 - 33 g (Fig.5.6C, F and I). This suggests that growing conditions in August – February are preferable for chilli. Similarly, ascorbic acid concentrations of Jalapeño planted in August and September were higher than in fruit planted in October (Fig. 6.3). In addition, high incidence of blossom end rot (BER) (approx 40 - 50 %) and cracking was found in Jalapeño plants planted in October, which resulted in low fruit numbers (fruit number = 134 fruit compared to 315 fruit in August) (section 5.3.1.2 and Fig. 5.8 and 5.19). Jalapeño plants planted in August apparently had sufficient assimilates to produce fruit with high resistance to disease (i.e. blossom end rot) and cracking while chilli

plants planted later were developing in the height of the summer and had more competition between rapidly maturing fruit and plant growth.

Further evidence for slower maturation of early planted fruit comes from analysis of colour. Jalapeño fruit planted in August tended to change colour more slowly at lower nodes than fruit at lower nodes planted in September and October and even more slowly than fruit at higher nodes in August planting (Fig. 5.13 - 5.15). This presumably relates to growing condition at that time (i.e. cooler temperature and higher RH) during fruit development. The first fruit (at lower nodes) from plants planted in August began to set in October when temperature is lower than fruit which were set later in summer (at higher nodes); this may result in slow growth rate and ripening. For chilli growing in NZ, the germination of Jalapeño in May - June allows the chilli plants to develop in a glasshouse. The first fruit will set in October and continue to grow over summer (from November - April) which is the preferred season for chilli growing.

There was a significant reduction in fruit weight across all nodes with later planting when fruit were harvested 10 WAFS but this was not found for fruit harvested at 6 or 8 WAFS although there was no overall significant difference in weight among time of plantings. Without the careful experimental design used here it would not have been possible to clearly demonstrate the benefit of early planting of chillies.

7.2.3.2 Position on plant, maturity at harvest and crop load

Although effects of pre-harvest factors (position on plant, maturity at harvest and crop load) have been reported previously, there is considerable interaction between them, so interpreting reported experimental results can be challenging. The experimental approach of this thesis allows us to discuss each factor (i.e. position on plant, maturity at harvest and crop load) independently and also interact among factors on a range of fruit attributes.

This research gives insight to the influence of sink/source relationship on fruit growth. Firstly, when all fruit were picked at once when fruit from the first node reached 6, 8 or 10 WAFS, the act of harvest did not lead to a progressive change in

the source and sink relationship. We can distinguish changing related to overall increasing plant maturity as each higher node is progressively younger and captive a plant and fruit development at certain time. This generated data from the range of maturities with the oldest fruit present at the lowest nodes (Fig. 7.2A - C). This approach is not similar to commercial reality as fruit were harvested while fruit and plant are still developing.

In the second approach, which is more commercially relevant, fruit were individually harvested from each node at 6, 8 or 10 WAFS, but now there is potential for the sink and source relationships to be changing as fruit are progressively removed from the plant with time (Fig. 7.2D - F and Fig. 7.3). Due to the effect of time of planting discussed previously, there was a lower number of fruit on plants planted in September and October due to blossom end rot. Therefore the trend for fruit planted in August will be mainly discussed in the further section. Note that this thesis was focused on the first flush of fruit production and allowed only one fruit per node at high crop load (and only one fruit at every fourth node for low crop load).

7.2.3.2.1 Fruit weight and shape

A strip pick experiment showed that Jalapeño grew rapidly in weight, volume and circumference through 6 WAFS, but fruit length reached maximum about 2 WAFS (Fig. 5.2. and 5.3). This indicated that fruit elongated fully during an initial stage of fruit development, and then fruit expansion began to occur (Fig. 5.2B and C). Plants bearing higher fruit number tended to produce smaller fruit, which may be explained by competition amongst the high number of fruit on the plant as well as competition with the maturing vegetative structure (George & Nissen, 1988).

When Jalapeño fruit were harvested individually from 6 - 10 WAFS, fruit weight, length and circumference tended to increase from node 1 to nodes 5 - 8 depending on maturity at harvest, and then fruit weight and size tended to decrease at higher nodes in both high and low crop loads (Fig. 5.6, 5.9 and 7.4). The reduction in size at higher nodes is similar to previous research in cherimoya (González & Cuevas, 2008) and peach (Corelli-Grappadelli & Coston, 1991).

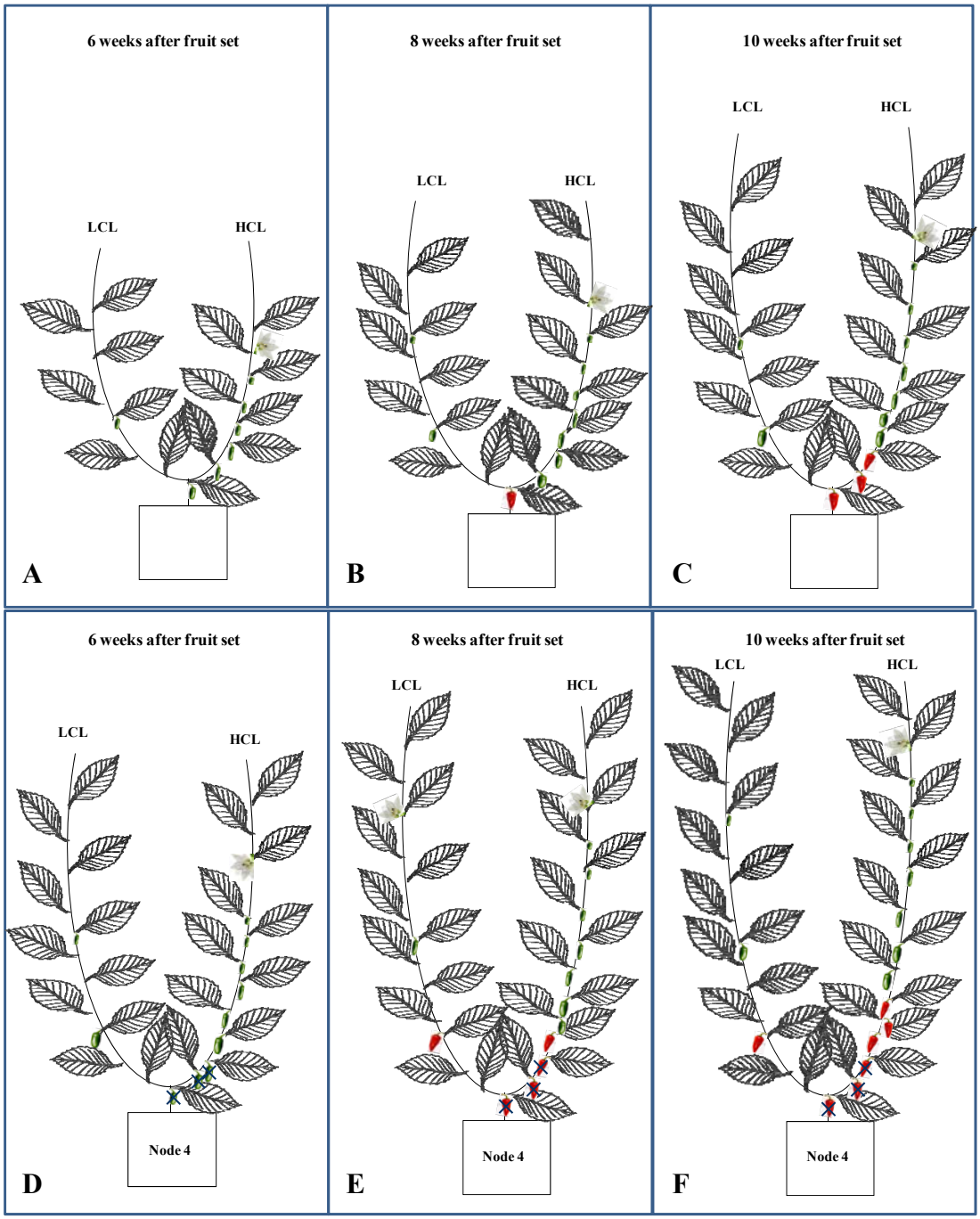


Figure 7.2 Diagrammatic representative of plant structure of Jalapeño; all fruit presented on the plant were harvested when fruit from the first node reached 6, 8 or 10 WAFS (strip pick experiment) (A - C) and fruit were progressively removed when individual fruit reached 6, 8 or 10 WAFS at node 4 (D - F). Fruit from nodes 1 - 3 were already harvested expressing by X. These experiments were conducted from high and low crop load plants. Plants were treated to have two leaders per plant and only one fruit was allowed to grow for each node. In practice, low and high crop load plants were separate but are shown in one plant for convenience.

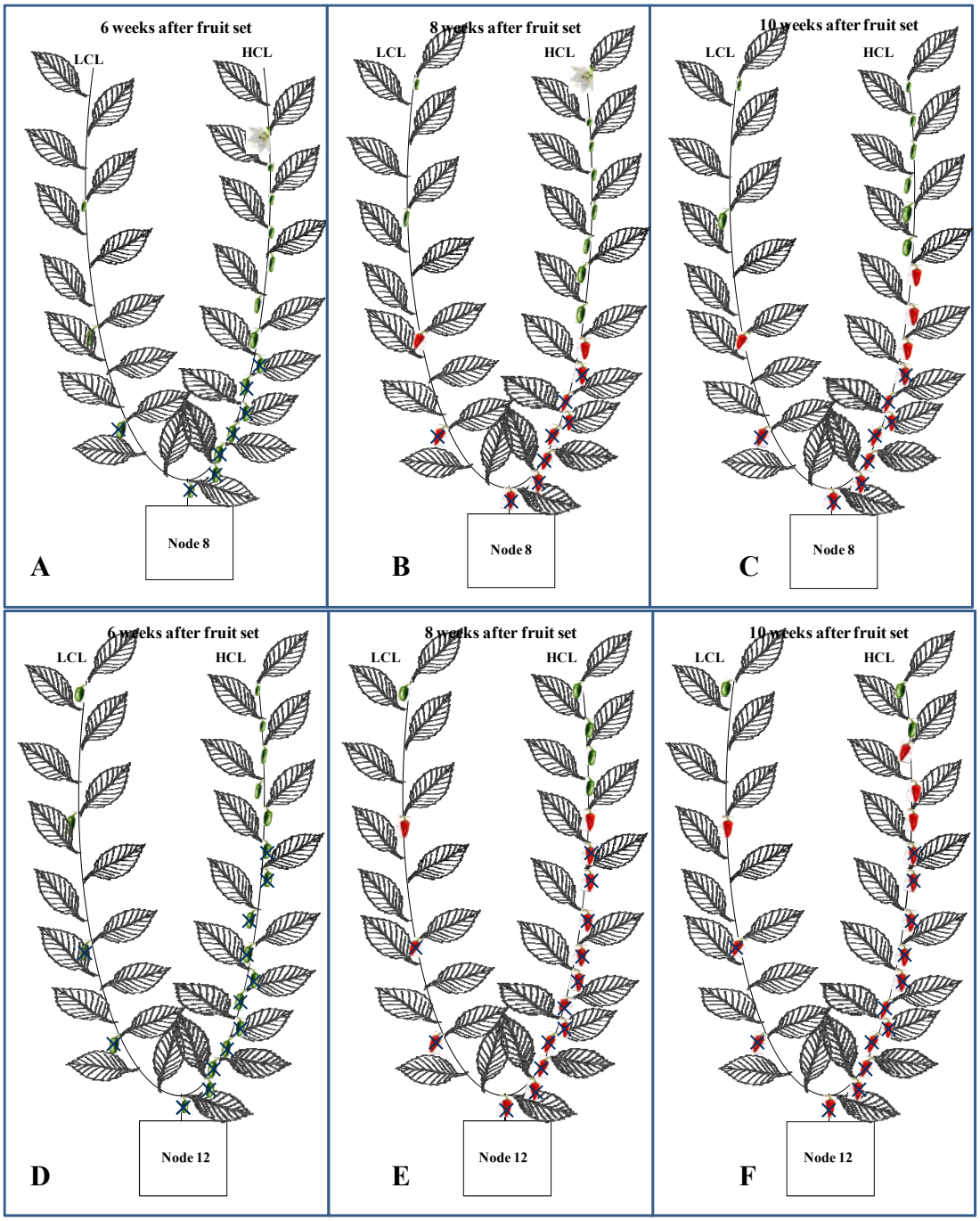


Figure 7.3 Diagrammatic representative of plant structure of Jalapeño which fruit were progressively removed when individual fruit reached 6, 8 or 10 WAFS from low and high crop load plants at node 8 (A - C) or node 12 (D - F). Fruit from nodes 1 – 7 in A – C and nodes 1 – 11 in D – F were harvested expressing by X. Plants were treated to have two leaders per plant and only one fruit was allowed to grow for each node. In practice, low and high crop load plants were separate but are shown in one plant for convenience.

Differences of fruit size at different positions on the plant can be explained by the combination of plant development and the competition for assimilates of fruit present on the plant. Fruit from nodes 1 - 3 were set at the initial stage of plant development therefore the competition for assimilate between plant growth and fruit development may result in small fruit size particularly in fruit harvested at 10 WAFS which had high competition from high number of younger fruit at higher nodes (Fig. 7.2D - F). Once plants were fully developed (with fruit from nodes 5 - 8 reaching full maturity), fruit size increased to a peak at nodes 5 - 8 (Fig. 7.3A - C) which may relate to high assimilate availability from the mature plant even though there was high competition from fruit both below and above the developing fruit. Fruit size tended to be smaller at higher nodes (node 8 onwards) particularly in fruit harvested at 6 WAFS due to the competition from fruit which remained growing at higher nodes, but this effect did not show a high impact in fruit harvested at 8 and 10 WAFS, which may be because fruit at higher nodes may already have reached their final size (Fig. 7.3D - F). However fruit at node 16 showed very small fruit size when harvested at any maturity. Small fruit at higher nodes may be explained by the increasing distance from water and nutrients supplied by roots (George & Nissen, 1988; Corelli-Grappadelli & Coston, 1991; González & Cuevas, 2008).

The competition amongst fruit on the plant can be verified by examining crop load treatments. At the same node, fruit from low crop load plants should be larger than fruit from high crop load plants. Interestingly, Jalapeño fruit at the same node did not differ in fruit weight from high and low crop load plants (Fig. 5.6). Similarly in cherimoya (González & Cuevas, 2008) and peach (Corelli-Grappadelli & Coston, 1991), there was no difference of fruit weight between control and thinned treatments. The lack of response of fruit mass to crop load manipulation may be explained by an abundant availability of resource (e.g. assimilates or nutrients) which was sufficient for fruit development despite the high number of sinks (fruit). That is the demand of assimilates for fruit growth has not exceeded the capacity of the source (Blanke & Holthe, 1997; González & Cuevas, 2008). This may imply that the increasing competition, which reduced fruit size at higher nodes, was more influenced by the competition for water and nutrients supply across the whole plant rather than solely competition with other fruit.

When fruit weight of fruit harvested at 6 - 10 WAFS from each individual node were compared between high and low crop load treatments, fruit harvested at 6 WAFS and planted in August did tend to be larger in low crop load plants (Fig. 5.6A). This difference was not found in fruit harvested at more mature stages. This suggests that the competition between fruit primarily affects the rate of fruit growth rather than the final fruit size and fruit harvested at 8 and 10 WAFS had fully developed and reached their full size.

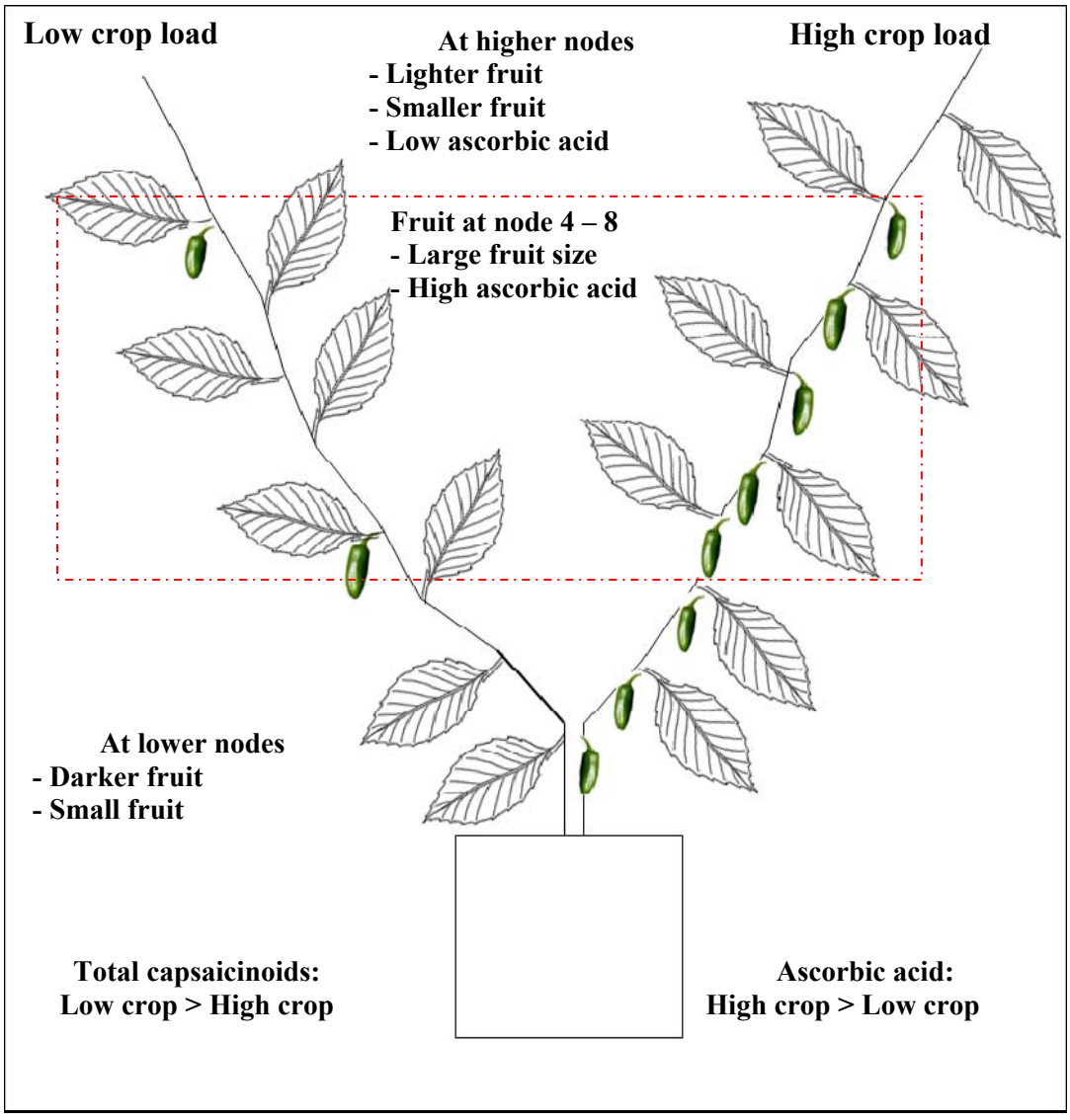


Figure 7.4 Overall conclusions on effects of position on plant and crop load on fruit characteristics and chemical compositions; there were no differences on total capsaicinoids, antioxidant activity (AOX) and total phenolic concentration (TPC) along the plants.

7.2.3.2.2 Colour

In the strip pick experiment, colour of Jalapeño began to change from green to red at 6 WAFS and colour change was delayed when more fruit remained on the plant (Fig. 5.12). Like fruit weight, when plants bear a high number of fruit, fruit developed more slowly due to the competition of fruit and plant growth which limit of assimilates and nutrients or water.

Colour change in Jalapeño from green to red colour during ripening occurred due to a combined contribution of chlorophyll degradation and of carotenoid synthesis (Fig. 5.17 and 5.18). Maturity at harvest is considered as a significant influence on Jalapeño colour (green or red). Jalapeño fruit harvested at 6 and 8 WAFS from lower nodes tended to change colour slower than fruit from higher nodes particularly in fruit planted in August, but this evidence was not found in fruit harvested at 10 WAFS as they were already fully mature and had developed red colour by the time of harvest (Fig. 5.13 - 5.15 and Fig. 7.2D - F). Slow ripening of fruit from early plants was found at lower nodes (Fig. 5.13A - B, Fig. 5.14A - B, Fig. 5.15A - B and Fig. 7.4), but not in fruit planted at other times. The delayed maturation of fruit at lower nodes may be explained as fruit were developing in the cooler conditions therefore effect of position on plant may not show a high impact on colour change in Jalapeño across all seasonal plantings.

This result is identical to earlier findings; time of fruit set during season affects colour changes of Jalapeño. This can be observed in fruit which were set and grown during mid season, which showed 18 - 34 % of fruit harvested at 6 WAFS changing colour at harvest, while more than 80 % of fruit harvested at 8 WAFS changing to a red colour (Table 5.1). By the end of the season fruit remained green when harvested at 6 WAFS and only 10 % of fruit were red at harvest when fruit were harvested at 8 WAFS (Table 3.5). Fruit developing in cooler spring or autumn conditions ripened considerably more slowly than fruit maturing in summer.

Based on these results, the combination of maturity at harvest and growing conditions were major influences on colour changes of Jalapeño. Although actual maturity indices (e.g. weeks after flowering or weeks after fruit set) can indicate the maturity

accurately, it is not practical to measure these properties for commercial application. Since fruit begin to set from the bottom of the plants, harvesting in a sequential order from the lower nodes to the higher nodes would assist in ensuring that harvested fruit have a similar maturity. In addition, to meet the consumer demand for uniform colour fruit, Jalapeño fruit can be harvested at the turning colour stage to ensure fruit will change to red colour after harvest. Pre- or postharvest treatments (such as ethylene application) may be investigated to reduce time to reach fully red stage for fruit maturing in cooler conditions. If consumers want green fruit, fruit should be harvested when they reach a desirable size and should not be left on plant beyond 6 WAFS. However, these fruit may change to red colour during storage at high temperature (i.e. 20 °C), but storage at 8 °C should mean > 90 % remaining green after 21 days of storage. Fruit can be harvested before 6 WAFS, but they will be a yield penalty as fruit keeps increasing in size until 6 - 8 WAFS.

7.2.3.2.3 Ascorbic acid

Vitamin C (both ascorbic acid and dehydroascorbic acid) biosynthesis occurs in mitochondria with the precursors being mannose or galactose (Giovannoni, 2007; Asensi-Fabado & Munne-Bosch, 2010) (Fig. 7.5). In this case, the source strength for ascorbic acid synthesis depends on the sugar source which is produced from photosynthesis. Ascorbic acid concentration increased with maturity stage and peaked at 6 - 7 WAFS in the strip pick experiment (Fig. 6.2B). Unlike fruit weight and colour, plants bearing higher fruit numbers (i.e. 8 and 10 WAFS) tended to have higher ascorbic acid concentration than 6 WAFS plants. Plants from which fruit were harvested when fruit from the first node reached 8 or 10 WAFS were more mature (Fig. 7.2A - C) and may have had a higher photosynthetic production (i.e. more leaf surface area). In addition, high competition for assimilates may somehow enhance vitamin C production. The effect of crop load supports this hypothesis as Jalapeño fruit harvested at the same node showed higher ascorbic concentration in fruit from high crop load plants than fruit from low crop load (Fig. 6.3 and Fig. 7.4). Similarly, Buendia et al. (2008) found higher ascorbic acid in peach from commercial crop load than fruit from a low crop load treatment and attributed high fruit numbers as a positive influence on photosynthetic activity of leaves, which may enhance vitamin C production. In non-green fruit, two hypotheses can be established; sugar is

produced by photosynthesis in leaves and imported to fruit for vitamin C synthesis or vitamin C is synthesized in leaves and imported to fruit (Osuna-Garcia et al., 1998). Either of these can be possible in Jalapeño, but as fruit are green; fruit photosynthesis may also directly contribute to vitamin C production. In order to test the relative contribution of fruit photosynthesis, the adjacent leaf and the rest of the plant, further experiments would be required, for example in selective leaf, removal or bagging of individual fruit.

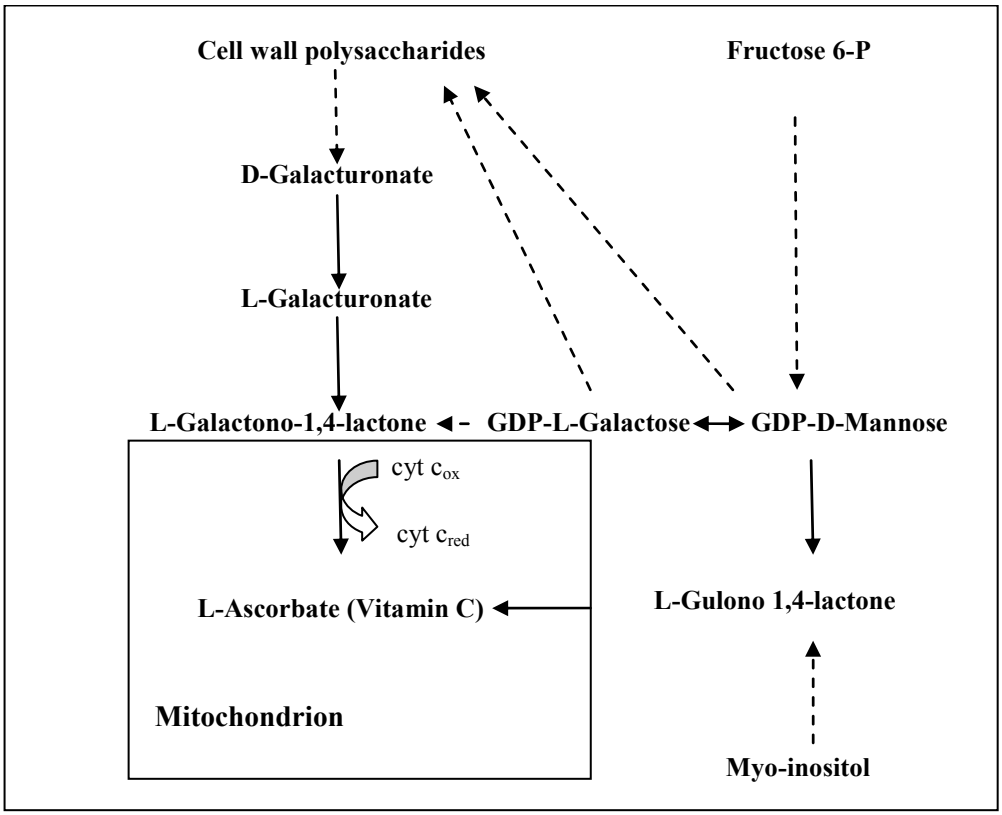


Figure 7.5 The biosynthesis of vitamin C in plants -modified from (Asensi-Fabado & Munne-Bosch, 2010).

Kiwifruit (Remorini et al., 2007) and starfruit (Zabedah et al., 2009) which were exposed to the sunlight showed higher ascorbic acid concentration than shaded fruit but this was not found in this research where assuming that the potential mechanism for fruit at the top and bottom of the plant was different from the amount of exposure to light that they received during growth (Fig. 6.3, 7.2D - F and 7.3). This is similar to Gautier et al. (2005) who also found that distal cherry tomato (fruit 8 - 14) truss had lower vitamin C concentration than the proximal fruit (fruit 1 - 7) and Asrey et

al. (2007) who found that guava fruit from upper canopy, which contained higher total sugar content, showed lower vitamin C (ascorbic acid and dehydroascorbic acid) than fruit from the middle and lower canopies. This indicates that there is no positive correlation between sugar and vitamin C accumulation.

This research seemed to show that high competition within plant enhanced ascorbic acid concentration: Jalapeño fruit from lower nodes had high ascorbic acid concentration, which may be explained by high competition from a higher number of fruit presented on the plant (Fig. 7.2D - F and 7.3A - C). Ascorbic acid concentration tended to drop at higher nodes, which might be caused by the lower number of fruit above the node (Fig. 7.3D - F). However, ascorbic acid of Jalapeño fruit planted in August and harvested at 6 WAFS remained high at node 12 (Fig. 6.3A). This may relate to competition for assimilates between fruit growth and production of secondary metabolites in these older plants on which fruit develop. These particular fruit develop more slowly, so perhaps synthesis of secondary metabolites is accelerated when fruit growth is slow (Plas et al., 1995). Jalapeño fruit at node 12 were relatively small at 6 WAFS (Fig. 5.6A), so possibly fruit from this node may accumulate more ascorbic acid. However, fruit from node 16 showed low ascorbic acid concentration in all maturity stages, which may depend on the distance from nutrients and water sources (George & Nissen, 1988; Corelli-Grappadelli & Coston, 1991; González & Cuevas, 2008). In addition, plant age or season (light and temperature) may have an influential role as late season or upper node mature fruit produced low ascorbic acid concentration. For example fruit planted in October and harvested at 10 WAFS showed lower ascorbic acid even at node 8.

Only ascorbic acid was measured in this research and it has been observed that more than 30 % of total ascorbic acid of Jalapeño was in dehydroascorbic acid form in both fresh and frozen fruit (Tan, 2011). The effect of pre-harvest factors on the ratio of ascorbic acid and dehydroascorbic acid in Jalapeño has not been tested, and all the above conclusions may need to be revisited if dehydroascorbic acid concentration varied with (for example) maturity.

7.2.3.2.4 Total capsaicinoids

In the strip pick experiment, total capsaicinoid concentration increased at the early stage of fruit development (< 3 WAFS) (Fig. 6.5 - 6.6) while there was no significant difference ($P > 0.05$) when fruit were harvested at 6 - 10 WAFS in a PGU glasshouse (Fig. 6.7) indicating that once total capsaicinoid concentration in Jalapeño was established by 4 WAFS then the concentration remained stable throughout maturation. Previously research has reported an increase of capsaicinoid from 4 - 7 WAFS (Sukrasno & Yeoman, 1993; Estrada et al., 1997; Contreras-Padilla & Yahia, 1998; Pandey et al., 2010).

The number of fruit on the plant affected total capsaicinoids as fruit from low crop load plants had higher capsaicinoid concentration than fruit from high crop load plants (Fig. 6.7), which may be explained by high competition for nitrogen sources from high fruit number on the plant. The application of nitrogen to chilli plants has been previously reported to increase capsaicinoid content in both Habanero and Jalapeño fruit (Johnson & Decoteau, 1996; Monforte-Gonzalez et al., 2010), because the amino acids, precursors for capsaicinoid biosynthesis are controlled by the availability of nitrogen. However, total capsaicinoid concentration seemed to be consistent along the plant even in a small fruit at node 16 as there was no significant difference of capsaicinoids in fruit from different nodes (Fig. 6.7). This trend was different from fruit size and ascorbic acid concentration. However, this disagrees with results of Zewdie & Bosland (2000) who showed higher pungency in peppers from the lower or earlier nodes, which they explained as a result of less competition from previously formed fruit while Estrada et al. (2002) found a higher capsaicinoid concentration in fruit from apical zones, which they suggested was a result of light exposure stimulating capsaicinoid accumulation. Accumulation of capsaicinoids is dependent on two pathways; fatty acid metabolism and phenylpropanoid metabolism (Fig. 1.5) therefore light, temperature and nitrogen fertilization may all be essential for capsaicinoid formation.

Capsaicinoids are synthesized by the capsaicin gland in placenta tissue (Iwai et al., 1979; Rowland et al., 1983; Zamski et al., 1987; Thiele et al., 2008; Broderick & Cooke, 2009); therefore capsaicinoid concentration mainly relates to the

development of placenta. In this case, it was assumed that fruit containing a high proportion of placenta were expected to have high capsaicinoid concentration. In this research, although placenta weight was correlated to fruit weight and number of seed of Jalapeño (R^2 = 0.7; P < 0.05 and 0.8; P < 0.05) respectively) (Appendix II) but capsaicinoid concentration did not increase with fruit weight. Therefore the model was developed to explain the variation of total capsaicinoid among fruit in this research which can be explained by the different proportion of tissues (i.e. placenta, pericarp and seed) in the sample (0.5 g) leading to different concentrations of total capsaicinoid (appendix I). This topic will be discussed in section 7.2.4.

7.2.3.2.5 Antioxidant activity (AOX) and total phenolic concentration (TPC)

In chillies and peppers, AOX arises from ascorbic acid, pigments (carotenoids and chlorophyll), phenolic compounds (TPC) such as capsaicinoids, and flavonoids (Howard et al., 2000; Materska & Perucka, 2005; Howard, 2006), therefore changes of AOX should relate to changes of these compounds. AOX and TPC of Jalapeño in a strip pick experiment were reasonably consistent in fruit harvested at different maturities (Fig. 6.9). However, low AOX was found in plants bearing low fruit number while TPC was high indicating that there was no correlation between AOX and TPC (Fig. 6.9, the blue line).

It was expected that Jalapeño fruit containing high ascorbic acid would show high AOX, but this trend was not found as AOX seemed to be consistent along the plant (Fig. 6.10A - C) and only a weak correlation was found between AOX assessed by FRAP and ascorbic acid (Fig. 6.11B). This indicates that ascorbic acid may not contribute significantly to AOX in Jalapeño and it may not be well extracted by ethanol for AOX measurement by FRAP. Similar to AOX, TPC also remained stable along the plant (Fig. 6.10D - F), but no correlation between TPC and capsaicinoids was found. In particular there was no significant difference (P > 0.05) of TPC among pericarp, placenta and seed of Jalapeño (Table 6.9), while capsaicinoid concentration in placenta was 50 times higher than in pericarp (Table 6.5). A similar result was observed by Conforti et al. (2007) who also found no correlation between

capsaicinoids and TPC in pepper. This may indicate that capsaicinoids are not well extracted in the TPC measurement process which should be tested in future work.

Overall, AOX in mature Jalapeño harvested at 8 and 10 WAFS was higher than younger fruit (Fig. 6.10A - C). These results mimic those of Howard et al. (2000), Materska & Perucka (2005), Navarro et al. (2006), Conforti et al. (2007), Deepa et al. (2007) and Sun et al. (2007) who all showed an increase of AOX in chillies and peppers during maturation. Unlike AOX, TPC remained stable among fruit harvested from 6 - 10 WAFS (Fig. 6.10D - F). Earlier reports showed either increase (Lee et al., 1995; Howard et al., 2000; Deepa et al., 2007) or decrease (Estrada et al., 2000; Materska & Perucka, 2005; Navarro et al., 2006; Conforti et al., 2007; Deepa et al., 2007; Menichini et al., 2009) of TPC during ripening. These differences may be compromised by different chemical compositions in each chilli variety and different extraction solvents.

One explanation for the weak correlation between AOX and TPC and AOX and ascorbic acid may be that chlorophyll and carotenoids show antioxidant activity and may be extracted by ethanol (Lichtenthaler, 1987). Colour was indeed observed in these extracts. It may be possible that a single extraction of 50 % or acidified ethanol may not extract these compounds very well (as colour still remained in the sample). Interestingly, Deepa et al. (2007) did not observed AOX (by FRAP) in carotenoids extracted from pepper with acetone. From this research AOX in Jalapeño may come from a combination of compounds that can be extracted by ethanol.

7.2.4 The accuracy of capsaicinoid measurement

The model which was developed from capsaicinoid concentration in each individual tissue showed that the proportion of placenta in each extract highly influences total capsaicinoid concentration of the sample (Appendix I). This means that if a large proportion of placenta is presented in the extracted sample (0.5 g), it will result in high capsaicinoid concentration. This finding partially explains the large variability of total capsaicinoid concentration (150 - 2000 $\mu g.gDW^{-1}$) across the whole crop.
Although some authors such as Collin et al. (1995) have noted that the need to use larger sample of chilli material to reduce the problem of variability in capsaicinoid

measurement, no one has previously described the important of including specific proportions of placenta, seed and pericarp in the sample. Most research follows (with some modifications) Collins et al. (1995) who measured capsaicinoids by HPLC with fluorescence detector from dried whole fruit sample (Estrada et al., 1997; Contreras-Padilla & Yahia, 1998; Estrada et al., 1999a; Estrada et al., 1999b; Estrada et al., 2000; Zewdie & Bosland, 2000; Gnayfeed et al., 2001; Estrada et al., 2002; Pandey et al., 2010). However in other works, placenta and seed were removed before extraction from the dried pericarp (Kozukue et al., 2005; Materska & Perucka, 2005) and in theory these data should be more consistent. Some groups measured from fresh samples (Kirschbaum-Titze et al., 2002a; Kirschbaum-Titze et al., 2002b; Deepa et al., 2007). The sub-sampling pattern is more affected in hot chilli varieties than in mild varieties, which was not shown such large differences among tissues. In addition, in this research, total capsaicinoid concentration was measured from one half of Jalapeño fruit (another half was frozen for vitamin C and AOX measurement) which may contribute to variability since an unequal proportion of placenta may be found in each half of fruit. This method followed Contreras-Padilla & Yahia (1998) who measured capsaicinoids in one half of chilli and froze another half for enzyme analysis. Overall, the chilli variety and the eaten part should be considered for the accuracy of capsaicinoid measurement, for example for whole fruit consumption there is no problem for mild variety to measure capsaicinoids because there would be less difference of capsaicinoid concentration among the tissues. While for medium or hot varieties, further study should be considered on the difference of capsaicinoid concentration in each tissue and the acceptable amount of sample which is required for a desired precision of measurement (sample size ranged from 1 - 10 g). Kirschbaum et al. (2002) found that combining five fruit replicates resulted in an acceptable precision for capsaicinoid measurement (for the whole fruit). Since some people consume pericarp only, measuring capsaicinoids only in the pericarp could also be considered.

It is difficult to compare the capsaicinoid concentration to previous publications as they mostly studied different varieties or cultivars. Previous research also showed inconsistencies of capsaicinoids observed in each chilli species (Zewdie & Bosland, 2001) even in the same variety, capsaicinoid concentration also varied with

environmental factors (Estrada et al., 1999a; Kirschbaum-Titze et al., 2002b). The model developed in this research (Appendix I) confirmed that the variability on capsaicinoid concentration came from the varying proportions of each tissue in the sample. An error can be introduced from the amount of placenta contained in sample suggesting the hotter the chilli, the larger error there will be in capsaicinoid concentration. Overall, it is difficult to measure capsaicinoids precisely in chilli as there is inevitable variability in measurement.

7.2.5 Fruit to fruit and plant to plant variation

The variation in Jalapeño attributes was found in the commercial glasshouse although fruit were harvested at known maturity stages. For example the variation of capsaicin concentration from fruit harvested at 7 WAFS from the same plant showed capsaicin concentration between 280 and 610 $\mu g.gDW^{-1}$ (Table 6.2). Even in the experiment conducted by growing Jalapeño (F1 seeds) in the PGU glasshouse and harvesting fruit at a known maturity defined by weeks after fruit set, considerable variation in fruit attributes still remained. For example, ascorbic acid concentration of Jalapeño fruit from low crop load treatment planted in August and harvested at 6 WAFS from node 8 varied from 40 - 1240 $\mu g.gFW^{-1}$. Variation can be observed even in fruit from the same plant. For example, capsaicinoids of fruit harvested from one plant at the same node (node 4) but different leaders were 19.8 and 42.8 g while fruit from node 5 were 51.2 and 50.5 g.

Open or cross-pollination of different plants or cultivars may affect variation on fruit quality. Generally, self-pollination can occur when chilli flowers are moved by wind. Pollen can be transferred by birds, bees, butterflies, etc. to fertilize in a flower or between flowers causing both self-pollination and open-pollination (Andrews, 1984). Effects of variable pollination have been found in other fruit such as plum (Hassan et al., 2007), blueberry (Bieniasz, 2007) and apple (Stino et al., 2002) in terms of fruit size, shape, firmness and/or chemical contents. Therefore cross pollination by other cultivars (e.g. growing in the field or growing many cultivars in the same glasshouse) should be avoided to reduce the variation. However, in this research only Jalapeño were planted in the PGU glasshouse, therefore cross pollination to other varieties should not be a major problem. In addition, a positive correlation between

fruit size and seed number was found in sweet pepper, which indicates that a procedure to ensure good pollination, such as hand pollination may increase fruit weight (Shipp et al., 1994). However Marcelis & Baan Hofman-Eijer (1997) did not find a weight increase of pepper with supplementary pollination, although their naturally pollinated fruit already had high seed numbers. In this research, a correlation between seed number and fruit weight was found in Jalapeño from a PGU glasshouse (R^2 = 0.5) (Appendix II), so an additional pollination may have been beneficial to maximise fruit size. However, Bakker (1989) did not found the relationship between seed number and fruit weight of individual pepper fruit which may explain that there are other factors relating on fruit weight.

To reduce fruit to fruit variation, it is very important to work with the F1 seeds that produce plants with uniform fruit. It was noteworthy that despite that this was the case in this research; there were occasional plants which produced very small fruit. New cultivars which can deliver uniform fruit characteristics should be developed to reduce variation among plants and fruit.

7.3 Conclusion

The achievements of this research are to determine the optimum storage temperature of chilli, to define factors affecting water loss in chillies and to develop understanding of pre-harvest factors which contributed to variability on fruit size, colour and phytochemical compounds at harvest to deliver consistent high quality fruit.

Habanero and Jalapeño can be stored at 8 °C for 4 - 5 weeks as fruit show low respiration rate and delayed loss of firmness without displaying chilling injury symptoms during or after storage at low temperature. Meanwhile Paprika requires warmer storage temperatures because a dramatic loss of firmness was found during storage at 8 °C, although overall appearance was still marketable.

Water loss in Jalapeño, known as a major problem for postharvest quality, occurred through fruit skin, calyx and pedicel. Cracking on the fruit skin was demonstrated as a significant path of water loss. A mathematical model was developed to describe the

factors affecting water loss, which can be used to predict water loss in Jalapeño during storage at different conditions.

Effects of pre-harvest factors such as time of planting, position on plant, maturity at harvest and crop load were shown to exert a major influence on Jalapeño quality (i.e. fruit size, colour and phytochemical composition). In brief, differences in fruit size, colour and ascorbic acid were found in fruit from different time of plantings which related to growing conditions affecting plant and fruit growth. In addition, high incidence of cracking was found in fruit planted later. Therefore, the suggestion from this work is to plant chilli plants at a single time early in the season to obtain fruit with similar characteristics. Fruit size and ascorbic acid concentration of Jalapeño harvested at the same maturity varied with the position on the plant. Different fruit size can be explained by the competition between plant and fruit growth and also the distance from nutrients and water supply while ascorbic acid accumulation in fruit was related to high competition between fruit on the plant and also plant age. For colour, maturity had a major effect on colour at harvest but colour change was also influenced by position on plant and growing conditions. However, capsaicinoids, antioxidant activity and total phenolic concentration seemed to be more consistent along the plant. Total capsaicinoid measurement can be affected by the sub-sampling error influencing by the proportion of each individual tissue (i.e. pericarp, placenta and seed) contained in the sample. Meanwhile a weak correlation was found between AOX and TPC or AOX and ascorbic acid indicating that ascorbic acid or TPC was not a major contributor of the AOX in Jalapeño.

Thinning leaders during production is essential for decreasing the risk from plant collapse due to weight. Based on this research, fruit thinning is not needed as there is no benefit to generate a low crop load. Chilli growers should aim to produce as high yield as possible that does not cause damage to the plant (due to weight) with the knowledge that striving for high yield per plant has little influence on fruit quality attributes. It is important to note that this conclusion is applied to plants grown with two leaders per plant with a single fruit per node and only the first flush of fruit production.

From the observed results, to produce larger Jalapeño fruit with high level of health beneficial compounds such as ascorbic acid, Jalapeño plants should be pruned to not more than 12 nodes. However, fruit yield from limited size Jalapeño plants is likely to be lower than yield from normal height plants (~ 16 - 20 nodes in this research), but these fruit may have higher marketability due to larger fruit size and with high level of beneficial compounds. An alternative strategy would be to allow plants to grow normally to maintain fruit yield but fruit from the middle nodes could be sold separately at a higher price.

In future research, the effect of more leaders, a higher number of fruit per node and the second flush of fruit production should be studied to understand the real condition in a commercial glasshouse. It would be helpful to seek input from a chilli grower to define the differences between the research and commercial glasshouses in terms of production of consistent quality fruit with high health benefits. In addition, Jalapeño fruit obtained from pre-harvest controlled experiment should be studied after harvest. They may well be significant differences in storage potential - for example, do fruit harvested from node 4 which contain high ascorbic acid continue to have high ascorbic acid after storage, and does that ascorbic acid contribute to fruit storability?.

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Appendix I

Estimation of Sampling Error Contribution to Capsaicinoid Content Determination for Jalapeño.

A brief methodology description

Dr Andrew East

The experimental work presented in Figure 6.6 and 6.7 identified that a large amount of variability was being observed in the measurement of capsaicinoid concentration, which could not be attributed to measurement error. However, there is a large spatial distribution of capsaicinoids within a Jalapeño (Tables 6.5 - 6.6) in which the range of concentration differences are approximately 50-fold. This large spatial concentration difference introduces the possibility that in sampling from the powdered tissue of the Jalapeño, the composition of the random set of particles that makes up the desired sample size (in mass) may have a significant influence on the capsaicinoid in the sample and hence the capsaicinoid measurement. This appendix briefly discusses how this possible sampling error can be investigated through the use of a mathematical technique known as Monte-Carlo simulations.

Monte-Carlo simulations are a methodology of determining a result through repeated sampling from populations of known variability. In this case in creating a sample of 0.5 g for subsequent capsaicinoid analysis, particles which may have originated from pericarp, placenta or seed are obtained. In addition, each particle comes from a distribution of potential particle sizes as influenced by the powder preparation (particle formation) process. By mathematically sampling individual particles to create a 0.5 g sample a prediction of the average capsaicinoid concentration of the entire sample can be made.

Monte-Carlo simulations become a powerful tool when sampling error is required to be estimated. As a mathematical process, simulation of creation of numerous samples (usually 1000 as a minimum) can be constructed in limited time, resulting in a population of potential results which can be interpreted as the sampling error. In this case 1000 potential samples can be simulated from the same fruit resulting in

quantification of the sampling error caused by the random mix of particles chosen to create the 0.5 g sample which is later analysed for capsaicinoid concentration.

In conducting the Monte-Carlo sampling error estimation, we assumed that an "average" 40 g Jalapeño was being measured. This average Jalapeño has the following attributes (Table A1).

Table A1. Component attributes of an average Jalapeño.

Component	Proportion of Mass (%)	Average Capsaicinoid Concentration (μg.g DW^{-1})	Density (g $DW.m^{-3}$)
Pericarp	87.2 %	120	1200
Placenta	10.4 %	6000	1040
Seed	2.3 %	900	720

When sampling from this Jalapeño, we assumed that the Jalapeño is perfectly halved (i.e. the same amount of seeds etc. are in each half). At this point the fruit is oven-dried and then turned into a powder (Section 2.6.4). Given that the physical nature of the seeds, placenta and pericarp are different it is entirely possible that each of these components is broken up in a different manner during the grinding process resulting in a different size distribution. In order to investigate this possibility, sample of each of the component only (e.g. seed only) were created, with each of these component samples put through the same grinding process. The size distribution of the resulting powders were analysed by Rattanawan Jansasithorn by using Mastersizer. The size distribution is measured based on average diameter and hence conversion to mass requires an assumption of spherical shape and conversion of the resulting volume to mass with the use of the knowledge of particle density (Table A1). Particle density (g $DW.m^{-3}$) was measured for each component.

The size distribution data collected indicates that for a 10 second process each Jalapeño component was broken down to a wide distribution of particles ranging from approximately 10-1000 μm (Fig. A1). Most notable, the seed component was

observed to be more difficult to break down than the other components of the Jalapeño.

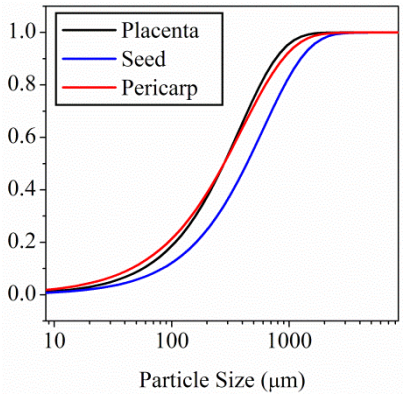

Figure A1 Cumulative distribution of particle size (average diameter) for each component of Jalapeño after grinding process.

Given this pool of information, then the Monte-Carlo simulation can begin. A Monte-Carlo simulation is a mathematical experiment whereby effects of sampling from a population of known variability on a final result can be analysed. The power of the mathematical experiment revolves around the ability to rapidly conduct sampling from the same population and hence estimate the sampling error itself.

In this case the Monte-Carlo simulation was established to create a 0.5 g sample of Jalapeño flesh as it was constructed by a mix of particles. This was conducted by individually collecting particles until a 0.5 g sample was established (Figure A2). Each of the particles will have an inherent capsaicinoid concentration based on the source of the material (pericarp, placenta, and seed).

In this Monte-Carlo simulation two "decisions" are required to be made by random sampling (Figure A2) through the use of random number generation. The first random number generated determines the component of the Jalapeño (seed, placenta or pericarp) which is randomly selected based on the proportion of these materials in the fresh product. The second random number generated determines the size of particle acquired based on the particle size distribution that is created in the grinding process and information of the Jalapeño component as informed by the fruit random number.

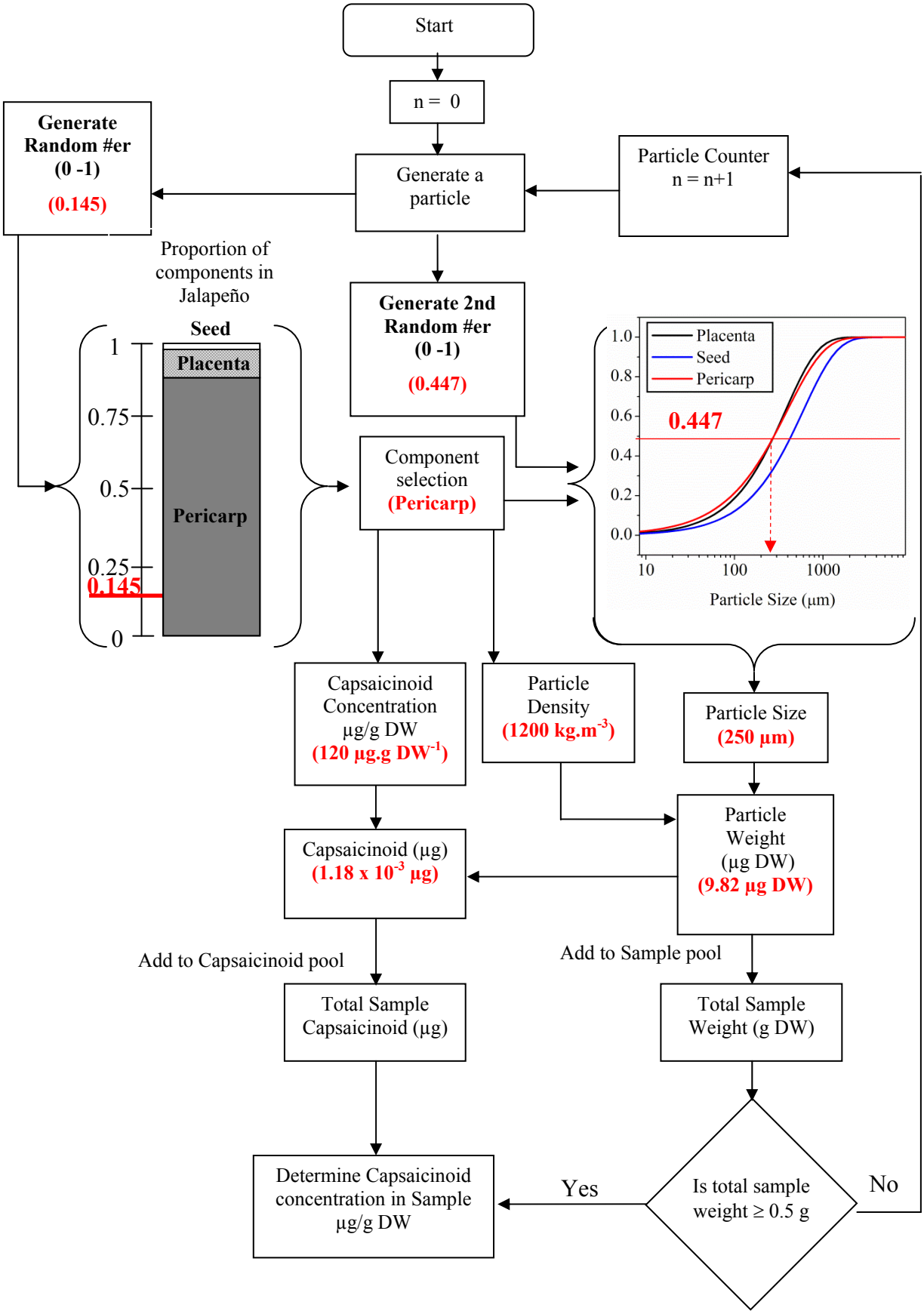

Figure A2 Flow diagram for the Monte-Carlo simulation of creating a 0.5 g sample of particles from a powdered Jalapeño source. Numerals and lines in red, provide an example of how the simulation of the creation of a single particle is conducted.

Once these 2 processes of information (component and size) are generated using the defined properties for the components and the assumption of spherical particles, a single particle are generated which contributes to the sample mass and content of capsaicinoid. Given that in practice a 0.5 g was used this process continues to generate new particles until the summation of the particles results in a 0.5 g sample. Once a 0.5 g sample has been generated the capsaicinoid concentration is the summation of the capsaicinoid in each particle while the concentration becomes the content divided by the sample weight. The particle counter allows the total number of particles contributing to the 0.5 g sample, which in itself will be variable due to sampling. In this case between 1760 and 3000 particles were required to construct a 0.5 g sample.

The above process will provide a result for a single sample. Conducting this process 1000 times results in 1000 potential samples (and associated capsaicinoid concentrations). The distribution of the potential capsaicinoid content from a single fruit is provided in Figure A3.

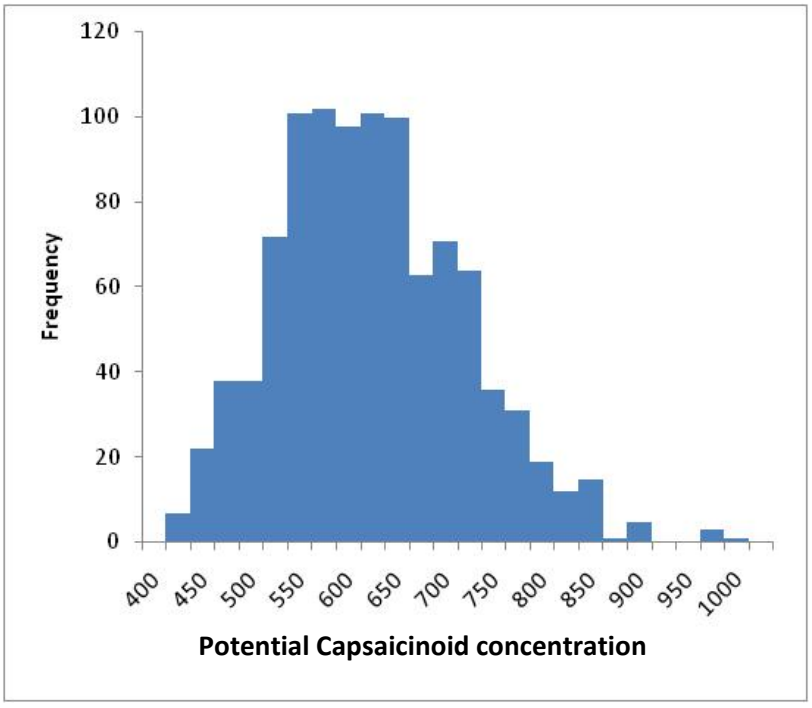

Figure A3 Distribution of potential capsaicinoid concentration caused by sampling error.

Appendix II

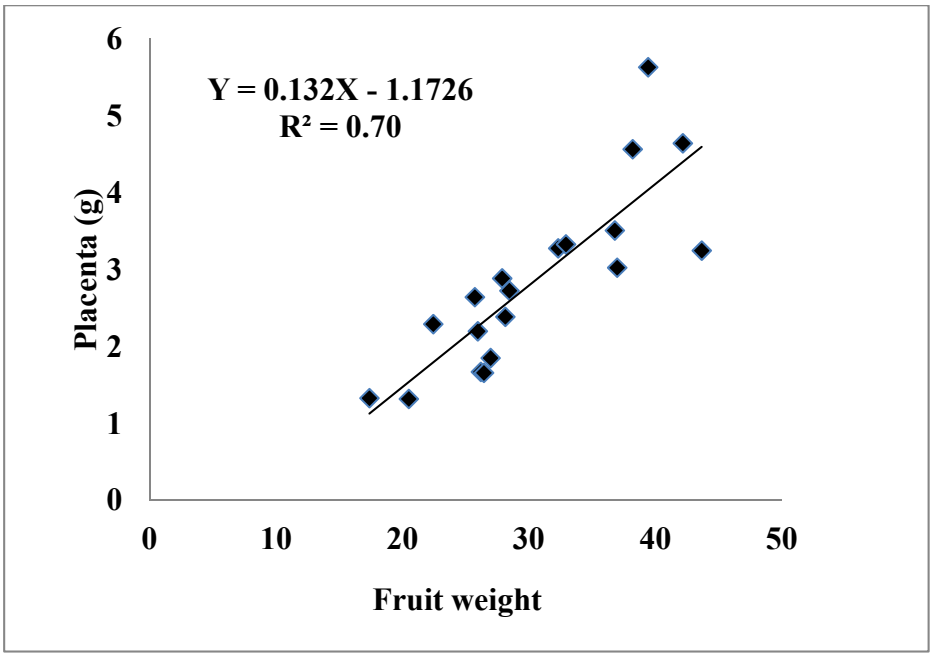

Figure A4 The correlation between placenta weight and fruit weight of Jalapeño.

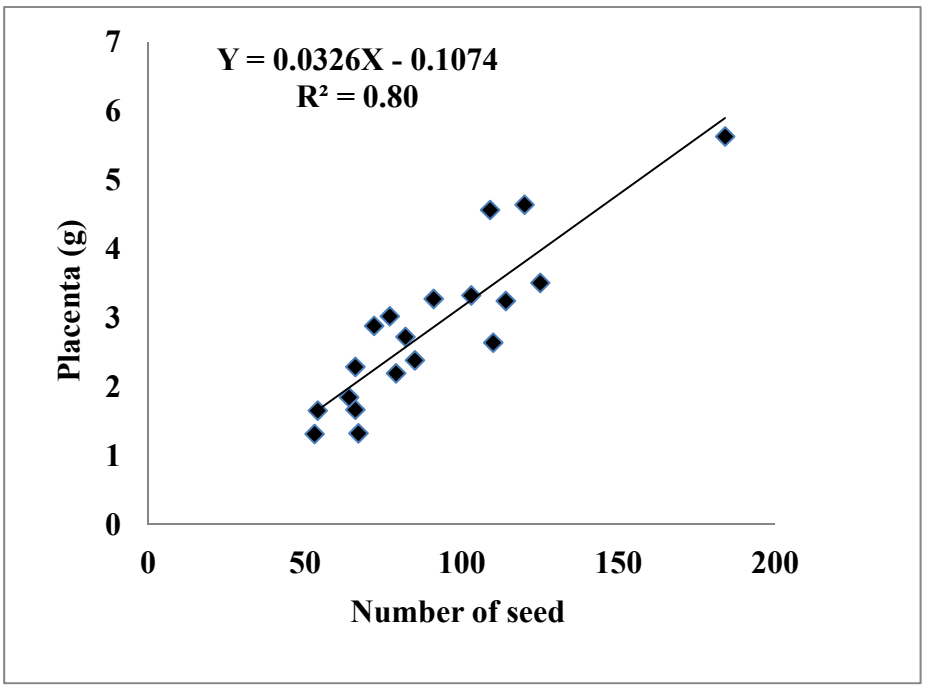

Figure A5 The correlation between placenta weight and seed number of Jalapeño.

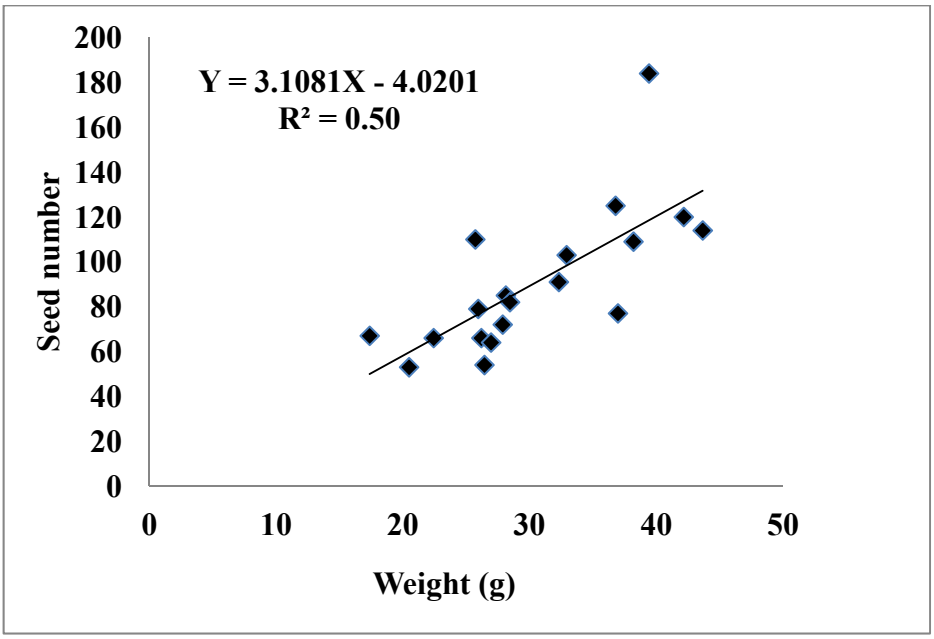

Figure A6 The correlation between seed number and fruit weight of Jalapeño.