Improving the chilli and paprika spice production system from field to processing

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Capsicum annuum L., from red fruit to spice powder.

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Abstract

In recent times, there has been a significant increase in the consumption of capsicum spices in Australia, mostly from imported products. There is an opportunity for the development of a capsicum spice industry in Australia if growers are able to supply products of consistent colour and pungency, and if production costs can be reduced through machine harvesting. However, knowledge of production, harvesting, and processing conditions is limited. Therefore, this thesis investigated the most critical control points of this spice production system to maximise yield and quality, and to allow once-over machine harvesting. These points were identified to be growing conditions (water stress and air temperature), harvesting, post-harvest handling and processing for a given, suitable cultivar.

Two cultivars were selected, one was the sweet 'PS72285' paprika and the other the pungent 'Caysan SPS705' cayenne chilli. Both types belong to *Capsicum annuum* L. They also have a similar upright growth habit and single fruit per node as preferred for machine harvesting.

Experiments were conducted from late 1996 to early 2000 with container-grown capsicums located either in a shadehouse or glasshouse, depending on experimental requirements at the Waite Campus, Adelaide University.

To determine the effect of air temperature on yield and spice quality, plants were grown under two different regimes: a lower temperature range of 22/17°C (day/night) was compared to 30/25°C. Neither fruit yield components nor colour content were affected by temperature. Dry weight of only the vegetative parts of mature paprika plants was reduced by 41% at the higher air temperature, although this was not observed for chilli plants. Internodal length was very short for both cultivars at the lower air temperature, which also reduced pungency of chilli by 29%, but not for paprika.

The effect of water stress was investigated by subjecting the plants to irrigation frequencies of 2, 4 or 6 days. Treatments were applied after plants had obtained the maximum fruit set at the fourth node until harvest. Irrigating every 4 or 6 days induced water stress as shown

by a marked reduction in both leaf water potential and stomatal conductance. This resulted in a reduction of total fruit yield and plant dry weight by 40 to 50% for both cultivars compared to irrigating every 2 days. A lower fruit number induced by water stress (up to 50% reduction compared to irrigating every 2 days) was the major component responsible for the yield reduction for both cultivars. However, water stress had no effect on individual fresh fruit weight. Neither colour nor pungency of the spice powder was affected by irrigation frequency.

The effect of water stress was further investigated using a split root system to apply 'Partial Rootzone Drying' (PRD). The PRD technique maintained one half of the root system in a drying state, while the other half was irrigated. PRD induced some of the symptoms of water stress, as shown by a small reduction in stomatal conductance, but without any concomitant change in leaf water potential. PRD had no effect on marketable fruit yield, colour or pungency. However, fruit colour development was slightly delayed. The PRD technique could supply the total water needs of the container-grown *Capsicum* plant while reducing water usage.

To increase the efficiency for once-over machine harvest, plants were treated with ethephon (ethylene-releasing ripening agent) with the aim of synchronising maturation. Ethephon solutions of 0, 1000, 3000 or 5000 $\mu L.L^{-1}$ were applied once as foliar applications. Ethephon accelerated fruit maturation of both cultivars by increasing the percentage (by number) of red marketable fruit for chillies and decreasing the percentage of green paprika fruit. The intensity of extractable red colour in chilli fruit was increased by 16% for 1000 $\mu L.L^{-1}$ ethephon, while pungency increased by 46% and 48% for 1000 and 3000 $\mu L.L^{-1}$ ethephon, respectively. However, red marketable fruit yield, measured as fresh and dry weight, decreased in chillies due to a large number of abscised fruit. Paprika quality and yield were unaffected, as fruit were harvested before much abscission took place. Ethephon also induced defoliation and fruit skin damage.

To increase the percentage of red marketable fruit after machine harvesting, post-harvest ripening of fruit after ethylene application was examined. Fruit at seven different ripeness stages; light green, deep green, breaker (slight colouration), breaker red (some red colour), bright red (100% red), deep red and succulent, and deep red and partially dried were harvested on the same day. Fruit of each stage were then allowed to ripen at room temperature with or without the addition of 100 $\mu L.L^{-1}$ ethylene in the storage atmosphere. Ethylene treatment had no effect on colour development associated with fruit ripening for both cultivars. Paprika fruit underwent a respiratory climacteric as long as they were attached to the plant, but this was not distinct for chilli fruit. However, once harvested fruits were left to ripen, the climacteric behaviour was absent for both cultivars. This suggested that *Capsicum* fruit ripening did behave differently on and off the plants, and may also vary with cultivar. However, green or deep-green harvested fruits failed to fully colour, while fruit that were harvested at or near breaker stage completed their ripening, visually developing a fully red colouration within six to seven days for both cultivars. Only fruit that were of deep red colour and partially dry at harvest were consistently of the highest colour intensity, 194 colour units based on the American Spice Trade Association (ASTA) for paprika powder and 120 ASTA colour units for chilli powder. Pungency did not change between ripeness stages for chilli, ranging from 16,000 to 22,000 Scoville heat units (SHU).

The influences of drying conditions on colour were investigated only for paprika. A drying temperature at or below 60°C prevented browning and red pigment loss. However, drying was slow, whether using a low humidity heat-pump drier at 40°C or a step-wise drying process using a hot air drier at 60°C for six hours followed by 40°C until products were dried.

To decrease drying time, dips in drying oil were applied to fruit before drying, and compared to cut or uncut fruit. The dips were made up of an oil (10% weight by volume) containing mostly ethyl esters of $C_{14}/C_{16}/C_{18}$ fatty acids, and emulsified in aqueous potassium carbonate (2.5% volume by volume). Drying temperature was continuously set at 45°C in an industrial air drier. Both oil dips and cutting accelerated drying. Hot oil dipping (10% oil + 2.5% K_2CO_3, 65°C) of whole fruit halved the drying period compared to untreated fruit, but cutting fruit into small sections accelerated drying most, reducing

drying times by 81%. Combining oil with fine cutting did not further accelerate drying. Oil dips changed the structure of the surface wax, increasing water permeability; cutting opened alternative routes to the cuticle for water movement. Colour intensity and stability increased slightly for cut fruit, but was unaffected by oil. The aroma of water-dipped samples was fresh and hay-like, while that of oil-dipped samples was slightly nutty. These results can be utilised to improve the production, harvesting and processing system for chilli or paprika spice production, resulting in good crop yield and quality.

Statement

This thesis contains no material which has been accepted for an award of any degree or diploma in any University and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text.

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M. Krajayklang
10/05/01

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List of Abbreviations

ABA	abscisic acid
AOAC	Association of Official Analytical Chemist
ASTA	American Spice Trade Association
a*	green to red colour components
b*	yellow to blue colour components
°C	degrees Celsius
C	capsaicin
C*	Chroma
C_2H_4	ethylene
$Ca(OH)_2$	calcium hydroxide
CIE	Commission Internationale de l'Eclaire
CO_2	carbon dioxide
cm	centimeter
Ct	control
2D, 4D or 6D	irrigation every 2 days, 4 days or 6days
DAS	number of days after sowing
DAT	number of days after transplanting
DHC	dihydrocapsaicin
e.g.	for example
g	gram
gs	stomatal conductance of leaves ($mmol.m^{-2}.s^{-1}$)
h	hour
h°	hue angle
HC	homocapsaicin
HCl	hydrochloric acid
HDHC	homodihydrocapsaicin
HPLC	high pressure liquid chromatography
i.e.	that is
K_2CO_3	potassium carbonate
kg	kilogram
KOH	potassium hydroxide

kPa	kilopascal
kW	kilowatt
L	left
L*	lightness coefficient
LSD	Least Significant Differences
m	meter
M	Molar concentration
max/min	maximum and minimum
1-MCP	1-methylcyclopropene
MC	moisture content
mg	milligram
min	minute
mL	milliliter
mm	millimeter
mol	molecule
MPa	megapascal
NDHC	nordihydrohocapsaicin
nm	nanometer
ns	not significant
PRD	partial rootzone drying
R	right
RDI	regulated deficit irrigation
RH	relative humidity
SE	standard error of the mean
SEM	Scanning Electron Microscopy
SHU	Scoville heat unit
SICU	Standard International Colour Unit
SWC	soil water content (volumetric: %)
T	treated
TDR	time domain reflectometry
UC	University of California potting mix (Waite Version)
v/v	volume by volume
w_f	final weight
w_t	weight at the time of drying

WUE	water-use efficiency
w/v	weight by volume
Ψ_L	leaf water potential (MPa)
μ	micro
#	number
%	percent
Ψ_{soil}	soil water potential (kPa)

Chapter One

General introduction and aims

1.1 Introduction

With changing eating patterns in Australia, a marked increase has been observed in the consumption of pungent capsicums in Asian ethnic food products and Mexican cuisine. Imports of dried chillies (pungent capsicums) almost doubled to A$ 2.2 million over the 4 years to 1994 (Miles, 1994), but the unreliable quality of imported materials causes difficulties for processors in sourcing a consistent product (Murison, 1995). An opportunity for developing the capsicum spice industry exists if Australian growers are able to supply products with consistent quality.

Capsicum spices are produced from dried red fruit made from any *Capsicum* species, and can be sold in flakes, crushed or powdered. Spices are classified based on their colour and pungency (hotness) depending on their fruit type. Therefore, colour and pungency are both important for evaluating quality and for classification of these spices in the spice industry.

For economical *Capsicum* spice production, it is necessary to select the right cultivars that allow machine harvesting (Somos, 1984; Philp and Burne, 1988) with a large pendulous single fruit born per node and showing upright growth habit. This reduces labour cost for spice production compared to traditional hand harvesting which requires intensive time and labour. Several factors have been shown to influence fruit colour and pungency levels throughout the spice production system. Selection of a desirable cultivar alone does not ensure good quality of final spice products. It is therefore important to identify the other critical factors affecting spice quality, and to appropriately manage these factors for spice production. This information is limited for Australian spice producers.

According to the spice production system, factors that show a high potential for affecting spice yield and quality can be identified and broken down into the following five categories (Figure 1.1); cultivar selection, growing conditions (including growth temperature and water management that were investigated in this thesis), harvesting, post-

harvest ripening and processing. These will be the main factors to be discussed and investigated in this study.

1.2 Research aims

The overall aim of this research was to better understand pre- and post-harvest factors affecting yield, colour and/or pungency of *Capsicum* spices for the selected cultivars (*Capsicum annuum* L.) available in Australia, in order to develop suitable procedures for growing, harvesting and post-harvest handling for the *Capsicum* spice industry.

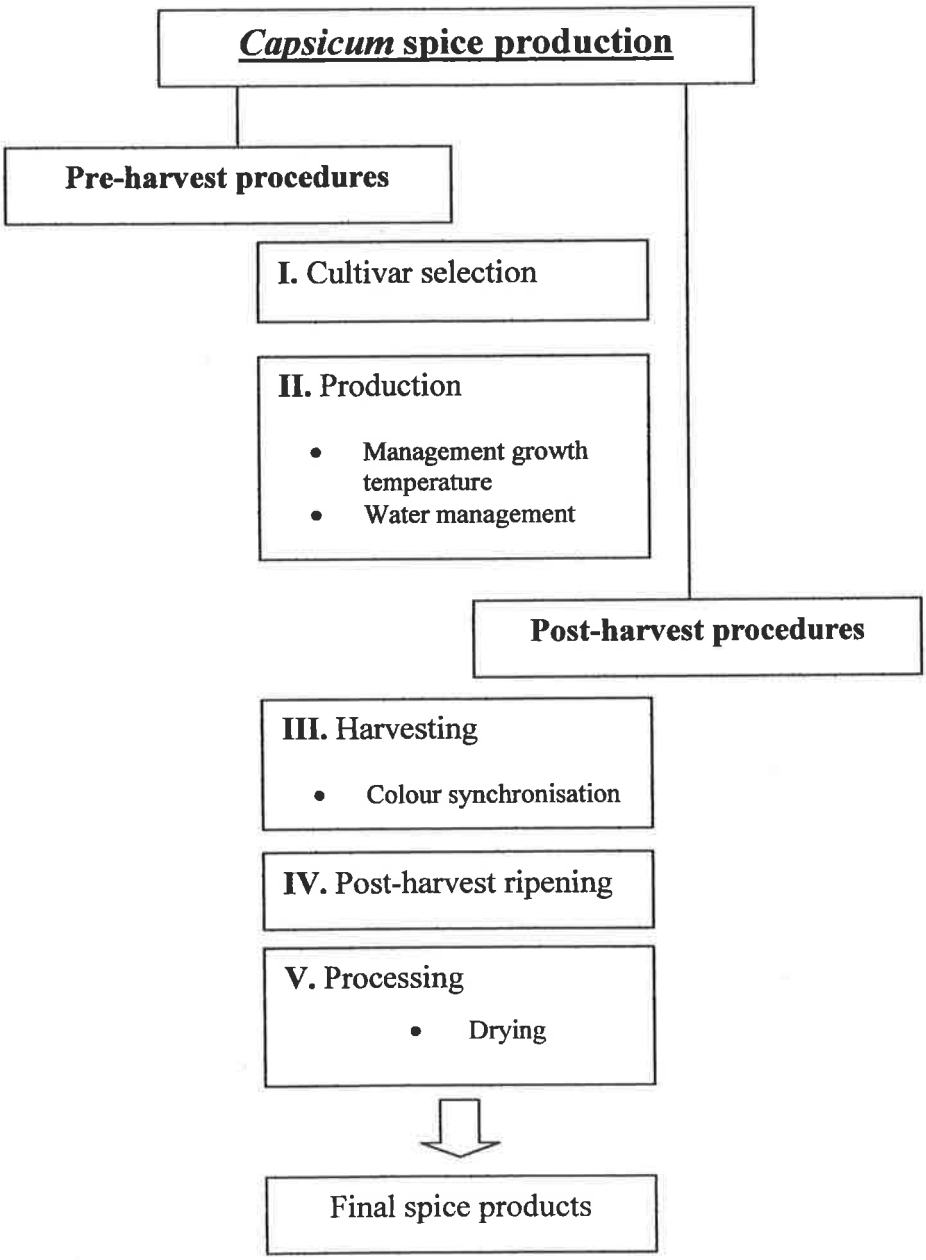


Figure 1.1 Diagram shows the main procedures involved in the *Capsicum* spice production system that affect yield and quality, and were investigated in this study.

Chapter Two

General literature review

2.1 *Capsicum* spices

Capsicum is a nutritious food, being low in calories, but high in vitamins A and C (Martin, 1984). The fruit can be used not only as fresh material in salads and in cooked dishes, but also as processed dehydrated spices for their colour, flavour and hotness. They can also be processed by canning or freezing.

The genus *Capsicum* originated in Central and South America (Martin, 1984), and subsequently was introduced to Europe, Asia and British colonies. The nomenclature of *Capsicum* is not clear, and various varieties have been produced in different countries under different names. However, the cultivated forms belong to five species: *Capsicum annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. pubescens* (Wien, 1997). The most important species is *C. annuum*, which includes most of the main commercial types such as sweet capsicum (bell pepper), paprika (sweet pepper), and chillies (chilli pepper) such as cayenne or bird's eye.

Literature has stressed the ambiguities and confusion in the names currently used for different types of capsicums and chillies. Some *Capsicums* in Australia are also called chillies, but the term 'capsicum' entirely refers to the sweet or non-pungent types while 'chilli' refers to the pungent types. There is also an argument over spelling. For the record 'chilli' (singular) and 'chillies' (plural) are the standards in the United Kingdom (Larkcom, 1995), and Australia but 'chile' and 'chili' dominate in the United States of America, and they are also referred to as 'peppers'. In this study *Capsicum* encompasses all pungent and non-pungent types in this genus, 'paprika' refers to mild types, and 'chilli' refers to hot or very hot types. However, for a specific use, the adjective 'sweet', 'hot', 'non-pungent' or 'pungent' may also be used.

The extent of production of *Capsicum* spices in Australia is unknown, but it appears to be relatively small, and is mainly confined to Queensland. However, the total Australian market can be estimated from import statistics. For example, in 1974/75 Australia imported

216 tonnes of paprika out of the total import of *Capsicum* spices of 284 tonnes (Ellis, 1980). It is estimated that paprika comprises about 70% and chillies 30% of imported *Capsicum* spices in Australia (Ellis, 1980). The Department of Economic Development survey of wholesalers and processors revealed that 110 tonnes of *Capsicum* spices were consumed in 1978, while almost 270 tonnes of capsicum spices was consumed in Australia in 1980 (Ellis, 1980). In recent years, there has been a marked increase in the consumption of *Capsicum* spices through an increased popularity of Asian ethnic food products and Mexican cuisine. Imports of dried chillies have almost doubled to A$ 2.2 million over four years to 1994 (Miles, 1994), and the total chilli industry (fresh and processed) is expected to grow to A$ 30 million (Miles, 1994). Estimated Australian production of the fresh green capsicums (sweet bell pepper) and chilli alone is about 31,000 tonnes in 1999 (FAO, 1999) which increased from 23,000 tonnes in 1995. Although it is not clear whether this includes *Capsicum* production for processed products, domestic demand for processed *Capsicums* was estimated to be 600 tonnes in 1998/99 with an increasing trend (J. Small, pers. comm., 1999).

In general, most processing outlets in Australia now require large volumes of product with a consistent, standard quality. Originally, *Capsicum* spices were mostly imported from overseas sources (Miles, 1994). The largest producers of paprika are Spain, Hungary, Bulgaria, Mexico and Yugoslavia. Asian countries including China, India, Pakistan and Indonesia are major chilli producers (Ellis, 1980). The unreliable quality of imported materials and a demand for different levels of hotness that are not available in Australia has caused difficulties for processors in sourcing a consistent product (Murison, 1995). There is, therefore, a good opportunity for developing a *Capsicum* spice industry in Australia if growers are able to supply a consistent quality at reasonable cost. In order to succeed, two critical factors have been previously stated for Australian producers (Miles, 1994). The first is a reduction in the production cost through mechanical harvesting, and this will be discussed later in section 2.4.4. The second is the need for high quality of product that can be competitive with imported materials.

Among the processed forms of *Capsicum*, dehydrated and ground spice products will be the focus of this thesis, and will be the focus of this review.

2.2 Quality attributes of dehydrated capsicums

The quality of dried red chilli and paprika products is based on visual and extractable red colour, pungency, and to a lesser extent, nutrition (Bosland, 1993). Extractable red colour and pungency will be the main quality factors examined in this study, as they are the most important quality components for the manufacture of dehydrated capsicums.

2.2.1 Spice colour

Colour is one of the most important attributes of dehydrated capsicums. Chemical compounds named carotenoids are the main pigments responsible for red and yellow colour in capsicum fruit, especially in types which are red when ripe. Fruit of *Capsicum annuum* may contain almost 60 different carotenoids (Matus *et al.*, 1991) (cited in Britton and Hornero-Méndez, 1997). The red colour in *Capsicum* comes from capsanthin (Figure 2.1a) and capsorubin (Figure 2.1b), while the yellow-orange colour is from beta-carotene and violaxantin (Coultate, 1996).

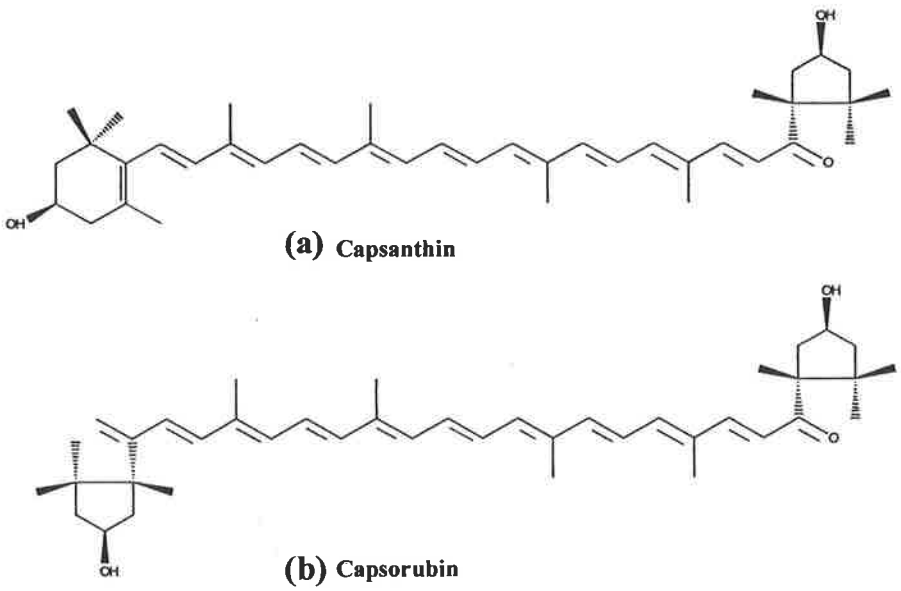


Figure 2.1 Chemical structures of major carotenoids; (a) capsanthin, (b) capsorubin.

Capsanthin is the major carotenoid which contributes up to 60% of the total carotenoids in ripe fruit (Bosland, 1993). Capsanthin is noted as an unusual carotenoid (Britton and Hornero-Méndez, 1997), as it is found only in fruit of the *Capsicum* species (Coultate, 1996). Carotenoids are fat-soluble compounds (Britton and Hornero-Méndez, 1997), and

their biosynthetic sites are located within the chromoplast during the ripening process (Britton and Hornero-Méndez, 1997). The amount of carotenoids in fruit tissue at harvest depends on factors such as cultivar, maturity stage and growing conditions (Bosland, 1993). Colour of dehydrated *Capsicums* is commercially analysed as extractable red colour as its market value depends largely on this colour. Extractable colour is measured by spectrophotometry, using a standard procedure developed and registered by the Association of Official Analytical Chemist (AOAC) and the American Spice Trade Association (ASTA, 1985). Colour values are expressed in the American Spice Trade Association (ASTA) units. For example, colour extracted from red bell-shaped capsicum averages at a low 80 ASTA, while extractable red colour from paprika varieties, which are a brighter red, averages at more than 120 ASTA units (Bosland, 1993). The ASTA colour units are the worldwide industrial standard unit for measuring red colour. Another unit for describing red colour in oleoresin, a red oily mixture of carotenoids, is the standard international colour units (SICU), where 100 ASTA colour units equal 4,000 SICU (Bosland, 1993).

In the case of *Capsicum* spices, quality standards have been set to aid trade, both national and international, but grade specifications of physical and chemical parameters of spices vary between types and countries. Based on the US Government Standard, *Capsicum* spices have been classified into three types corresponding to commercial and consumer preferences (Purseglove *et al.*, 1981):

red 'pepper' is defined as the dried fruit of any species of *Capsicum* that processes to a characteristic red to brown-red colour and perceptible pungency in three out of five people as tested using the organoleptic Scoville method;

paprika is defined as the sweet, non-pungent or slightly pungent dried red fruit of *C. annuum* L.; and

chilli pepper is defined as a blend of Anaheim and Ancho varieties of *C. annuum* (Gooindarajan, 1985b).

Cayenne is not separately typified, but it is a form of ground red pepper which conforms to the definition for that type (Purseglove *et al.*, 1981). Apart from the cleanliness specifications and some analytical parameters that are not the focus in this review, the three types of spices must satisfy the requirements shown in Table 2.1.

Table 2.1 Requirements of the US Government Standard for capsicum spices in the trade.

Characteristics	Commercial spices		
	Paprika	Red pepper (ground or crushed)	Chilli pepper (powder)
Extractable colour (expressed as ASTA colour units), not less than	110	-	70
Pungency (expressed in Scoville units)	-	30,000-55,000	-

From: Purseglove *et al.* (1981); - = not defined in standard or no minimum limit.

For paprika products the minimum acceptable level of colour is about 110 ASTA, while 70 ASTA is required for chilli powder (Table 2.1). However, the level of colour between 140 to 300 ASTA is also used for specification in the American trade (Larkcom, 1995), while 148 ASTA and above is acceptable in the Australian market (Scalzo Food Industries, Victoria, unpublished data, 1993). Paprika cultivars are usually higher in ASTA colour than pungent chilli cultivars. The price differential between high ASTA and low ASTA products can be an incentive for Australian growers and processors to produce high-colour products. However, the minimum colour acceptance varies with capsicum types, manufacturer and processor. Furthermore, price of products in Australia is not generally determined by the colour value as production is insufficient for domestic demand, unlike for American producers (J. Small, pers. comm., 1997). In this study, the minimum level of extractable red colour for paprika and chilli (cayenne type) powders will be considered to be 140 and 110 ASTA units respectively.

2.2.2 Spice pungency

Pungency is also an important quality component, especially for the pungent spices. The pungency or hotness sensation of *Capsicum* fruit is due to a group of compounds called capsaicinoids. They are amino amides of vanillylamine and C_9-C_{11} branched fatty acids (Quinones-Seglie *et al.*, 1989). Five analogues of capsaicinoids have been reported: capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin (Quinones-Seglie *et al.*, 1989). Of these, capsaicin (*N*-vanillyl-8-methyl-6-nonenamide) (Figure 2.2) and dihydrocapsaicin (*N*-vanillyl-8-methyl-6-

nonanamide) (Figure 2.2) which has a fully saturated side chain) are the major compounds in most *Capsicum* species (Coultate, 1996).

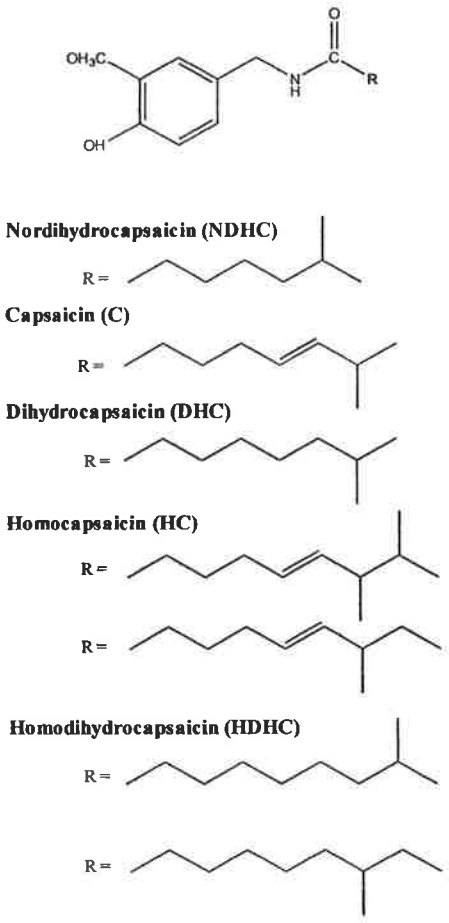


Figure 2.2 Chemical structures of capsaicinoids; the pungent principles of *Capsicum*.

Capsaicin accounts for about 70% of the pungency in *C. annuum* and 47% in *C. frutescens*, while dihydrocapsaicin concentration is as high as 30% and up to 53% in *C. annuum* and *C. frutescens*, respectively (Cotter, 1980). However, both are the most active (most pungent) compounds, and they account for nearly 90% in regards to total sensory heat (Gooindarajan and Sathyanarayana, 1991; Todd *et al.*, 1977). The ratio of capsaicin to dihydrocapsaicin in *Capsicum* is generally between 1:1 and 2:1, depending on species and cultivars (Gooindarajan and Sathyanarayana, 1991).

The major site of capsaicinoid synthesis is the placenta (Iwai *et al.*, 1979) and capsaicinoids accumulate in the vacuoles of placental epidermal cells (Suzuki *et al.*, 1980) until they are metabolised to lignin-like substances in the cell wall (Bernal *et al.*, 1995) of pungent *Capsicum* fruit. Capsaicinoids have no flavour or odour (Rowland *et al.*, 1983), but act directly on pain receptors in the mouth and throat. The crystalline capsaicinoids melt at 64 to 65°C, are very sparingly soluble in water, but are readily soluble in fats and oils (Gooindarajan and Sathyanarayana, 1991). Capsaicinoids are very stable, and their content is measured in 'Scoville Heat Units' (SHU). Wilbur Scoville developed the first quantitative organoleptic test in 1912, with SHU defined as the reciprocal of the highest dilution (threshold) at which a panel would definitely recognise a pungent sensation (Scoville, 1912). In recognition threshold tests, capsaicin and dihydrocapsaicin evoke equal pungency with a thresholds of 16×10^{6} $mL.g^{-1}$, while other homologs and analogs varied with pungency levels of 30 to 70% of the major components (Gooindarajan and Sathyanarayana, 1991). More recently, the content and composition of capsaicinoids in pungent *Capsicums* is measured in parts per million (ppm or $\mu g.g^{-1}$) on a dry weight basis using High Pressure Liquid Chromatography (HPLC). This method is the most accurate method for measuring pungent components in *Capsicum* spices, although it is more costly than the organoleptic analysis. The heat contribution of each component can be converted to SHU by multiplying the concentration (in ppm) of that constituent by the threshold values; 16 million for both capsaicin and dihydrocapsaicin (Todd *et al.*, 1977). The SHU are commercially known as the industry standard for levels of hotness. However, trade specifications for capsicum oleoresin (spice-extracted oil) in some countries uses the content of capsaicinoids, for example 1% capsaicin = 150,000 Scoville units.

Pungency of *Capsicum* fruit varies according to types and variety. For example, among paprika cultivars pungency can range from 0 to 5000 SHU, among mild chillies from 10,000 to 30,000 SHU, among hot to very hot red chillies from 50,000 to 195,000 SHU (Table 2.2), and Habaneros are the hottest chillies ranging from 200,000 to 300,000 SHU. Furthermore, the hottest chilli has recently been discovered in India. This is the 'Naga Jolokia' chilli (*C. frutescens*) from Assam. Its pungency is recorded at 855,000 SHU (Telegraph, 2000). This compares with 16 million SHU for pure capsaicin (Gooindarajan and Sathyanarayana, 1991).

Table 2.2 Trade types of capsicum spices.

Trade name, Type	Source	Classification Pungency (Scoville heat units)	Total capsaicinoids (%)	Principal use
Naga Jolokia	India	855,000	>5.0	
Habanero	-	200,000	>1.0	
		Extremely high		
Mombasa chillies	Uganda Tanzania	150,000	0.8-1.0	Oleoresin
Bird's eye chillies	Papua New Guinea	Very high	0.5-1.0	
Fukien rice chillies	China	120,000	0.5-0.95	
Jwala	India	High	0.6-0.7	
Bahamian	U. S.	75,000	0.5	
Tasbasco	U. S.	Medium	~ 0.5	Chilli powder
Funtua	Nigeria	60,000	0.4	
Sannam	India	-	0.3	
Jalapeno	Mexico	Low to medium		
Dandicut cherry	Pakistan and Bangladesh	30,000	0.2	Pickle
Ancho	Mexico	low	<0.1	
Paprika, pungent	Hungary (pink)	15,000	0.05-0.1	
Paprika, sweet	Hungary (special)	5000	≤0.03	Paprika Powder
		Very low		

From: Govindarajan (1985b).

In the Australian market, the minimum acceptable level of hotness of pungent spices is about 10,000 SHU (J. Small, pers. comm., 1999), while the specification according to the US Government Standard is as high as 30,000 to 50,000 SHU (by organoleptic test) for red pepper (Table 2.1). Combining processed non-pungent and pungent spices to provide intermediate powders is also common in the market. In some countries such as India, a very hot chilli spice is most highly valued in the market (Tewari, 1990), with the price depending on its hotness. In this study, the minimum acceptable level of pungency therefore will be 10,000 SHU for chilli, whereas no minimum level exists for paprika.

2.3 A system approach to improve dehydrated capsicum spice production

Capsicum spices are widely traded, and a consistent high quality with minimal production cost is essential. Plant breeders continue to strive for new cultivars with superior genetic potential for yield, protection against production hazards, and improved quality (Bosland, 1993). However, an appropriate cultivar is not sufficient on its own to improve spice production. Appropriate management throughout the production cycle is necessary for maximizing yield and quality of the final product. Therefore, pre- and post-harvest procedures within the production system must be considered as a whole. There are several factors that show the highest potential for improving capsicum spice production. Cultivar selection, environmental growth conditions, harvesting, post-harvest handling, and processing have been indentified as the main factors, and they will be discussed below.

2.3.1 Selection of cultivar

To be profitable, growers need to produce specialised cultivars for drying, and have an intensive approach to spice production. Dehydrated capsicums are produced from many cultivars that can be grouped into hot and sweet types (Philp and Burne, 1988). For instance hot cultivars are Anaheim, long red Cayenne, Thai hot and Tabasco types. Long red sweet and paprika types are examples of sweet cultivars. These are available in Australia (Philp and Burne, 1988), but there are now new *Capsicum* types sold as fresh product in both niche and super markets. These include Red Cherry (mild), Super Cayenne (hot), Tabasco (very hot) and Habanero (extremely hot) types. Larkcome (1995) suggested that these are also suitable for dehydration. However, supply quantity is low, and seed suppliers are not readily identifiable in Australia. Therefore, the selection of cultivars is limited in Australia at this stage.

For ideal management, a 'broad acre' approach to production is needed for dehydrated capsicums (Philp and Burne, 1988). Growing requirements are generally the same as for tomato production, and mechanised planting, irrigation and harvesting are required to reduce labour costs.

For economical spice production, cultivars must have high yield as well as high colour content and/or pungency (Gooindarajan, 1985a). *Capsicum* cultivars vary in height and

type of the shoot system, with multiple stems and upright growth habit being preferable for machine harvesting (Somos, 1984). This has an advantage of reducing labour cost for spice production (Miles, 1994). However, a single fruit produced per node, a large fruit size and a pendulous fruit position are also preferred as those fruit are easiest to harvest. These are normally the types found in the genus *C. annuum* L., whereas bushy plants are more typical of *C. frutescens* where there is a large number of small fruit produced per node thus creating difficulties during harvest. In addition, cultivars with virus resistance are recommended (Ed. Furkovic at South Pacific Seeds, NSW, pers., comm., 1997). According to these characteristics, the Hunter Valley Herb Farm (Merriwa, NSW), the commercial co-operator of this study, selected two main capsicum types; paprika 'PS72285' and the Cayenne chilli 'Caysan SPS705'. They were the most appropriate commercially available cultivars at that time, and they were used throughout this study.

2.3.2 Growing conditions

Capsicum is a warm-season plant, and requires a long growing season, approximately six to seven months, to reach full yield potential. In general, irrigation, growth temperature and fertilisation from planting and throughout the growing season appear to be the main factors directly influencing plant growth and yield, and therefore affect the spice quality and yield.

Impact of growing conditions on plant growth and development

Air temperature is one of the most important factors affecting plant growth since *Capsicum* is naturally frost sensitive. For ideal seed germination, air temperatures in the range of 20 to 25°C are required (Grattidge, 1990). Temperatures within the range of 17 to 32°C are preferable for flowering and fruit setting of *Capsicums* (Philp and Burne, 1988). However, very hot days (above 32°C) and dry weather (low humidity) often cause abscission of flower buds, flowers and immature fruit, and finally affect crop yield.

Drought conditions are also commonly noted as a factor affecting crop yield in all horticultural commodities. The most common cause of yield reduction under drought conditions is through poor pollination (Rylski, 1986). Severe fruit abscission also often occurs when high temperature is combined with moisture stress (Wien, 1997). For capsicums, total yield reduction (by up to 30% depending on cultivar and stage of plant

development) was observed under drought conditions throughout the growing season in the studies of Techawongstien *et al.* (1992). The sensitivity of *Capsicum* plants to moisture stress appears to be dependent on the stage of plant development and the individual cultivar. Salter and Goode (1967) (cited in Techawongstien *et al.*, 1992) suggested that flowering is the most sensitive stage to water stress, and fruit and seed development to a lesser extent. From this point of view, it is necessary to manipulate irrigation practices for maximum yields of the final product.

Excessive nitrogen (N) fertiliser was reported to reduce yield (Bosland *et al.*, 1994) due to poor fruit set under field conditions. However, yield reduction at high nitrogen levels might be caused by salt injury rather than a direct effect on fruit set (Wien, 1997). Heavy application of nitrogenous fertiliser may also increase vegetative growth and delay maturity (Rajput and Parulekar, 1998). Pre-plant fertiliser application of 60 kg of nitrogen (N), 70 kg of phosphorus (P) and 60 kg of potassium (K) per hectare is recommended for field-grown *Capsicum* (Grattidge, 1990), without any further fertiliser application thereafter. Although nutritional information is now well documented, poor management can cause major reduction in yield and quality.

Capsicum is susceptible to a number of pests and diseases. These can affect crop yield and fruit quality by causing root rots, leaf blights, discoloured fruit or abnormally shaped fruit. Pest and disease incidence can be minimised through proper cultural management. Soil fumigation and soaking seed with a recommended bleach solution may be necessary for successful crop production in the field in order to avoid damage from nematode and infected seeds from bacteria, fungi or viruses (Grattidge, 1990). Foliar diseases and pest can be controlled by chemical sprays, but misuse of chemical application can damage crop and contaminate the dry product. More information on spray application technology can be found in the QDPI publications (Banks *et al.*, 1989; Persley, 1989). Crop rotation between seasons is recommended for reducing any built up soil-borne diseases (Grattidge, 1990). Knowing the right chemical and how to use it effectively and safely are all parts of an effective *Capsicum* spice growing operation.

Fruit infection can also occur under poor conditions such as hail, sunburn or mechanical damage. Strong wind exposure can reduce yield (Hodges *et al.*, 1995). *Capsicums* are usually planted in double rows for better support of the plants and better protection from

sunburn (Grattidge, 1990). In some areas, a double wire trellis may be necessary for reducing wind-damage and sunburn. Increasing plant density has the advantage of providing shade that can limit fruit sunburn, although it may also reduce fruit size (Rubatzky and Yamaguchi, 1997). Recommended plant density varies with cultivar, but frequently is in the range of 25,000 to 30,000 plants per hectare (Grattidge, 1990; Rubatzky and Yamaguchi, 1997), but more than 30,000 plants per hectare may be desirable for machine harvesting (J. Small, pers. comm., 1996). Although growing conditions vary for each region, careful management of crop production is necessary throughout the growing season.

Impact of growing conditions on pungency

Variation in capsaicin content between cultivars and species is initially under genetic control (Ahmed *et al.*, 1987) and depends on maturity stage (Estrada *et al.*, 1997), but its accumulation also appears to interact with several environmental factors (Bosland, 1993; Cotter, 1980; Estrada *et al.*, 1998; Estrada *et al.*, 1999). For example, variability of capsaicin content has been observed between geographical areas, between plots in the same field and even among individual plants from the same plot (Lindsey and Bosland, 1995).

Although the capsicum plants appear to be relatively insensitive to soil-applied nutrients, increased nitrogen levels were reported to reduce fruit pungency (Cotter, 1980). Several levels of nitrogen rates were observed in the study of Johnson and Decoteau (1996). Higher nitrogen rates (at 22.5 and 30.0 mM in Hoagland's nutrient solution) reduced the pungency (by ~20%) in 'Padron' fruit (*C. annuum* L.), compared to the medium rate (15.0 mM) which was favourable for the highest pungency (~4,000 SHU), when nutrient treatments began at transplanting (Johnson and Decoteau, 1996). In the same studies, no potassium rate (1, 3, 6, 9 or 12 mM K in Hoagland's nutrient solution) affected fruit pungency when application was initiated at transplanting or flowering. Mineral nutrition (1 g per pot of 13N-40P-13K fertiliser during vegetative growth and 15N-11P-15K during flowering for once a week) has also been highlighted recently as a factor affecting capsaicin content in 'Jalapeno' fruit (*C. annuum* L.) (Estrada *et al.*, 1998). The nature of interrelationship between fertiliser and fruit development has been previously studied, and therefore nutrient application was not included in this study.

Temperature and soil moisture were also reported to influence pungency levels of *Capsicum* fruit. High temperature at the time of fruit ripening (Cotter, 1980) has increased fruit pungency in some cultivars of *C. annuum*. Under a growth temperature regime of 35/21°C (day/night), capsaicin content (% as dry weight basis) was significantly increased by 50% relative to that at 25/10°C (Levy *et al.*, 1989). Since the recommended air temperature range of 25±5°C has been previously noted for all growth phases of *Capsicum* (Somos, 1984), little is known about how this temperature range could influence the final quality of *Capsicum* spices. Therefore, further information is still required for this factor.

With less irrigation, fruit often contain higher pungency levels than with more frequent irrigation (Cotter, 1980; Estrada *et al.*, 1999; Levy *et al.*, 1989; Quagliotti, 1971). The response of pungency to drought conditions is inconsistent, with differences observed between plant species or even plant cultivars. Each stage of plant growth responds differently to drought conditions (Techawongstien *et al.*, 1992). While yield is affected differently at different stages of plant development (Techawongstien *et al.*, 1992), little is know about the interaction of development and drought and their effect on pungency. The relative importance of different growing conditions for manipulating pungency, especially of different cultivars, needs to be clarified.

Impact of growing conditions on colour

In general, any condition that has an effect on plant growth may also affect fruit colour quality. Although visual appearance of fruit is not important for spice production, any fruit damage, for example sunburn or fungal infection, can dilute colour content of the final product. Environmental factors such as temperature, available water, soil quality and environmental pollution, have also been suggested to affect the level of fruit colour development (Cotter, 1980). The best fruit colour development occurs between 18 to 25°C air temperature (Murison, 1995), but fruit colouring is not sensitive to light or darkness (Philp and Burne, 1988). There is, however, very little information on specific factors, and how they influence final colour content.

2.3.3 Harvesting

Fruit maturity at harvest directly affects spice quality. Two stages of maturity are mainly harvested depending on market requirements; green fruit for fresh consumption and red

fruit for fresh consumption and spice production depending on cultivar. *Capsicum* takes about 30-35 days from fruit set to complete development of fruit for harvest at the green mature stage. The fruit start ripening 80-90 days after fruit set, and can first be harvested at the red stage at 13 to 16 weeks after planting, depending on weather conditions and cultivar. Fully red-coloured fruit are required for dried spice production (Lease and Lease, 1956), as the high colour content and/or pungency of this stage are desired by consumers. While hand harvesting is mainly used for the fresh market with several harvests per growing season, it is not economically feasible for spice production. Machine harvesters thus can be used for spice production, especially for a large broad acre production. Because fruit appearance does not negatively impact on marketing in this case, machine harvesters can reduce production costs. A machine harvester has been used at the Hunter Valley Herb Farm, NSW (J. Small, pers. comm., 1998) for spice production, therefore it will not be described here. The once-over operation of the machine harvester, however, requires uniformity of fruit maturity on the bush. In order to increase the efficiency of harvesting and to reduce production costs, harvesting aids such as 'ethephon', a chemical ripening agent, to synchronise fruit maturity has been used in the USA for spice production.

Ethephon (Ethrel®, 2-chloroethyl-phosphonic acid) has been used to de-green *Capsicum* fruit (Sims *et al.*, 1970; Sims *et al.*, 1974). Ethephon is generally stable in aqueous solutions below pH 4. It acts by releasing the plant hormone ethylene on contact with plant tissues, as the cytoplasm of plant cells has a pH greater than 4 (de Wilde, 1971). Foliar applications of ethephon to *Capsicum* plants have successfully induced red colour associated with fruit ripening in chilli (Sims *et al.*, 1970; Sims *et al.*, 1974), pimiento (Sims *et al.*, 1974; Batal and Granberry, 1982), bell (Sims *et al.*, 1974) and paprika (Worku *et al.*, 1975). However, there is still controversy surrounding the use of this chemical, as it had little value as a fruit-ripening agent in some studies (Knavel and Kemp, 1973; Cooksey *et al.*, 1994), or resulted in different responses in various types of *Capsicum* (Singh *et al.*, 1992). Furthermore, ethephon can cause fruit abscission resulting in yield reduction (Conrad and Sundstrom, 1987). Therefore, further study of ethephon use is required, but factors affecting this approach need to be considered.

Important factors for ethephon effectiveness are time of application, air temperature and concentration of the chemical. Optimal time of application has been suggested to be when 20% of fruit have reached the mature red stage (Sims *et al.*, 1974). An average air

temperature less than 21°C during day time was found to be too low for ethephon to induce fruit colour development (Knavel and Kemp, 1973). In a experiment where plants were sprayed with ethephon, increasing concentration of ethephon up to 15,000 $\mu L.L^{-1}$ gave a higher red fruit yield (Conrad and Sundstrom, 1987). Ethephon concentrations of 50 to 5000 $\mu L.L^{-1}$ were shown to be effective in inducing fruit maturity in various *Capsicum* cultivars (Sims *et al.*, 1970; Worku *et al.*, 1975). However, its effectiveness still varies with cultivar (Worku *et al.*, 1975). The possibility of using ethephon to synchronise fruit maturity therefore needs to be investigated for commercial cultivars in use in Australia.

Treatment with ethephon may have effects on the final quality of the spice powder. Previous studies have mentioned that ethephon applied during the growing stage not only stimulated fruit colour development, but also increased the total extractable colour of paprika fruit (Worku *et al.*, 1975; Batal and Granberry, 1982). For example, the capsanthin content of one treated cultivar was increased with increased concentration of ethephon (0 to 500 $\mu L.L^{-1}$). The capsanthin content (mg per g fruit dry weight) was 33 to 100% higher compared to the untreated paprika fruit depending on cultivar (Worku *et al.*, 1975). The ethylene released by ethephon may or may not influence some enzymes involved in the carotenoid biosynthesis pathway which would alter pigment concentration; however, the actual mechanism of this effect is not clear. Furthermore, the effect of ethephon on pungency is not known. Further research on these quality parameters, and how they are affected by ethephon, needs to be carried out.

2.3.4 Post-harvest handling

Capsicum fruit are frequently born singularly at each node, and sequentially develop, and then ripen at different times from the lower through to the upper branching nodes; up to 10-11 nodes may develop depending on the length of the growing season. This results in a mixture of different coloured fruit (both ripe and unripe) after once-over harvesting with a mechanical harvester. In general, unripe fruit may account for more than 30% of total harvested fruit. These include immature, green or breaker (chocolate or green with slight red coloration) fruit stages which are not acceptable for spice production. Instead of being discarded, the colouration of unripe fruit may possibly be further improved by using ethylene, a natural ripening hormone. This would increase yield as the numbers of the red fruit after harvest would be increased. Little is known about how different coloured fruit

may further develop their red colour before processing, and whether this could affect their spice quality.

In order to increase red fruit numbers by using an application of ethylene, an understanding of fruit colour development associated with fruit ripening after harvesting is essential. In general, once spice capsicum fruit have reached their full mature size, a change in colouration will occur from green to dark green, to breaker or chocolate and finally to red. Colour development is normally associated with fruit ripening that is also accompanied by various metabolic processes such as changes in respiration and ethylene production, fruit firmness, sugar levels and flavour (Wills, 1998). While this is true for capsicum fruit that are still attached to the mother plant (Tadesse *et al.*, 1998), it is not clear whether unripe harvested fruit may continue their colour development.

Fruit ripening is normally classified into two different ripening patterns, known as climacteric and non-climacteric (McGlasson, 1985; Wills, 1998). Non-climacteric fruit show no significant increase in respiration or ethylene production after harvest, and examples are citrus, strawberry and grape. Climacteric fruit, for instance banana and apple show a marked peak of ethylene production and also an increase in respiration associated with ripening (McGlasson, 1985). For climacteric fruit, treatment with exogenous ethylene leads to autocatalytic biosynthesis of ethylene and accelerates fruit ripening (McGlasson, 1985).

Classification of *Capsicum* fruit as climacteric or non-climacteric fruit remains controversial. Several varieties in this genus have been classified as non-climacteric (Rhodes, 1970; Lu *et al.*, 1990; Pretel *et al.*, 1995) as their colour change could not be regulated by ethylene (Pretel *et al.*, 1995). However, Gross *et al.* (1986) reported that hot chillies (*C. annuum* cv. Chooraehong) do exhibit a respiratory climacteric during their ripening. The rate of ethylene production was low throughout, being only 0.7 $\mu L.kg^{-1} h^{-1}$ at the climacteric peak when the surface colour was 30 to 40% red. Lurie and Ben-Yehoshua (1986) also found a rise in respiration as colour changed in 'Maor' bell pepper. It is inconclusive therefore whether *Capsicums* undergo the climacteric-type ripening, and this may vary among cultivars.

For spice quality, the extractable colour and pungency levels are directly dependent on the final colour intensity of the *Capsicum* fruit prior to processing. The full red colour stage has been shown to have the highest intensity of colour (Lease and Lease, 1956) and/or pungency (Lease and Lease, 1956; Gooindarajan, 1985b) in most *Capsicum* types tested. However, little is known to what extent post-harvest treatments influence these qualities. One report found that pungent principles (capsaicinoids) were induced in fruit of non-pungent capsicums (*C. annuum* L. var. *grossum*) during post-harvest storage at 28°C under continuous light (Iwai *et al.*, 1977). After 10 days the capsaicinoid contents in placenta increased to 19 μg per 100 mg dry weight in non-pungent capsicum which was more than 10-fold of that prior to storage, although this effect was not report in pungent *Capsicums*. A traditional practice of post-harvest ripening termed 'curing' in Hungary has shown to increase the red pigment content of harvested red paprika (Gooindarajan, 1985b; Gooindarajan, 1986). Total carotenoid content was increased (on dry weight basis) by 50 to 100% for most paprika types over 6 weeks storage in the sun or shade after fruit were harvested at the fully red ripe stage (Gooindarajan, 1986). The process of this colour intensification was further modified and split into 3 weeks of drying on the plant after fruit reached the red stage and 3 weeks of storage after harvest in the shade (Gooindarajan, 1986). However, the benefit of curing to increase red pigment content could be a varietal characteristic, similar to variation between cultivars in the stability and formation of colour pigments during fruit ripening on the plant prior to harvesting. Little information of this practice is available for Australian spice producers.

2.3.5 Processing

Generally, pungency is little affected by the drying process, but colour pigment loss often occurs during and after drying due to their chemical properties. Therefore, less emphasis will be placed on pungency in the processing of *Capsicum* spices.

Dehydration

Capsicum fruit at harvest normally have a moisture content in the range of 65 to 85%, depending on whether they were partially dried on the plants or harvested while still succulent. Traditionally, sun-drying is the most common drying process and is immediately performed after harvesting (Cotter, 1980). Although it is excessively slow, non-uniform and unhygienic, it is still a widely used method throughout Asia, Africa,

Central, and South America and USA by numerous small growers (Purseglove *et al.*, 1981). However, sun drying has many disadvantages in regards to product quality.

Artificial drying techniques have advantages over traditional sun-drying, as they produce better quality products, take less time and minimise product losses. Various drying conditions have been developed depending on *Capsicum* type and drier. Since initial spice colour is dependent on the drying process (Purseglove *et al.*, 1981), careful control of drying conditions during this operation is essential.

For commercial processing in the USA (Purseglove *et al.*, 1981), fruit are normally brought to a drying centre, washed, inspected and spread out on trays either as whole fruit or sliced into 2.5 cm long pieces. Fruit are then dried in heated buildings or in tunnel driers that expose the fruit to a forced air current at 50 to 60°C, until the moisture content is reduced to 7 to 8%. This system has also recently been used by an Australian processor (J. Small, pers. comm., 1997). Some processors in the USA have introduced two-stage drying, firstly drying the fruit to a moisture content of 12-20% and then storing them at a temperature of 0°C; when required for grinding, the drying is continued until fruit contain 7 to 8% moisture (Purseglove *et al.*, 1981). This appears to preserve colour and pungency of dehydrated products for longer storage. Although various procedures for capsicum dehydration are available, new technology is still required for faster drying and better spice quality preservation.

Drying rates have been noted to be dependent on various drying parameters (air temperature, air flow rate and air humidity) and the properties of products (moisture content, surface-to-volume ratio, area of cut surface and rate of moisture loss) (Sigge *et al.*, 1998). Increasing temperatures and decreasing RH generally increase the drying rate (Sigge *et al.*, 1998). However, at 80°C pungency, initial colour and colour-retention properties were reduced (Lease and Lease, 1962), especially combined with a low product moisture content of 6% (Purseglove *et al.*, 1981). The optimum drying temperature was found to be 60 to 65°C, but higher temperature may be used initially for a few hours (Li *et al.*, 1994; Wall, 1994) when the moisture content of fruit is high (Wall, 1994). A final moisture content of 8-12% is recommended for capsicum spices, but this is also dependent on producer specifications (Lease and Lease, 1962; Li *et al.*, 1994; Wall, 1994). Although

much effort has gone into optimising conditions for *Capsicum* drying, little work has investigated the effect on final quality.

In order to select the appropriate conditions for dehydration, a minimal drying time is preferred due to energy savings (Purseglove *et al.*, 1981). Although high drying temperatures can reduce drying time, they are not suitable for preserving product quality as they induce caramelization, pigment degradation and ascorbic acid oxidation (Purseglove *et al.*, 1981). A technique, which combines modified drying conditions and conventional cutting to increase fruit surface area, may be useful. This is practiced for *Capsicum* spices in the USA (Purseglove *et al.*, 1981). For example, drying at 65°C to a final moisture content of 7-8% was reduced to 8 hours for sliced fruit, compared to 12 hours for whole fruit (Lease and Lease, 1962). However, drying may take 24-36 hours depending on drier and conditions (Purseglove *et al.*, 1981; Dahlenburg and Tugwell, 1995; J. Small, pers. comm., 1997).

Another factor determining water loss is the fruit cuticle (Lownds *et al.*, 1993). Although this extends storage life of fresh *Capsicums* on the market shelf, it appears to be the main barrier to slow moisture transfer during drying. In order to increase moisture transfer, a new approach of using dips in a drying oil prior to drying, similar to one used for dried Sultana grapes, may be useful. The drying oil is a mixture of fatty acid ethyl esters (2% v/v) and potassium carbonate (K_2CO_3) (2.5% w/v) in water (Fogerty and Burton, 1981). Application of drying oil by spraying or dipping successfully increased drying rates, but also improved colour quality of dried Sultana (Fogerty and Burton, 1981). Water permeability of the cuticle increased due to the rearrangement of the fruit surface waxes by the dipping oil (Grncarevic, 1963), reducing drying time by up to 50%. However, no information is available for this technique with *Capsicum* spices.

Several types of drier have been investigated for dehydrated *Capsicums*, mainly for reducing drying time and energy cost. Among these, heat pump driers have shown a good potential for colour retention, and reduced energy costs compared to hot air driers (Chou *et al.*, 1994). The benefit of an air-recirculating system with a heat pump is that of faster drying rates and lower energy consumption (Dahlenburg and Tugwell, 1995). The heat-pump drier consists of a chamber containing the product to be dried, an air circulating system and an electrically-driven heat pump which uses the basic components of a

refrigeration system. The air is heated before entering the drying chamber with a low RH, it then passes over the product, picks up moisture and returns to the dehydrator to be retreated and recirculated (Chou *et al.*, 1994; Dahlenburg and Tugwell, 1995). With a heat pump drier, it takes about 12-18 hours for drying raisins to reach a final average moisture content of 15% with an average drying temperature of 55°C (Dahlenburg and Tugwell, 1995). The energy consumption is about 600-1500 $kW.h^{-1}$ for each ton of dried crops (Hesse, 1990), and this consumption is only one third to one sixth of the energy used by other heated air driers for the same product. Recently, heat pump drying has been trialed for both dehydrated herbs and vegetables in Australia, but little information is available for commercial dehydrated capsicums (J. Small, pers. comm., 1997).

Grinding and storage conditions

In general, paprika is always produced in powder form, while chillies can be produced as whole fruit, flakes or powder depending on producer specifications. Grinding causes some colour loss, and affects colour stability (Purseglove *et al.*, 1981). Several factors have been noted to affect colour loss during and after grinding. Different grinding equipment may result in varying colour loss of products during storage (Gooindarajan, 1986). Although a stone mill is desirable for least colour loss (Gooindarajan, 1986), up to 14% of colour loss was observed during grinding by a stone mill under controlled conditions (medium grind; less than 0.08 mm particle size) for Spanish paprika (Gooindarajan, 1985b). At low moisture levels the heat generated during milling is high enough to adversely affect colour (Gooindarajan, 1986), with 10% moisture content considered optimum during grinding (Gooindarajan, 1985b). A minimum proportion of 5% seeds mixed with dried pericarp is also considered essential for satisfactory milling of paprika (Gooindarajan, 1985b). It has been noted that the seed oil helps to uniformly distribute colour (Gooindarajan, 1985b), and improve colour stability during storage (Márkus *et al.*, 1999).

Colour retention is important during the storage stage. Dehydrated *Capsicums* are stored in various forms such as whole fruit, flakes or powder. Ground *Capsicum* fruit lose colour the fastest, compared to flakes or whole fruit, due to the high surface area (Wall, 1994). However, the flaked product requires less space than whole fruit for storage, so producers often store the products as flakes before final grinding (J. Small, pers. comm., 1997). Differences in colour stability have been observed between cultivars, and cultivars with high initial colour content did not always retain colour effectively during storage (Lease

and Lease, 1956). Apart from cultivar, various factors that influence colour retention have been previously reviewed. Among these, storage conditions (temperature, relative humidity, atmosphere, light) have shown the greatest effect on colour retention during storage (Lease and Lease, 1956; Wall, 1994). Colour loss is faster under high temperature storage, and this is an additive effect with exposure to light and oxygen (Wall, 1994). The recommended storage conditions are 0-5°C in the dark. The moisture content of products should be about 10-15% to prevent mould growth, and a relative humidity in storage of 60-70% is desirable (Purseglove *et al.*, 1981). Recently, alternative methods to improve storage life of dehydrated capsicums have been investigated in the study of Klieber and Bagnato (1999), for example. Storage conditions will, therefore, not be investigated in this study.

Summary

Cultivar, environmental growing conditions, season, stage of ripeness and post-harvest handling all influence the final quality of dehydrated *Capsicum* fruit. A cultivar's potential for high yield and quality is only realised under optimum management, but little consistent information is available. To provide a framework for quality assurance and production management of processed *Capsicum* fruit in Australia, it is necessary to better understand the factors affecting yield, colour and pungency of the final product.

Therefore, this study focuses on the following, and has been divided into appropriate experimental chapters:

Chapter 4: Effect of air temperature on *Capsicum* spice yield and quality.

Chapter 5: Effect of water stress on *Capsicum* spice yield and quality.

Chapter 6: Effect of partial rootzone drying on *Capsicum* spice yield and quality.

Chapter 7: Effect of pre-harvest ethephon application on *Capsicum* spice yield and quality.

Chapter 8: Effect of post-harvest treatment with ethylene on *Capsicum* spice yield and quality

Chapter 9: Effect of dehydration treatments on *Capsicum* spice yield and quality.

Chapter Three

General materials and methods

3.1 Introduction

This chapter describes the materials and methods that were common to most of the experiments described in this thesis. Materials and methods that were specific to a particular experiment will be described in the relevant chapters. All experiments are summarised in Appendix I.

3.2 Plant materials

Cultivar selections: Two cultivars of *Capsicum annuum* L (Figure 3.1) were selected on the basis that they were the most appropriate types for mechanised spice production in Australia.

Paprika 'PS 72285' is a semi-pungent type with carrot shaped fruit that are about 13 to 16 cm long and 2 to 3.5 cm broad. Commercially, it is the best type for spice powder based on its deep red coloration, and is always sold as powder.

Cayenne chilli 'Caysan SPS705' is a pungent type with smooth and long red fruit. Fruit are about 10 to 17 cm long and 15 to 20 mm broad. Cayenne chilli is also the most common type for producing spice powder.

Figure 3.1 Characteristics of selected cultivars at 104 days after transplanting (DAT).

Production of seedlings: Hybrid seeds of both cultivars were obtained from the Peto Seed Company (Sydney) through the Hunter Valley Herb Farm (Merriwa, NSW) prior to each growing season. Seeds were sown in 10 cm diameter pots containing the 'UC' mix (Figure 3.2, details in section 3.3), using two or three seeds per pot depending on the percentage of seed germination expected (Figure 3.3).

Figure 3.2 The 10 cm diameter pots containing the UC mix for seeding.

Figure 3.3 Sowing of two or three seeds in one pot.

Preparation for seedlings was conducted in the greenhouse at the Adelaide University, Waite Campus. The greenhouse was held at 25 ± 5°C for both day and night, without humidity control, but supplemental lighting was supplied during day time in winter (approximately from June to October of each year) with a total photon flux of 525 ± 40 $\mu mol.m^{-2}.s^{-1}$ at the level of bench surface. In general, seeds took about seven to ten days to germinate, depending on greenhouse temperature. Seedlings were thinned to one per pot seven days after germination. Approximately 30 to 45 days after sowing when seedlings had reached the transplanting size (the fourth leaf stage, Figure 4.4a, b) they were transplanted to 20 cm diameter polyethylene pots containing the UC mix. Uniform plants were then randomly assigned into experimental Units, and relocated to either the greenhouse or the shadehouse depending on the experiment. Experimental designs and treatments will be described in each experimental chapter.

Figure 3.4 Seedlings of transplanting size; (a) located in the greenhouse and (b) seedling at the fourth leaf pair stage.

A slow-release fertiliser (25 g of Osmocote Plus, 15N:4.8P:10.8K:1.2Mg) per pot was placed as topdressing at first flowering (approximately three to four weeks after transplanting for both cultivars), and again six weeks after the first application. With the exception of experiments described in chapters 3 and 4, irrigation was generally provided as required throughout the growing season by hand-watering. Pest and diseases were controlled with appropriate foliar chemical sprays when necessary.

3.3 Potting medium

The University of California Mix (UC mix, Waite version) was the standard soil medium for all experiments. The UC mix was used for both seeding and transplanting base media in all experiments. The mix consisted of two parts of coarse washed sand (Golden Grove, Adelaide), one part of peat moss, and included a preplant-incorporated application of calcium hydroxide, calcium carbonate and Nitrophoska (15N:4P:12K) fertilisers (more details are provided in Appendix IV). A 5 cm depth of coarse pine bark was also laid down at the bottom of the transplanting pots before planting, but there was no bark in the seeding pots.

3.4 Location of experiments

All experiments were conducted at the Waite Campus of the University of Adelaide using potted *Capsicum* plants between October 1996 and January 2000 (details in Appendix I). The plants in most experiments were placed on high benches located in a temperature-controlled greenhouse (25 ± 5°C) (Figure 3.5a) or on low benches in the shadehouse (Figure 3.5b). The plants used in the study of harvesting aid (Chapter 7) were grown on the ground in the shadehouse for the whole growing season after transplanting. The soil surface of each pot was covered in some experiments. Prior to flowering, stakes of 150 cm length were used to support plants (Figure 3.6).

Figure 3.5 Seedlings at one week after transplanting; located in the greenhouse.

Figure 3.6 Use of a stake to support plants.

3.5 Yield and yield components

The fruits from each individual plant were harvested by hand all at once when the maximum number of red fruits (>60%) was obtained on the bush, to simulate once-over harvesting. However, specific times of harvesting will be described in the relevant chapters. In general, harvested fruit were classified as marketable (all red coloured fruit) or non-marketable fruit (green to breaker and all defective fruit) (Figure 3.7).

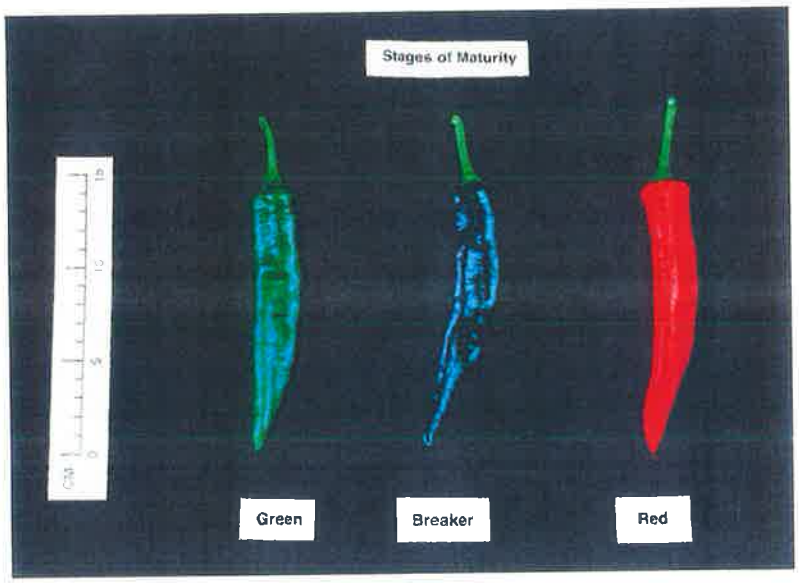


Figure 3.7 Colour classes of mature capsicum fruits.

Fruits from each colour category were counted (#.plant^{-1}), weighed, and yield was recorded (g.plant^{-1}). For some experiments individual plant were also cut off at the soil surface, and shoots and roots were separated. To obtain plant biomass, all plant parts, including fruits, were dried in an industrial hot air oven (Ward, Adelaide South Australia, Figure 3.8) at 45°C to a constant weight. Dry weight was recorded.

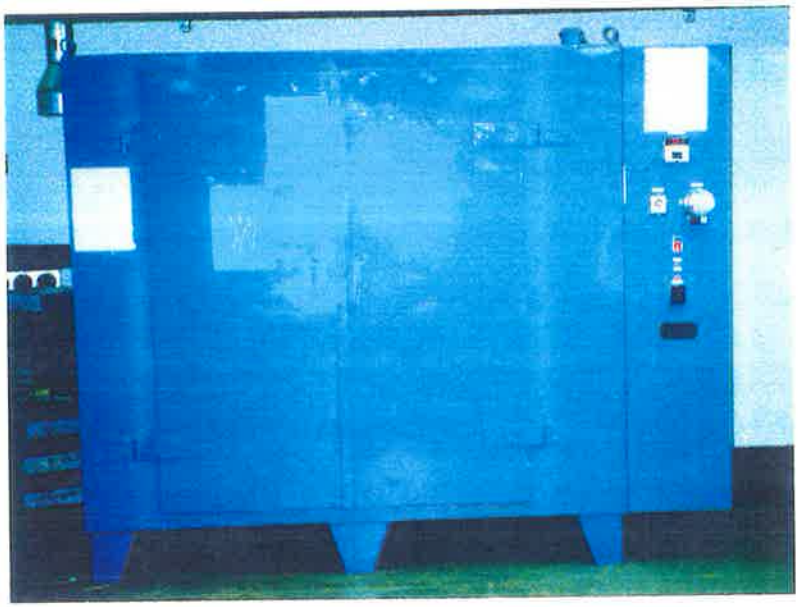


Figure 3.8 Hot air oven drier; front doors.

3.6 Fruit sampling and spice composition

3.6.1 Berry sampling

Only uniformity red coloured fruits (Figure 3.9) were used for final quality measurements. These fruit were cut into pieces to increase surface area before drying. Calyxes were removed from the dried red fruit (Figure 3.10), and fruit were ground into powder with a Culatti electric mill (1.5×10^{-8} m or 0.015 μm mesh size) (Figure 3.11). Ground samples (Figure 3.12) were placed in plastic bags, kept in the dark at room temperature (20-22°C) and used for colour and pungency measurement.

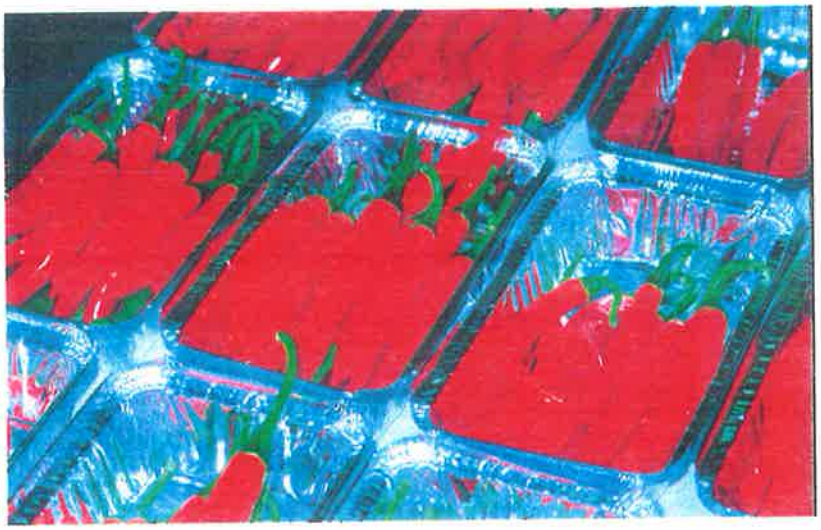


Figure 3.9 Red chilli fruit.

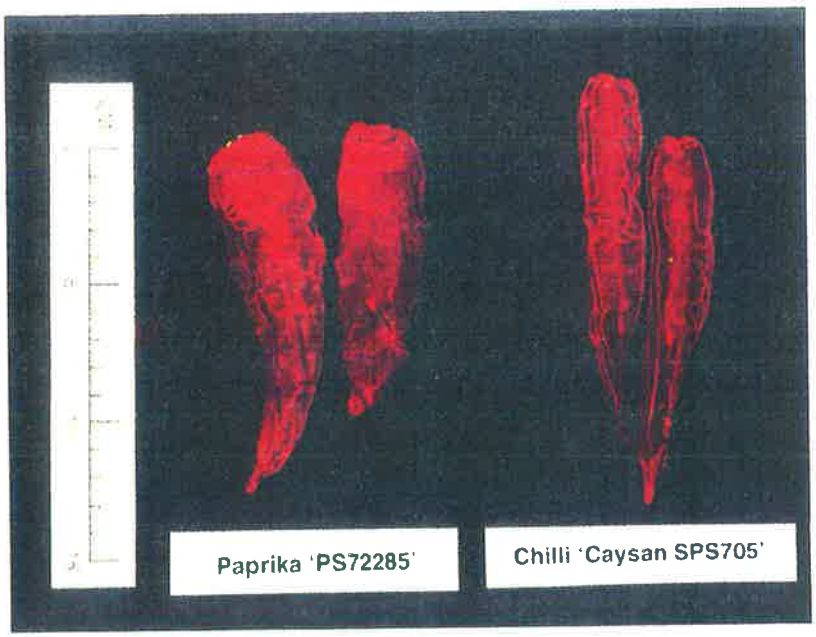


Figure 3.10 Fruits after drying.

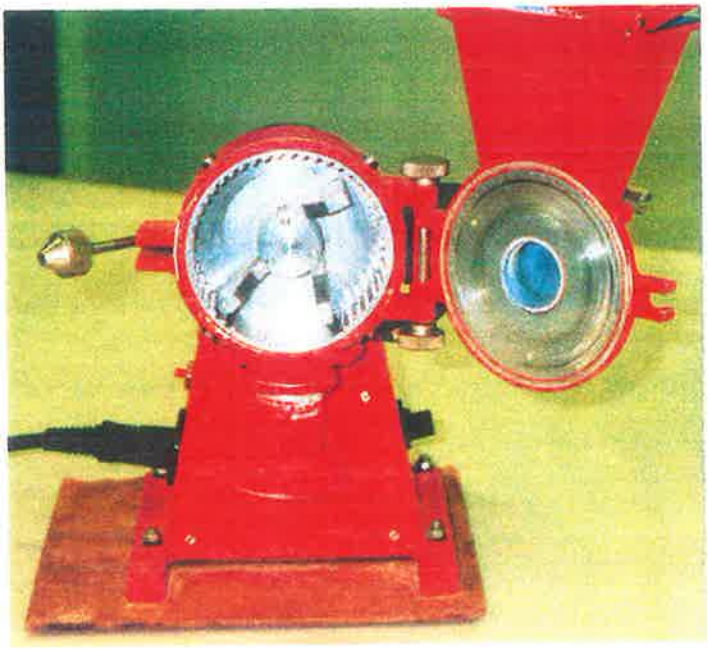


Figure 3.11 Electric mill for grinding fruit samples.

Figure 3.12 Samples after grinding.

3.6.2 Surface colour

For the experiment 3 in Chapter 9, surface colour of powders was measured once for each sample, as the reflected colour in the CIELAB (L*a*b*) colour space (CIE, 1986) using a Minolta model CR-300 Colorimeter (Osaka, Japan). All colour measurements are referenced to the CIE system (Commission Internationale de l'Eclaire), using the standard Illuminant C. The lightness coefficient, L*, ranges from black to white on a scale of 0 to 100. The a* value measures red when positive and green when negative; and b* value measures yellow when positive and blue when negative. The hue angle (h°) and chroma (C*) can be determined from the a* and b* values. Hue angle is defined as sets of colour, and equals the arctangent b*/a*. For example, a sample with a hue angle of 0°C is red, 90°C is yellow, 180°C is green and 270°C is blue (McGuire, 1992). Chroma represents colour saturation or purity, and varies from dull (low value) to vivid colour (high value). Chroma is calculated from the square root of the sum of $(a^*)^2$ and $(b^*)^2$.

3.6.3 Extractable red colour

Extractable red colour was measured in units of the American Spice Trade Association (ASTA) according to the ASTA official analytical method 20.1 (Woodbury, 1997). A representative ground sample (about 70 to 100 mg) was placed in a 100 ml volumetric flask and extracted by soaking in 100 ml of acetone for 16 h at room temperature (20-22°C) in the dark. A 10 ml sample of the supernatant was placed in a 1 cm cuvette, and the absorption was recorded at 460nm either with a Pye Unicam PU 8600 UV/Vis spectrophotometer (Philips, Sydney, Australia) or a Cary/1E UV-visible spectrophotometer (Varian-Optical Spectroscopy Instruments, Melbourne, Australia) depending on availability. A standard glass reference (SRM 2030a glass filter 30%-T26 (Figure 3.13), National Institute of Standard and Technology, Gaithersburg, USA) was used at 465nm to determine an instrumental correction factor for each measurement day (Mavrodineanu *et al.*, 1993).

Figure 3.13 Glass standard.

An acetone blank was used for calibration of zero at 460 nm. The final value for each measurement was calculated as:

$$\text{ASTA colour} = \frac{\text{absorbance at 460 nm} \times 16.4 \times \text{instrument correction factor } (I_f)}{\text{sample dry weight (g)}}$$

$$\text{where} \quad I_f = \frac{\text{declared absorbance of glass reference standard}}{\text{absorbance obtained at 465 nm on glass reference standard}}$$

3.6.4 Pungency

The pungency of each sample was estimated by determining the gross capsaicin and dihydrocapsaicin contents. These pungent compounds account for more than 90% of the capsaicinoids in most pungent capsicums and are primarily responsible for the hotness (Todd *et al.*, 1977). The amount of capsaicin and dihydrocapsaicin were evaluated as parts per million ($\mu g.g^{-1}$) using a high-performance liquid chromatographic (HPLC) procedure, and expressed as Scoville Heat Units (SHU). The SHU can be defined as the reciprocal of the highest dilution (threshold) at which a panel would definitely recognize the pungency sensation (Fisher, 1992). SHU are commercially known as the industry standard for levels of hotness.

A 1:10 (g: mL) ratio of dried ground sample to acetonitrile was placed in a 250 mL beaker and blended at 3500 rpm for 3 min using an Omni Mixer Homogeniser (Figure 3.14) (Lomb Scientific Pty. Ltd., NSW, Australia). A clean up step of capsaicinoids was performed using a C_{18} Sep-pak solid phase extraction cartridge (Figure 3.15) (Waters Associates, Sydney, Australia). A 1 mL aliquot was removed and diluted with 9 mL of double-distilled water. The diluted sample (10 mL) was injected into the Sep-pak column that had been pre-conditioned with 5 mL of acetonitrile followed by 5 mL of double-distilled water. The capsaicinoids retained on the Sep-pak were then eluted with 4 mL of acetonitrile followed by 1 mL of acetonitrile containing 1% acetic acid to reduce cloudiness, as previously described by Attuquayefio and Buckle (1987). The sample eluate was then filtered through a 0.45 μm syringe filter (25 mm Polypure Membrane, Alltech Associates, Sydney, Australia), and kept in the freezer until analysis.

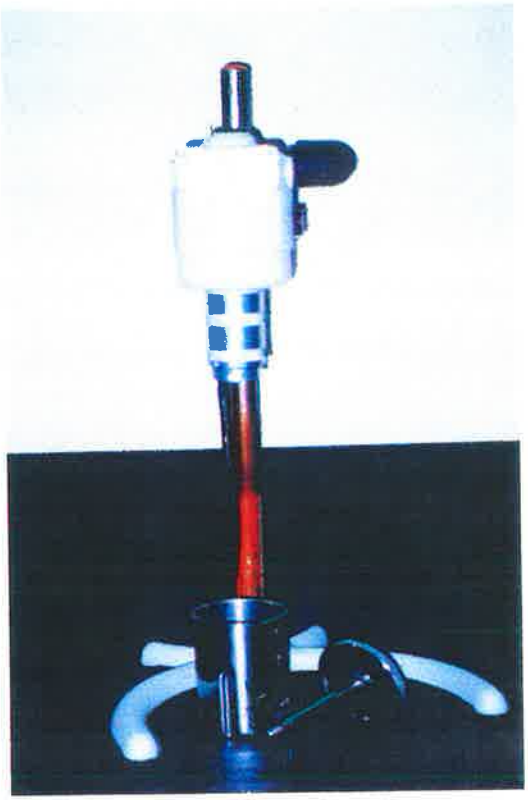


Figure 3.14 Omni homogeniser.

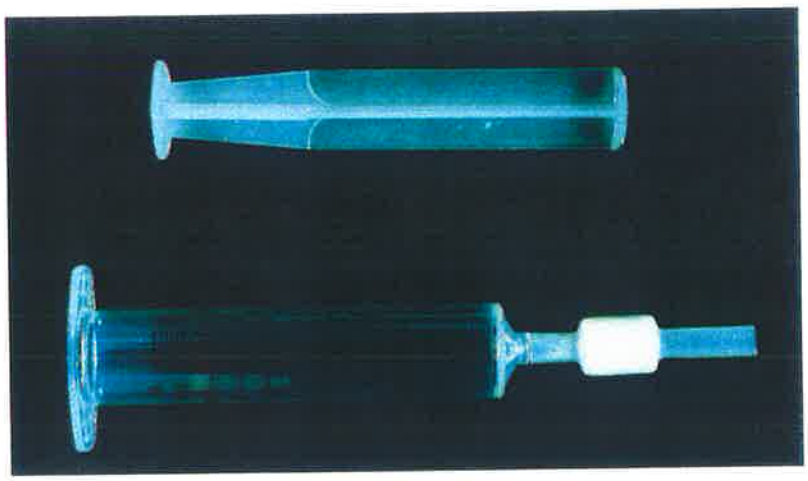


Figure 3.15 C_{18} Sep-pak column.

Capsaicin and dihydrocapsaicin compounds were analysed for estimations of pungency. A modified method of that developed by Attuquayefio and Buckle (1987) was used to quantify the content of both capsaicin and dihydrocapsaicin.

For the growing season of 1996/97, separation was carried out using a Shimadzu LC-10AT pump with a gradient mixer, an SIL-10AXL autosampler, an SPD-10A UV-Vis detector set at 280 nm and a CTO-10A column oven (40°C) (Figure 3.16a). Samples (20 μL) were analysed using a Cosmosil $5C_{18}$-AR (4.6 mm × 150 mm) column (Shimadzu Scientific Instruments, Japan), with a C_{18} guard column. The mobile phase was acetonitrile-water (45:55), with a flow rate of 1 $mL.min^{-1}$. The sample had a run time of 25 min with retention times of about 9.56 min and 13.98 min for capsaicin and dihydrocapsaicin, respectively.

For following growing seasons, a System Gold 126 NM pump, a 507e autosampler, a 168 UV-Vis detector set at 280 nm and a CH-30 column heater (40°C) (Beckman Instruments, Fullerton, CA, USA) (Figure 3.16b) was used. Samples (100 μL) were analysed using a Cosmosil $5C_{18}$-AR (4.6 mm × 150 mm) column (Shimadzu Scientific Instruments, Japan), with a C_{18} guard column. The mobile phase was 100% acetronitrile-1% acetic acid in water (55:45), with a flow rate of 1 $mL.min^{-1}$. The sample had a run time of 20 min with retention times of approximately 5.13 and 6.80 min for capsaicin and dihydrocapsaicin (Figure 3.17a and b), respectively.

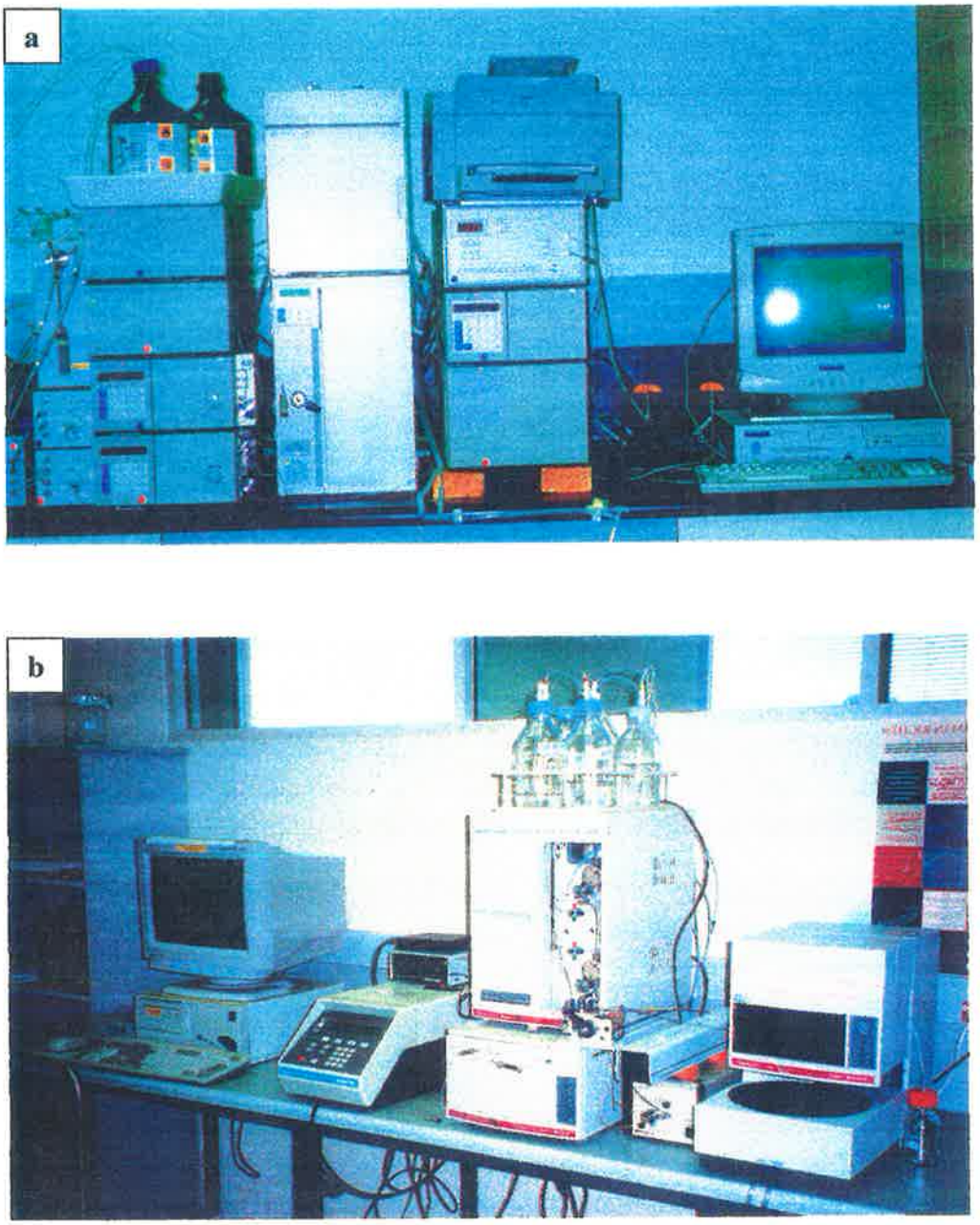


Figure 3.16 The HPLC systems;

(a) at the school of Food Science and Technology, the University of Newcastle, Central Coast Campus, NSW or

(b) at the Department of Horticulture, Viticulture and Oenology, Adelaide University, Waite Campus, South Australia.

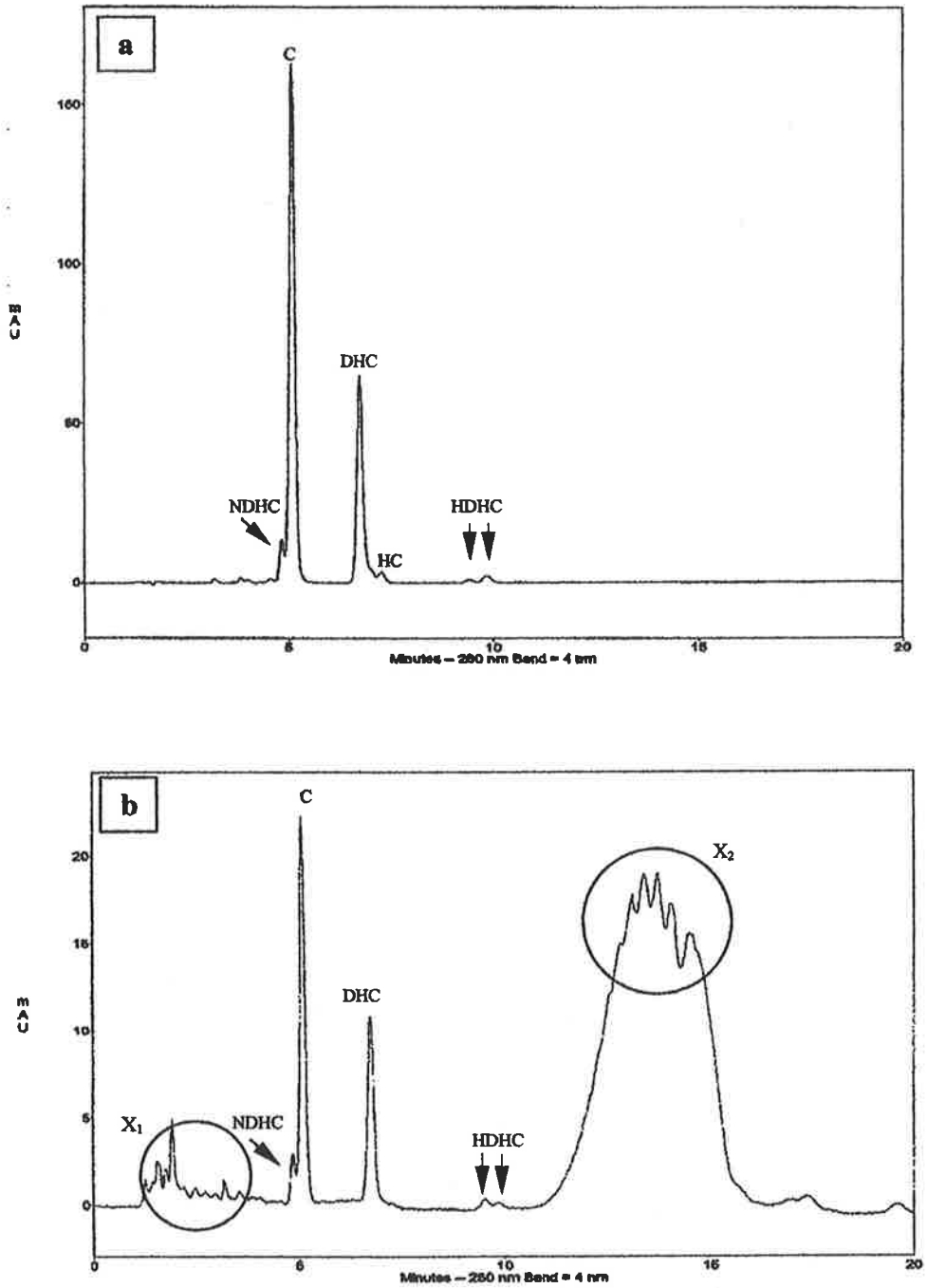
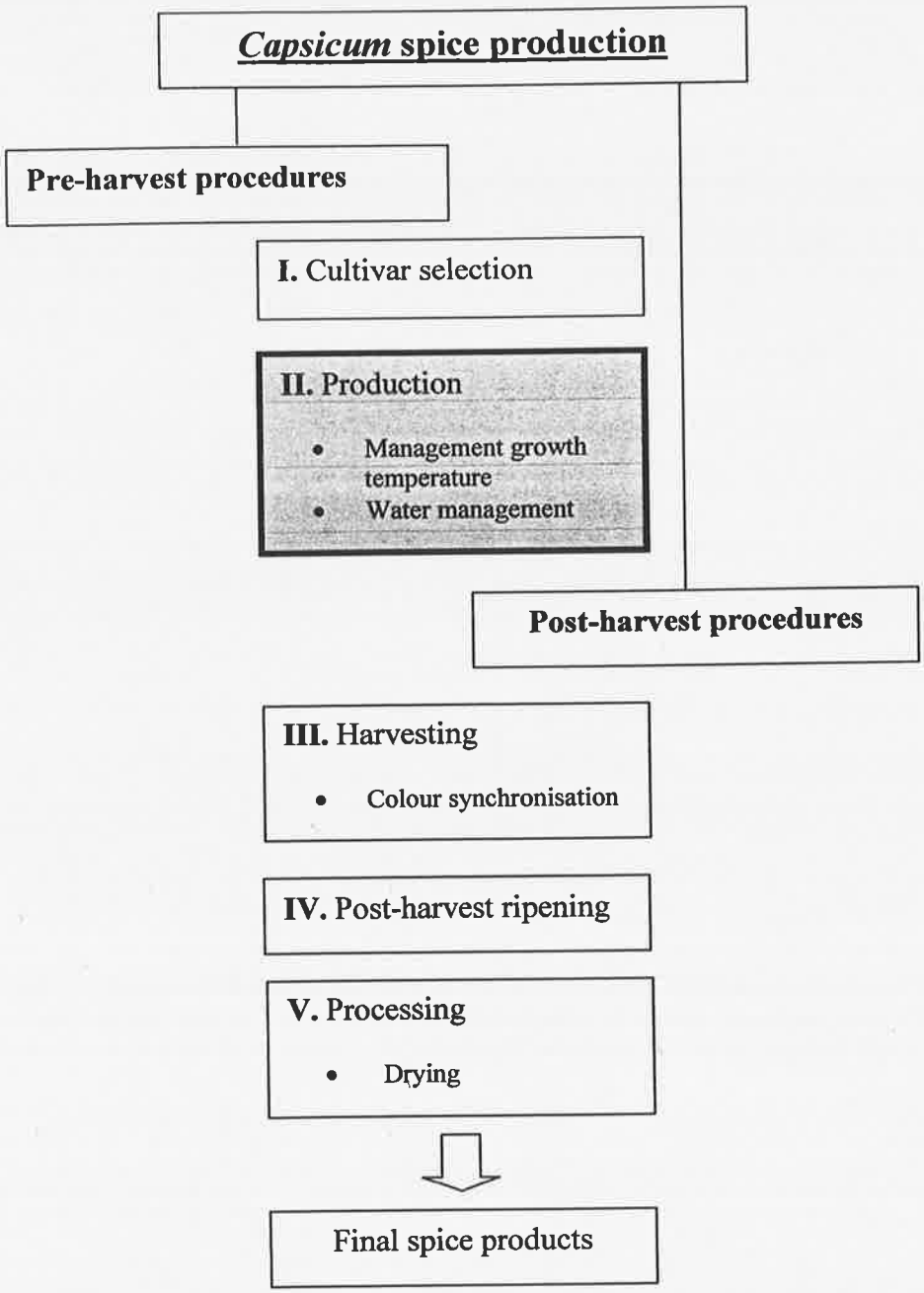


Figure 3.17 Chromatographs of capsaicinoid compounds; (a) standard peaks, and (b) peaks of unknown sample.
Key: NDHC, nordihydrocapsaicin; C, capsaicin; DHC, dihydrocapsaicin; HC, homocapsaicin; HDHC, homodihydrpcapsaicin; $X_1 - X_2$, unknown.

Calibration curves were produced for both compounds using a known standard of 8-methyl-*N*-vanillyl-6-nonenamide (capsaicin) and 8-methyl-*N*-vanillyl-6-nonanamide (dihydrocapsaicin) (Sigma Chemical Co., Sydney, Australia). System specific software was used for measuring peak area. Scoville values were calculated by multiplying the amount of capsaicin and dihydrocapsaicin (in μg) per gram dry weight by 16 million Scoville heat units for pure capsaicin and dihydrocapsaicin (Todd *et al.*, 1977).

3.7 Statistical analysis

All statistical analyses were conducted using GENSTAT 5 for Windows Release 4.1 (Genstat Software, Lawes Agricultural Trust, Rothamsted Experimental Station, England). A general analysis of variance with one-way or two-way analysis was used. The treatment means were compared using least significant differences at $P<0.05$. Each cultivar was evaluated as a separate experiment. Regression analysis was also performed for specific relationships that will be described in the relevant chapters.



The *Capsicum* spice production system.

Areas covered by this chapter are highlighted.

Chapter Four

Response to air temperature

4.1 Introduction

This study was conducted in late summer of 1998, as differences in spice fruit quality, especially of spice pungency, were observed between different growing seasons in previous experiments. For example, spice pungency was relatively high in the summer growing season of 1996/97 (Chapter 7) (20,000 SHU and higher), but lower in the summer of 1997/98 (approximately 10,000 SHU, Chapter 5). Air temperature may potentially be the factor that affected spice pungency as outlined below.

Capsicums are originally warm climate plants. Their growing season can last for more than six to seven months, depending on cultivar and weather conditions. Crop management, such as cultivar selection, and environmental factors that directly affect plant growth therefore affect final fruit quality.

Air temperatures around 25 ± 5°C are recommended for all growth phases of *Capsicum* (Somos, 1984). An absence of frost is also essential for *Capsicum* plant growth and development. Temperatures below 15°C slow growth and over 32°C interfere with fruit setting (Philp and Burne, 1988; Grattidge, 1990), directly influencing final crop yield. The expression of fruit pungency depends on the interaction between genotype and environment (Somos, 1984; Bosland, 1993; Lindsey and Bosland, 1995), and high temperature (~30°C) at the time of fruit maturation has been reported to increase fruit pungency in *Capsicum* (Cotter, 1980). However, little is known about the extent to which temperature can affect the level of fruit pungency while maintaining yield of *Capsicum*; also nothing is known about the effect of air temperature on colour quality. This study, therefore, aimed *to investigate the effects of air temperature, within the desirable range for plant growth, on 'PS72285' paprika and 'Caysan SPS705' chilli fruit yield and spice powder quality.*

4.2 Materials and methods

Experimental design and treatments: Approximately twenty-four days after transplanting when plants started flowering on February 16, 1998 (designated day 0 (D0)) two groups of nine plants of each cultivar were transferred into two separate growth chambers. Plants were randomly arranged in three rows in each chamber, with 30 cm × 30 cm spacing and 0.5 m allowed between cultivars (see Appendix II for plot layout of Figure A1). Air temperature in the first chamber was set to 22/17°C day/night ('low temperature') and in the second chamber to 30/25°C ('high temperature'). Each chamber had a 16 h photoperiod of 800 $\mu mol.m^{-2}.s^{-1}$ light (metal halide), and humidity ranged from 40 to 70% throughout the experiment. Each cultivar was evaluated as a separate experiment (a completely randomised design with nine replications).

Harvesting was performed on May 9, 1998 (D82) and June 27, 1998 (D131) for paprika grown under high and low temperature regimes, respectively. Chilli fruit were harvested on July 1, 1998 (D135) and July 21, 1998 (D155), respectively (see time frame in Appendix I). Dry weight of plant materials was determined. Spice powders from individual plant were then analysed for extractable red colour and pungency as previously described (Chapter 3).

4.3 Results

4.3.1 Plant responses

Plants responded to air temperature within the first week after start of treatments. Initially leaf colour changed for both cultivars, from green to darker green for plants grown under the higher temperature regime, and from green to light/or yellow-green colour under the lower temperature regime.

The soil water content was maintained above 25% volumetric water content throughout the study (Table 4.1).

Table 4.1 Soil water contents (SWC, %) of pot-grown *Capsicum annuum* plants; cv. 'PS72285' paprika and cv. 'Caysan SPS705' chilli grown under two different air temperature regimes. Data were taken during the period of May 4-12, 1999 (D78 to D85), see Chapter 5; section 5.2 for soil water measurement.

Treatments Min/Max	SWC (average, %)
'PS72285' paprika	
25/30°C	26.4 a[z] ±1.6
17/22°C	28.4 a ±1.1
'Caysan SPS 705' chilli	
25/30°C	26.6 a[z] ±1.2
17/22°C	28.6 a ±1.1

[z] The difference among means within the same column for each cv. based on Least Significant Differences (LSD) at $P<0.05$ (n=9; ± SE).

In addition, shoot elongation and internodal length were reduced at the low temperature regime for both cultivars (Figure 4.1a,b). All plants flowered heavily under either temperature regime and continued flowering throughout the experiment (Figure 4.1b, c). However, the harvesting date of both cultivars was delayed at low temperatures due to delayed fruit colour development from 106 days to 159 days after transplanting, and from 159 days to 179 days after transplanting for paprika and chilli, respectively.

Figure 4.1 Pot grown *Capsicum* cv. 'PS72285' paprika:

(a) plants were raised in the glasshouse at 25°C day and night prior to transfer to the growth chamber - normal green leaves and healthy shoot development were observed.

(b) plants after transfer to 22/17°C (day/night) with a constant 16 h photoperiod of 800 $\mu mol.m^{-2}.s^{-1}$ light - within one week leaves turned light green, plants had short internodes and massive flowering on the upper nodes within three weeks.

(c) plants after transfer to 30/25°C (day/night) with a constant 16 h photoperiod of 800 $\mu mol.m^{-2}.s^{-1}$ light - within one week leaves turned to dark green; but pale green afterward, plants had long internodes and also massive flowering.

4.3.2 Yield and yield components

Air temperature did not affect total or marketable fruit yield of either cultivar (Table 4.2), but unmarketable fruit yield was reduced in chilli grown in lower temperature. Mean fruit weight of marketable fruit was not affected by temperature, but unmarketable fruit were smaller at the low temperature for both cultivars. Shoot dry weight was increased at low temperature for paprika, but not for chilli (Table 4.2).

Table 4.2 Effect of air temperature regime on yield components of paprika cv. 'PS72285' and chilli cv. 'Caysan SPS705'.

Treatments	Fruit dry weight (g.plant^{-1})			Individual fruit dry weight		Shoot dry weight
				Marketable	Unmarketable	
(Day/Night)	Marketable	Unmarketable	Total	(g.fruit^{-1})	(g.fruit^{-1})	(g.plant^{-1})
	'PS72285' paprika					
30/25°C	14.6 a[z]	6.1 a	20.7 a	19.5 a	15.0 a	18.8 b
22/17°C	17.8 a	6.9 a	24.6 a	16.9 a	8.1 b	26.5 a
	'Caysan SPS 705' chilli					
30/25°C	24.9 a[z]	15.9 a	40.8 a	5.9 a	8.5 a	25.5 a
22/17°C	25.0 a	6.6 b	31.6 a	7.3 a	3.5 b	28.8 a

[z] Values are means for 9 plants. Different letters within a column for each cultivar show significant differences (P<0.05) based on Least Significant Differences.

Total fruit number or marketable fruit number was not affected by air temperature at the end of the growing season, but there were fewer green fruit and more immature fruit at low temperatures for both cultivars (Table 4.3).

Table 4.3 Effect of air temperature regime on fruit numbers on paprika cv. 'PS72285' and chilli cv. 'Caysan SPS 705' plants.

Treatments	Numbers of fruit by colour categories (#.plant^{-1})				
(Day/Night)	Marketable	Breaker	Green	Immature	Total
	'PS72285' paprika				
30/25°C	6.6 a[y]	1.9 a	2.4 a	0.3 b	11.2 a
22/17°C	8.0 a	1.7 a	0.0 b	2.8 a	12.5 a
	'Caysan SPS 705' chilli				
30/25°C	17.8 a[y]	2.0 a	14.4 a	0.4 b	34.6 a
22/17°C	20.3 a	2.4 a	2.9 b	7.2 a	32.8 a

[z] Shoot dry weight = stem + leaf + flower dry weight.
[y] Values are means for 9 plants. Different letters within a column for each cultivar show significant differences (P<0.05) based on Least Significant Differences.

4.3.3 Quality

Spice colour was not affected by air temperature (Table 4.4). However, marketable chilli fruit had 40% higher pungency in the spice when grown at the high temperature (Table 4.4). Paprika were only very mildly pungent, and this was unaffected by air temperature.

Table 4.4 Effect of air temperature regime on spice colour and pungency of paprika cv. 'PS72285' and chilli cv. 'Caysan SPS 705' fruit.

Treatments	Marketable fruit	
(Day/Night)	Colour (ASTA)[z]	Pungency (SHU)[y]
	'PS72285' paprika	
30/25°C	252 a[x]	544 a
22/17°C	230 a	550 a
	'Caysan SPS 705' chilli	
30/25°C	162 a[x]	13,366 a
22/17°C	181 a	9,534 b

[z]American Spice Trade Association colour units.
[y] Scoville Heat Units were calculated by multiplying the amount of capsaicin and dihydrocapsaicin per gram dry weight by 16 million Scoville units for pure capsaicin and dihydrocapsaicin.
[x] Values are means for 9 plants. Different letters within a column for each cultivar show significant differences ($P<0.05$) based on Least Significant Differences.

4.4 Discussion

4.4.1 Plant development

Although air temperature had no effect on dry matter partitioning in vegetative organs of chilli plants, more dry matter was partitioned into the shoots of paprika plants grown at lower temperatures. This is probably due to increased night respiration at the higher temperature regime (Somos, 1984), and therefore dry matter accumulated during the day was lost again resulting in a reduced net gain in dry matter. This also suggests that the response to air temperature varied with cultivar, as previously described by Philp and Burne (1988).

In the case of plants in the genus *Capsicum*, individual plant organs develop at the same time throughout the growing season. For example, once plants have branched, flowering begins to form. First flowering will occur on the bottom node; as the next nodes keep developing vegetatively, further flowering also will occur at those nodes in due course.

From this study, it appears that temperature had a greater effect on vegetative growth (node elongation, shoot dry weight) than on reproductive growth (fruit number, fruit weight, final fruit yield).

Overall, temperatures within the ranges of 17-22°C and 25-30°C were equally favourable for flowering, fruit setting and yield of both cultivars, although plant development was slightly affected at lower temperatures. Temperatures ranging from 16-27°C are generally recommended for flowering and fruit set of plants in the genus *Capsicum*; however, above 32°C plants did not set any fruit. High night temperatures of 16-21°C were found to induce massive flowering for *Capsicum* cv. 'Delaware Bell' and 'Pennwonder' (Somos, 1984). This may explain why heavy flowering was also observed for both cultivars under growth temperatures in this study.

4.4.2 Fruit colour development and fruit yield

Growth temperature significantly influenced fruit colour development. This was shown by an earlier harvest as fruit colour development was accelerated by higher air temperatures. Philp and Burne (1988) also found that temperatures above 25°C accelerated fruit colour development for most *Capsicum* spp. Although higher temperatures did not affect the fruit appearance in this study, fruit exposure to long periods above 27°C caused *Capsicum* fruit to develop a yellowish colour (Philp and Burne, 1988). Therefore careful management must be considered when plants may be exposed to high air temperatures.

Although fruit yield and total fruit number was not different between the two temperature regimes, plants grown at the lower temperature yielded more unmarketable fruit (e.g. immature fruit). This was perhaps due to a prolonged growing period and concurrent slower fruit colour development.

4.4.3 Spice quality

Although, the temperature regimes had no effect on spice colour for both cultivars in this study, pungency of chilli fruit was reduced under the low air temperature regime. While accumulation of pungent compounds varies with variety and season (Bosland, 1993), higher air temperatures (35/21°C compared to 25/10°C) also increased pungency in a study by Levy *et al.* (1989). Somos (1984) also found that pungency was increased at air temperatures of 30°C, but reduced at lower temperatures of 21-24°C in fruits of all *C. annuum* plants.

Recently, the expression of capsaicinoid pathway genes; *Pal*, *Ca4h* and *Comt*, have been monitored in developing *C. annuum* and *C. frutescens* fruit, and the expression levels for these three genes were found positively correlated with the accumulation of pungent compounds in placenta tissue of *Capsicum* fruit (Curry *et al.*, 1999). Since the expression of pungent compounds is responsive to environmental stress (Harvell and Bosland, 1997), it may be possible that the higher temperature regime in this study could influence these genes, and changes in levels of pungency might occur. Pungency of paprika fruit did not vary with air temperature, but it was very low due to the genetic background of this cultivar. More research at the molecular levels of enzyme expression involved in the capsaicinoid biosynthesis interacting with temperature effect may be useful.

Essential differences in pungency development were observed previously between *Capsicum* species (Somos, 1984). For instance, *C. pubescens* accumulated maximum pungency ten weeks after flowering, while in *C. annuum, C. frutescens* and *C. pendulum* this occurred three weeks after flowering (Somos, 1984). This supports the idea that the accumulation of pungency is sensitive to temperature effects at an early stage of fruit development. However, fruit colour content of both cultivars appeared to be very stable, as it was not affected by air temperature in this study.

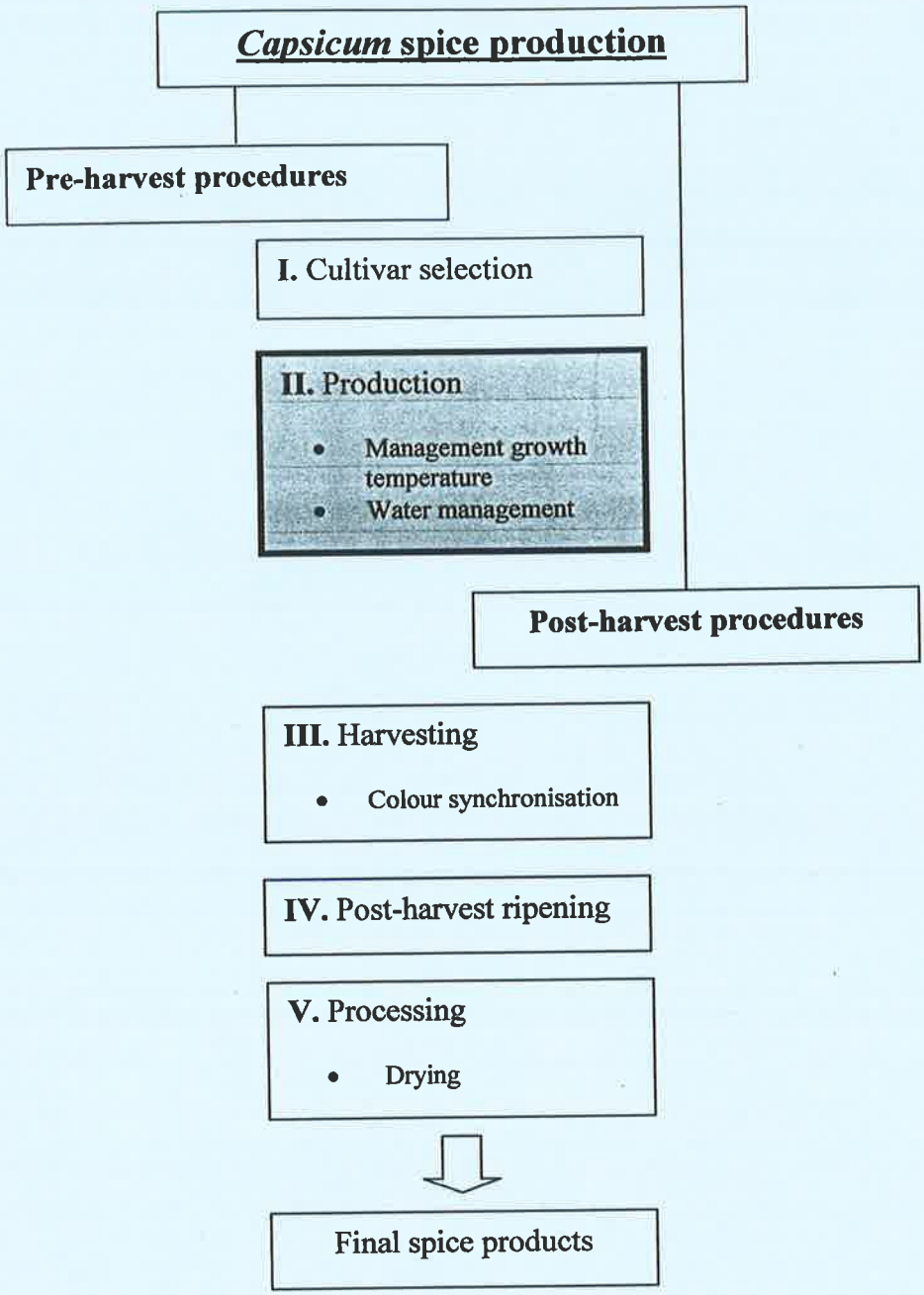
4.5 Conclusion

a) Air temperature treatments of 22/17 or 30/25°C (day/night) in this study were suitable for *Capsicum* fruit production, even though at lower temperatures plants appeared visually less healthy.

b) Neither total fruit weight nor fruit yield components were affected by temperature regimes of 22/17 and of 30/25°C (day/night). However, shoot dry weight of paprika was 29% lower at the higher temperature, although this was not observed for chillies. The internodal length seemed to be very short for both cultivars of *Capsicum* plants grown under lower air temperature, although no actual measurements were taken.

c) Pungency levels of chilli, but not of paprika, increased by 40% under higher air temperatures.

d) Colour pigment content for both cultivars was unaffected, but colour development was slower at lower temperatures.



The *Capsicum* spice production system.

Areas covered by this chapter are highlighted.

Chapter Five

Response to water stress

5.1 Introduction

The *Capsicum* plant is known to be sensitive to water stress (Simpson, 1981). In most cases, plant vigour and productivity are influenced by this stress factor (Simpson, 1981; Techawongstien *et al.*, 1992; Jaimez *et al.*, 1999). The sensitivity of plant response to water stress appears to be not only dependent on cultivar, but also on the stage of plant development when plants experience the stress. Salter and Goode (1967) (cited in Techawongstien *et al.*, 1992) stated that flowering is the most sensitive stage to water stress in most horticultural crops, with fruit and seed development being sensitive to a lesser extent. This also applies to *Capsicum* plants; however, an overlap between vegetative growth and reproductive processes is normally observed for a perennial plant like *Capsicum* (Techawongstien *et al.*, 1992). This is shown by the continuous flowering throughout their growing season; therefore variation among stages of reproductive growth commonly occurs on any one plant from mature fruit setting early at the first node, to flowering at the later nodes. If the water stress occurs during the reproductive growth stage, crop yield can be reduced and this is mainly due to flower and fruit abscission (Grattidge, 1990; DeWitt and Bosland, 1993). Although it is difficult to separate growth phases, water stress under experimental conditions has to be applied at a definable stage. Therefore, to reduce effects on yield, water stress was applied after fruit set had occurred at the fourth branching node. This represents an investigation of the reproductive phase, since it is a middle node of the plant and the duration from flowering to fruit setting are not much different from the first three nodes.

While yield reduction appears to be the final outcome of water stress, other earlier physiological responses have been shown: for example, reduction in leaf water potential (Ψ_L) (Aloni *et al.*, 1991), reduction in stomatal conductance (g_S) (Wullschleger and Oosterhuis, 1991), and some visible wilting of plant leaves (Somos, 1984). Crop quality is also commonly affected. Half irrigation compared to normal control irrigation increased pungency in some cultivars of *Capsicum* fruit (Quagliotti, 1971; Levy *et al.*, 1989), but not for the cv. 'Shany Matok' (Levy *et al.*, 1989). A short water stress prior to harvest

positively affected fruit quality in some crops such as apple (Kilili *et al.*, 1996). There may be a beneficial effect on *Capsicum* fruit quality if water stress can be applied to the *Capsicum* plant at the right growth phase. The effect of drought stress on spice colour and pungency is not fully understood, and information is inconsistent with differences between plant species or even plant cultivars (Quagliotti, 1971). The following experiment therefore *investigated the effects of soil water stress applied after fruit set at the fourth node, on spice colour, pungency and yield of pot-grown paprika cv. 'PS72285' and cayenne chilli cv. 'Caysan SPS705'.*

5.2 Materials and methods

Experimental design: Plants for each cultivar were randomly assigned into experimental Units of nine plants, using three rows with 45 cm x 45 cm spacing and 0.5 m allowed between cultivars (see Figure A2 in Appendix II for plot layout). Three replications of three plants each were used for each treatment. Experiments were all conducted in the glasshouse during the summer of 1997/98 (details in Appendix I).

Treatments: In order to induce different levels of water stress, three irrigation treatments were conducted that consisted of irrigation frequencies of every 2, 4 or 6 days (2D, 4D or 6D). Each pot was irrigated until water started running out from the bottom of that pot. Treatments commenced on March 24, 1998 (designated day 0 (D0)) and March 31, 1998 (designated day 0 (D0)) for paprika and chilli, respectively, when maximum fruit set had been obtained at the fourth node, and were continued until harvest.

Soil water measurement: Volumetric soil water content (SWC, %) was measured prior to each irrigation (16:00 h) using a time-domain reflectometer [TDR] (Trase, Soil Moisture Equipment Corp., Santa Barbara, CA, USA) with a 15 cm wave guide inserted vertically from the soil surface. Measurements were taken from D23 to D61 and from D16 to D55 for paprika and chilli, respectively.

Soil water release curve: Ten healthy pot-grown chilli plants (no damage from pests and diseases) at the reproductive stage (~40 days after transplanting (DAT) were randomly selected for determination of the soil water release curve. To measure soil water potential (Ψ_{soil}, kPa), a 15 cm long Jet Fill Tensiometer (Model 2725AR, Soil Moisture Equipment Corp., Santa Barbara, CA, USA) was permanently inserted into each pot vertically from the soil surface to a depth of 11 cm (Figure 5.1), and measurements were taken daily. Pots were then left to dry until plants showed signs of severe wilting (approximately 4 days, Figure 5.2). Watering was then resumed in all pots and continued for 12 days. Plant response and SWC using TDR (as previously described) were recorded daily. Plots of SWC versus Ψ_{soil} were performed to estimate the soil water release curve.

Figure 5.1 Tensiometer installation to a depth of 11 cm into the soil for measurement of the soil moisture potential.

Figure 5.2 Watering was applied for the soil water release study when plants showed severe wilting.

Leaf water potential: Leaf water potential (Ψ_L, MPa) was measured using a Scholander pressure chamber (Scholander *et al.*, 1965) at various times during the experiment. To obtain a reading, an individual leaflet was collected from each plant, covered by a plastic bag to reduce transpiration, and placed immediately into the chamber. A reading was recorded within a minute when xylem sap appeared around the vascular bundle of the petiole. One reading was taken for each plant to avoid plant damage from taking too many leaves, mainly between 10:00 to 11:00 h for each measurement. Measurements were taken on D51, D57 and D62 for paprika, and on D44, D50 and D55 for chilli.

Stomatal conductance: To measure the stomatal conductance (g_s, $mmol.m^{-2}.s^{-1}$), randomly selective three plants were used per treatment, and four fully expanded leaves per plant were randomly selected and labelled. A porometer (AP4-Delta-T Devices, Cambridge, UK) was used to measure the stomatal conductance on the abaxial surface of the leaves, mainly between 10:00 to 12:00 h (noon) for each measurement. Measurements were taken from D63 to D69 and from D56 to D62 for paprika and chilli, respectively.

Harvesting: Fruits were hand harvested on D70 and D111 for paprika and chilli, respectively. Each individual plant was also cut off at the soil surface on the next day after harvesting fruits. Shoots and roots were separated. Dry weight of all plant parts, including fruits, was recorded. A representative sample of spice powder was then analysed for chemical analysis following the same procedure as previously described in Chapter 3.

5.3 Results

5.3.1 Soil water status

Soil water release curve: For the sandy potting mix used in this study (described in Addendix IV), regression analysis showed a significant relationship ($P<0.001$, $r^2 = 0.67$) between Ψ_{soil} and volumetric SWC, with the best fitted model of:

$$SWC\ (\%) = 8.17 + 13.57\ (1.17)^{\Psi soil\ (kPa)} \qquad (1)$$

As shown in Figure 5.3, a SWC above 11% was found to be in the range of 'field capacity' as indicated by a Ψ_{soil} of >-10 kPa . However, the minimum amount of water in the soil must be maintained above 9%, that is a Ψ_{soil} of -20 kPa, to prevent plant damage. Below this point, plants suffered from wilting, and with prolonged soil drying, plants showed severe wilting when the SWC fell below 8%, that is a Ψ_{soil} of less than -40 kPa (Figure 5.3).

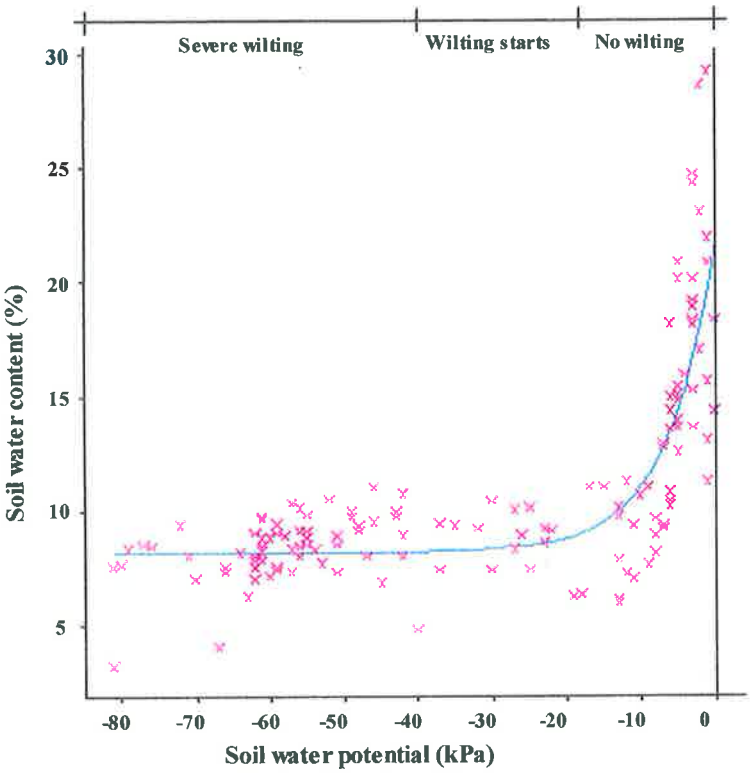


Figure 5.3 Relationship between soil water potential (Ψ_{soil}, kPa) and soil water content (SWC, %) for 'Caysan SPS705' chilli grown in sandy potting mix in a greenhouse. Data were collected on ten plants. $\Psi_{soil} = 8.17 + 13.57(1.17)^{SWC}$ ($r^2 = 0.67$, $P<0.001$).

Experimental water status: During the first four weeks of the experiment, 4D and 6D treatment plants showed signs of wilting at the end of each irrigation cycle when SWC declined below 8%. However, after rewatering SWC did not increase to a maximum of 30%, but remained lower than 20% for both treatments. Accordingly, approximately one month after commencement of the experiment, all plants were irrigated twice on the day of irrigation in order to re-establish the maximum SWC of approximately 30%.

The typical SWC in the latter half of the experiment for one cycle of a 2D, 4D or 6D irrigation treatment for paprika is shown in Figure 5.4; this was also representative of the chilli experiment. The maximum SWC was at approximately 30% for all treatments after rewatering (Figure 5.4). The soil was then left to dry resulting in a minimum SWC of 26, 19 or 15% for 2D, 4D or 6D, respectively, immediately prior to rewatering.

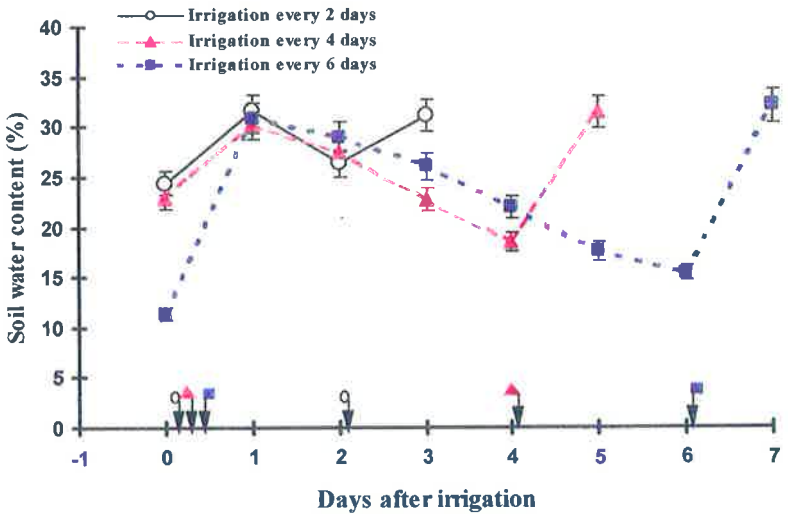


Figure 5.4 Typical soil water content of pot-grown *Capsicum annuum* plants (cv. 'PS72285' paprika pepper) under 2-day (O), 4-day (▲) or 6-day (■) irrigation frequency. Data are from days 38 to 46 after the start of the experiment and have been adjusted to the same time scale relative to the first application of irrigation. Arrows indicate time of irrigation for each treatment. Each data point is the mean of nine plants per treatment. Error bars show 2 x SE.

The average SWC maxima and minima throughout the paprika experiment were 29% and 21% for 2D, 28% and 15% for 4D, and 28% and 10% for 6D treatments. Similarly, in the chilli experiment, the values were 30% and 22% for 2D, 28% and 16% for 4D, and 27% and 11% for 6D treatments.

5.3.2 Plant response to water stress

Leaf water potential: Irrigation treatment had a significant effect on leaf water potential (Ψ_L) of both cultivars. The Ψ_L of 2D and 4D plants did not differ significantly throughout the experiment, ranging from -0.3 to -0.6 MPa for both cultivars. However, the 6D treatment had a much reduced Ψ_L on days when the SWC fell below that of the other treatments. Ψ_L of paprika declined to -1.0 MPa for the 6D treatment compared to –0.3 MPa for the 2D treatment. At this time SWC was 13% and 20% for 6D and 2D treatments, respectively. As the experiment progressed, Ψ_L increased to –0.5 to –0.6 MPa for the 6D treatment, and this was not significantly different to the other treatments.

A similar response was observed in chilli plants; Ψ_L initially declined to –1.2 MPa for 6D, significantly less than the 2D treatments (–0.5 MPa). This corresponded to a SWC of 10% and 16% for 6D and 2D treatments, respectively. As the experiment progressed, the Ψ_L did not differ between treatments and remained higher than –0.5 MPa, with the SWC 13% or higher.

Combined data for paprika and chilli experiments over two days of measurements were used to determine the relationship between SWC and Ψ_L. Separate fitted curves for each cultivar did not improve this relationship. The best fitted model could be described as

$$\Psi_L \text{ (MPa)} = -0.36 + (-12.00)\ (0.73)^{SWC(\%)} \qquad (2)$$

(Figure 5.5), ($r^2 = 0.39$, $P<0.001$).

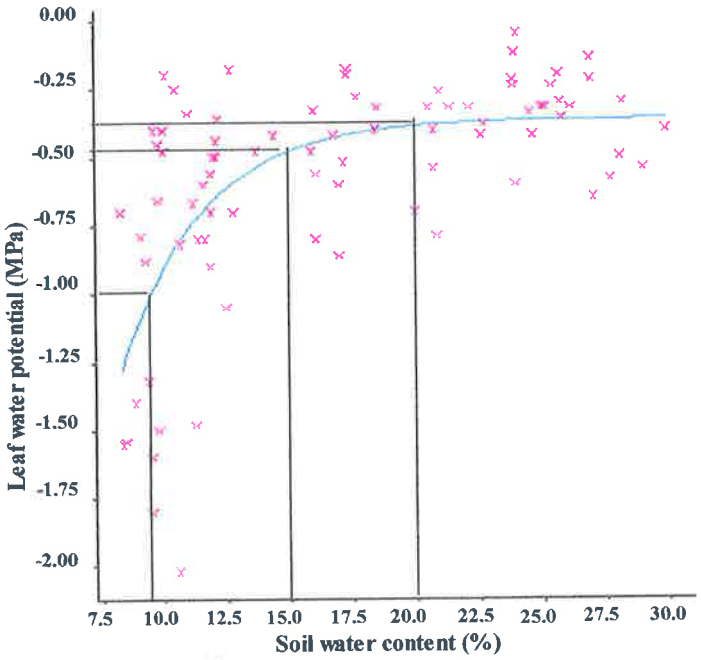


Figure 5.5 Relationship between SWC (%) and Ψ_L (MPa) of pot-grown *Capsicum* plants subjected to 2-, 4- or 6-day irrigation frequency. Data were recorded over two days (May 14, and May 20, 1999). $\Psi_L = -0.36 + (-12.0)\ (0.73)^{SWC}$ ($P<0.001$, $r^2 = 0.39$).

Ψ_L was maintained at -0.38 MPa and higher when SWC was greater than 20%. When SWC was between 15% and 20%, Ψ_L declined slightly to -0.47 MPa. However, a large decrease in Ψ_L occurred when SWC was less than 15%. At a SWC of 9%, plants showed signs of wilting and the Ψ_L was -1.0 MPa or lower.

Stomatal conductance: Changes in stomatal conductance (g_S) over an irrigation period are shown in Figure 5.6. The g_S increased immediately in response to irrigation, and then decreased for all treatments as water was withheld until the end of the irrigation period. For paprika, the g_S of 4D plants was 50% to 80% lower than that of 2D, and for 6D plants 60% to 90% lower than 2D over the irrigation period (Figure 5.6a). For chilli, the average g_S over the irrigation period for 4D and 6D plants was 50% and 53% lower than that of 2D plants (Figure 5.6b).

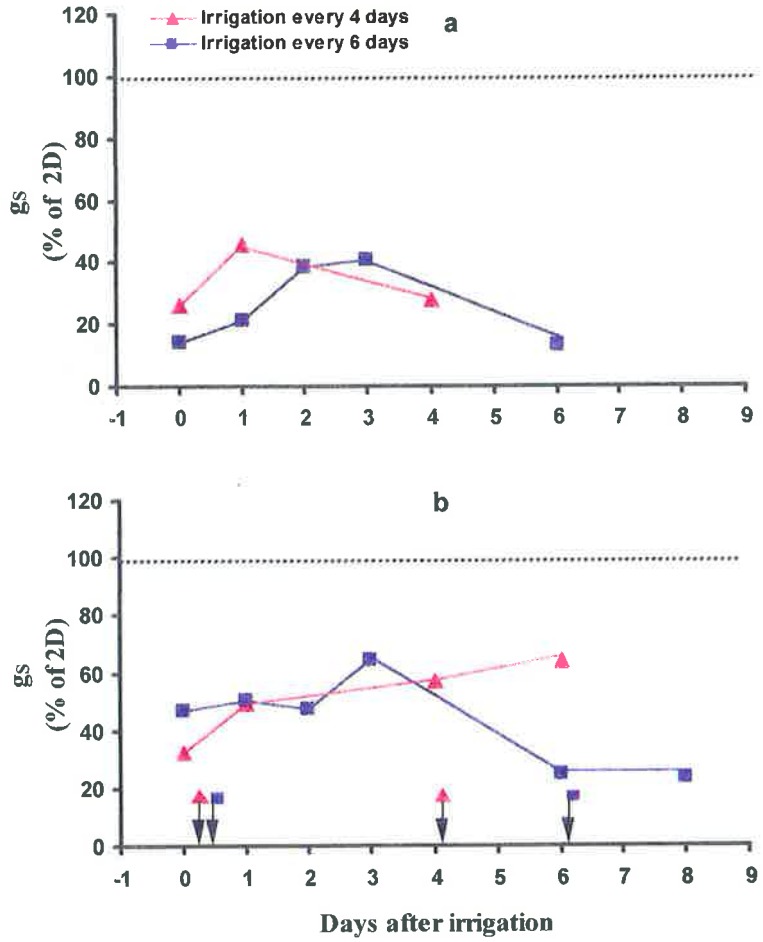


Figure 5.6 Stomatal conductance (g_S, mmol.m^{-2}.s^{-1}) of pot-grown *Capsicum annuum* plants; (a) cv. 'PS72285' paprika and (b) cv. 'Caysan SPS705' chilli. Irrigation frequencies were 2D, 4D or 6D. Each data point is the mean of three plants per treatment of 4D (▲) and 6D (■) irrigation treatments, and expressed as % of 2D. Data are from day 63 to 68 and day 56 to 62 after the start of experiment for paprika and chilli respectively and have been adjusted to the same time scale relative to the first application of irrigation. Arrows indicate time of irrigation of each treatment.

Growth habit: At the end of each irrigation cycle 4D and 6D plants of both cultivars showed signs of wilting; this corresponded to a SWC of less than 9% (Figure 5.7), or Ψ_L of -1.0 MPa or less. However, there was no obvious wilting of 2D plants. The severity of stress was dependent on irrigation frequency. From observation, flowers, flower buds and immature fruits on upper nodes (distal to node 4) abscised more on 6D and 4D plants than 2D, and this was more severe for paprika. A branched canopy with continuing flowering was observed in 2D plants, while both 4D and 6D plants had stunted, compact canopies, particularly the latter.

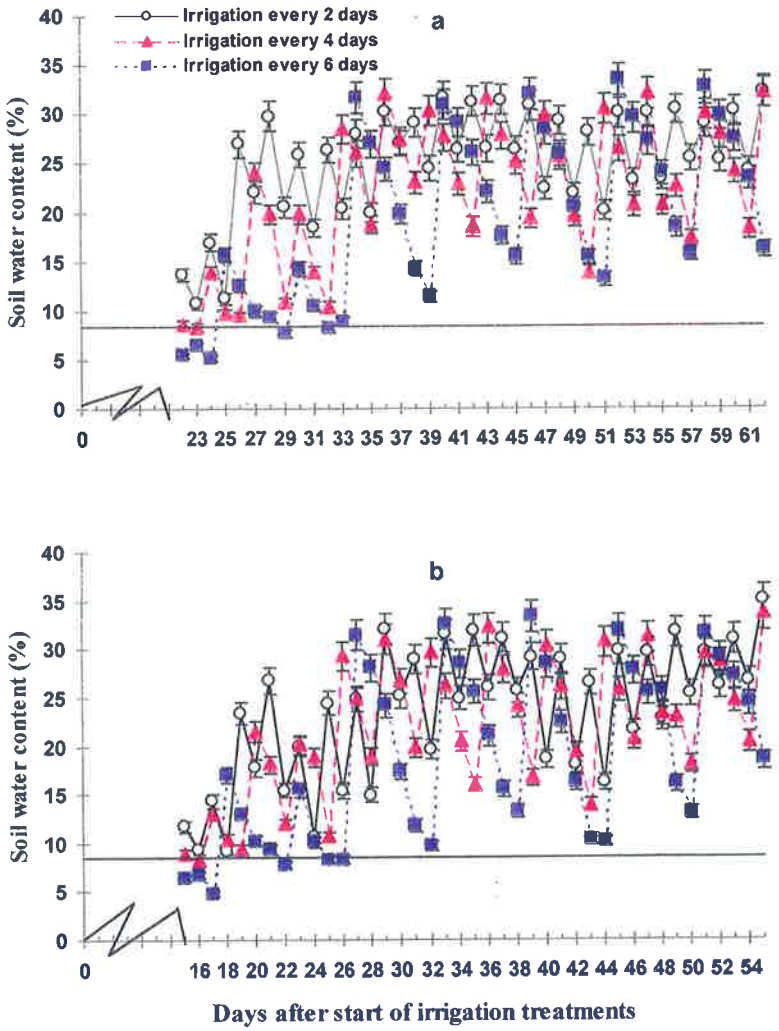


Figure 5.7 Time course of soil water content of pot-grown *Capsicum annuum* plants (a) cv. 'PS72285' paprika pepper and (b) chilli cv. 'Caysan SPS705' under 2-day (O), 4-day (▲) or 6-day (■) irrigation frequency. Measurements were taken from D22 to D62 and from D14 to D56 for paprika and chilli respectively. Each data point is the mean of nine plants per treatment. Error bars show 2 x SE.

5.3.3 Yield and yield components

Irrigation frequency had a significant effect on both fruit yield and plant dry weight for both cultivars (Table 5.1).

Table 5.1 Effect of irrigation frequency on yield of paprika cv. 'PS72285' and chilli cv. 'Caysan SPS 705' plants and fruits.

Irrigation frequency (Days)	Fruit dry weight ($g.plant^{-1}$)			Plant dry weight ($g.plant^{-1}$)		Shoot/root
	Marketable	Unmarketable	Total	Shoot	Root	
	'PS72285' paprika					
2	16.3 a[z]	8.7 a	25.0 a	39.3 a	15.8 a	3.2 b
4	14.3 ab	4.9 b	19.2 b	32.2 b	8.2 b	4.6 ab
6	11.0 b	3.1 b	14.2 c	26.5 b	5.2 b	5.2 a
	'Caysan SPS 705' chilli					
2	32.1 a[z]	12.6 a	44.6 a	38.8 a	15.6 a	3.0 b
4	19.4 b	6.7 a	26.1 b	27.0 b	7.8 b	4.4 ab
6	12.5 c	9.8 a	22.3 b	30.4 b	5.9 b	5.6 a

[z] The difference between means within the same column for each cv. is based on Least Significant Differences (LSD) at $P<0.05$.

For paprika, yields of marketable, unmarketable and total fruit dry weight decreased significantly as the period of soil drying increased. For 6D plants, a 33, 56 and 43% reduction of marketable, unmarketable and total fruit dry weights, respectively, was observed compared to that of 2D plants (Table 5.1). A reduced fruit number was the major component responsible for the yield reduction (Table 5.2). Dry weight of shoots and roots was also reduced for 4D and 6D plants relative to 2D plants. The ratio of shoot to root dry weights of 6D plants increased significantly compared to the 2D plants, but did not differ to the 4D plants (Table 5.1). Total plant dry weight (total fruit yield + shoot dry weight + root dry weight) was reduced by 26% and 43% for 4D and 6D plants, respectively, compared to 2D plants.

Table 5.2 Effect of irrigation frequency on yield components of paprika cv. 'PS72285' and chilli cv. 'Caysan SPS 705'.

Irrigation frequency (Days)	Numbers of fruit by colour categories (#.plant^{-1})					Individual fruit fresh weight	
	Marketable	Breaker	Green	Immature	Total	Marketable (g.fruit^{-1})	Unmarketable (g.fruit^{-1})
	'PS72285' paprika						
2	6.3 a[z]	6.8 a	5.3 a	6.8 a	19.0 a	18.4 a	8.9 a
4	6.0 a	4.8 a	1.3 b	4.8 a	12.7 b	17.0 a	6.8 a
6	5.0 a	0.9 b	0.6 b	0.9 b	7.7 b	16.5 a	7.7 a
	'Caysan SPS 705' chilli						
2	20.67 a[z]	2.8 a	7.6 a	0.56 a	31.6 a	7.9 a	8.9 a
4	10.67 b	1.2 a	3.8 a	0.11 a	15.8 b	8.2 a	9.7 a
6	7.89 b	1.3 a	6.7 a	0.56 a	16.4 b	7.8 a	7.9 a

[z] The difference between means within the same column for each cv. is based on Least Significant Differences (LSD) at $P<0.05$.

A similar response was observed for chilli plants. Yields of marketable, unmarketable and total fruit dry weights were reduced by 61, 22 and 50%, respectively, for 6D plants compared to 2D (Table 5.1). The yield reduction was due to lower fruit numbers rather than reduced individual fruit weight (Table 5.2). Shoot and root dry weights were also lower in 6D plants (Table 5.1). The shoot to root ratio was significantly increased for 4D and 6D plants compared to 2D plants (Table 5.1). Total plant dry weight of 4D and 6D plants was 38% and 41% lower than 2D plants.

5.3.4 Fruit quality

The individual fresh fruit weight was not affected by any treatment for either cultivar (Table 5.2). Unmarketable paprika fruit, including immature fruit, weighed less than red marketable fruits, but this was not obvious for chilli fruit (Table 5.2). The fresh weight of marketable paprika fruit ranged from 17 to 18 g per fruit, while for chilli it was about 8 g per fruit (Table 5.2).

The three irrigation frequencies in this study had no effect on colour intensity of paprika and chilli spices (Table 5.3). Average colour intensity ranged from 192 to 225 ASTA for paprika, and from 128 to 147 ASTA colour units for chilli (Table 5.3). However, variability of colour of paprika spices from individual plants was high, with a range of 120 to 284 ASTA. In chilli spices, there was less variability of 102 to 170 ASTA colour units.

Table 5.3 Effect of irrigation frequency on quality of spice paprika cv. 'PS72285' and chilli cv. 'Caysan SPS 705'.

Irrigation frequency (Days)	Marketable fruit	
	Colour (ASTA)[y]	Pungency (SHU)[z]
	'PS72285' paprika	
2	225.3 a[x]	807 a
4	204.3 a	770 a
6	191.6 a	905 a
	'Caysan SPS 705' chilli	
2	146.9 a[x]	10,557 a
4	127.9 a	9,425 a
6	132.4 a	11,554 a

[z] Scoville heat units were calculated by multiplying the amount of capsaicin and dihydrocapsaicin per gram dry weight by 16 million Scoville units for pure capsaicin and dihydrocapsaicin.
[y] American Spice Trade Association colour units.
[x] The difference between means within the same column for each cv. is based on Least Significant Differences (LSD) at $P<0.05$.

Similarly, pungency of mild paprika and hot chilli spices did not vary with irrigation frequency, ranging from 770 to 905 SHU for paprika and 9,425 to 11,554 SHU for chilli (Table 5.3). There was a large variation within treatments for both cultivars. For paprika spices, individual plants ranged from 239 to 2,198 SHU, and for chilli from 4,042 to17,486 SHU.

5.4 Discussion

5.4.1 Assessment of water stress during experiment

Water stress can be defined as a physiological reaction of plants to a limitation in supply of water (Goodwin, 1995). In the case of *Capsicum* plants, several physiological responses have been previously reported. These include the closing of leaf stomata (Aloni *et al.*, 1991; Wullschleger and Oosterhuis, 1991), reduced photosynthesis (Aloni *et al.*, 1991; Techawongstien *et al.*, 1992), and reduced cell division or loss of cell expansion (Leskovar and Cantliffe, 1992; Techawongstien *et al.*, 1992). All of these responses can potentially lead to severe plant damage as the level of water stress increases.

In this study, 4D and 6D irrigation treatments induced different degrees of water stress in *Capsicum* plants as shown by a reduction in g_S and Ψ_L compared to the well-watered 2D plants; g_S was reduced by 50% or more for both 4D and 6D plants. In addition, Ψ_L was reduced for both 4D and 6D plants, particularly for the latter. Previous studies support

these findings: the development of water stress in sweet bell pepper caused a significant reduction in both g_S (by 70%) and Ψ_L after water was withheld for 3 days (Wullschleger and Oosterhuis, 1991). Ψ_L of sweet bell pepper cv. 'Maor' also dropped from –0.5 (non-stressed) to nearly –2.0 MPa (stressed) when plants were subjected to drought conditions (Aloni *et al.*, 1991).

Irrigation frequency of every two days appeared to be the best practice for pot-grown *Capsicum* based on plant response in this study. However, irrigation scheduling for field management of spice production still needs more investigation since variation of soil water release curve often occur for each characteristic soil type.

5.4.2 Plant biomass

Frequent irrigation of 2D plants did not induce any water stress symptoms, and was optimal for plant growth in this study. However, the reduction of g_S with 4D and 6D treatments is likely to have had a negative effect on CO_2 assimilation rate and consequent biomass production. Aloni *et al.* (1991) found stomatal closure in response to water stress and subsequently *Capsicum* plant growth was suppressed due to reduced photosynthesis. Similar findings were noted for several types of fruit and vegetable plants in the study of Clarke and Durley (1981).

Another possibility is that abscisic acid (ABA), produced by drying roots, could be responsible for biomass reduction, since it has been shown that endogenous ABA plays a central role in controlling root and shoot growth as well as regulating stomatal closure (Davies *et al.*, 1986; Davies *et al.*, 1990). There was no measurement of ABA in this study, but a reduction of shoot and root dry weight was apparent for both 4D and 6D plants. The ratio of shoots to roots was also significantly increased as the level of stress increased. This indicates that water stress reduced root growth more than shoot growth. A similar reduction of shoot and root growth was found for all chilli cultivars tested (Techawongstien *et al.*, 1992), but this was less affected at the mature stage than at the seedling stage. Foliar application of ABA has been used to investigate the relationship between plant growth and ABA in 'Jupiter' bell pepper seedlings (Leskovar and Cantliffe, 1992), in addition to testing its effects as an antitranspirant. Treated plants showed a

reduction in transpiration rate that related to a reduction of g_S, but there were no effects on root growth (Leskovar and Cantliffe, 1992).

5.4.3 Fruit yield

Fruit yield was reduced for both 4D and 6D plants relative to 2D plants. Total paprika fruit dry weight was reduced by 27% to 57% for 4D and 6D plants respectively, and by 40% to 50% for chilli. Similar yield reductions have been found for other *Capsicum* plants under water stress conditions, for example in cv. 'Piccante di Caienna' chilli (Quagliotti, 1971), various cultivars of hot chilli (Techawongstien *et al.*, 1992) and cv. 'Jacq' sweet pepper (*C. chinense*) (Jaimez *et al.*, 1999). Plants in the genus *Capsicum* are relatively sensitive to soil water deficits.

Although the fruit yield decreased, there was no significant difference in individual fruit weight in response to water stress. Yield reduction from stressed plants was, therefore, solely due to a decrease in fruit number, which was the result of abscission of unmarketable and marketable fruits. A similar reduction of fruit number was also observed in the study of Techawongstien *et al.* (1992). The decrease in fruit number in this study appeared to be due, in part, to abscission of new flowers or immature fruits on the upper nodes from observation, because water stress was introduced after fruits on the 4th node had set, but before fruit had set on more distal nodes. This suggests that *Capsicum* plants of both cultivars need to be kept free from water stress at any stage of development after first flowering. Yield can be significantly reduced if plants are subjected to sustained stress at this stage (Somos, 1984; Techawongstien *et al.*, 1992). However, just before harvest, when all fruits have set, less irrigation may be applied to the plants in order to allow fruits for spice production to partially dry on the bush prior to harvest (Lease and Lease, 1956; Kanner *et al.*, 1977).

5.4.4 Colour

Spice colour of both cultivars was unaffected by any treatment. Previously, growing conditions such as fertiliser rates and plant establishment have been reported to affect the carotenoid synthesis in *Capsicum* fruit (Reeves, 1987; Príbela *et al.*, 1992; Bosland, 1993). Soil water status potentially is an important factor affecting *Capsicum* colour, because any change in nutrient translocation, especially potassium, may alter fruit pigment biosynthesis. A decrease in potassium concentration in the nutrient solution caused lower carotenoid contents in ripening tomato fruit at any stage of fruit ripening on the plant (Trudel and Ozbun, 1970). While this study found no significant difference, the timing of water stress may determine its effect on fruit quality. Fruit carotenoid content changed in response to stress when it was applied before anthesis (Trudel and Ozbun, 1970; Príbela *et al.*, 1992). In this study, water stress was applied after anthesis at node four, and fruit stressed pre-anthesis would have abscised. Overall, colour intensity of all treatments was acceptable for commercial trade, according to the minimum acceptable colour level of 140 ASTA for paprika and 110 ASTA for pungent chilli types (J. Small, pers. comm., 1996).

5.4.5 Pungency

There was no effect of treatment on spice pungency for either cultivar in this study. In other reports, pungency levels in fruits of 'Jalapeno' chilli (Levy *et al.*, 1989) and 'Padron' pepper (Estrada *et al.*, 1999) increased by 13 and 50%, respectively, when plants were subjected to water stress at an early stage of fruit development. The timing of water stress during the growing season has been shown to have an effect on fruit quality. At an early stage of fruit development, such as at anthesis (Levy *et al.*, 1989; Estrada *et al.*, 1999), stress had more of an effect on fruit pungency than when introduced after anthesis, as was the case in this study. Water stress was applied after anthesis of fruit on the 4th node, in order to reduce flower and fruit abscission due to water stress and to attempt to maintain good yields in this study. Also the pungency of fruit that did abscise was not investigated. Therefore, it is not clear whether the pungency of fruit that would have set after commencement of treatments changed, as mostly these fruit abscised. The level of pungency may possibly be increased by reduced irrigation at this earlier period of fruit development, but fruit yield would be reduced by 50% or more. Therefore, pungency is better regulated by selecting appropriate cultivars.

Plant to plant variation within treatments appeared to be high. This suggests that there are factors other than environmental effects causing this variation. The differences in positions of individual fruit that were harvested from different plants could potentially lead to this variation, as Zewdie and Bosland (1996) showed that there are significant differences in pungency between fruit from different node positions of 'CaGC87' and 'Sandia' chilli plants. The acceptable hotness level for both sweet and semi-pungent paprika is between 250 and 750 SHU (J. Small, pers. comm., 1996). While the pungency of all treatments was slightly higher than recommended, it was still acceptable for commercial trade. For the chilli spices, the preferred treatment was the two-day irrigation frequency in order to ensure a minimum pungency level of at least 10,000 SHU (J. Small, pers. comm., 1996) with acceptable yields.

5.5 Conclusions

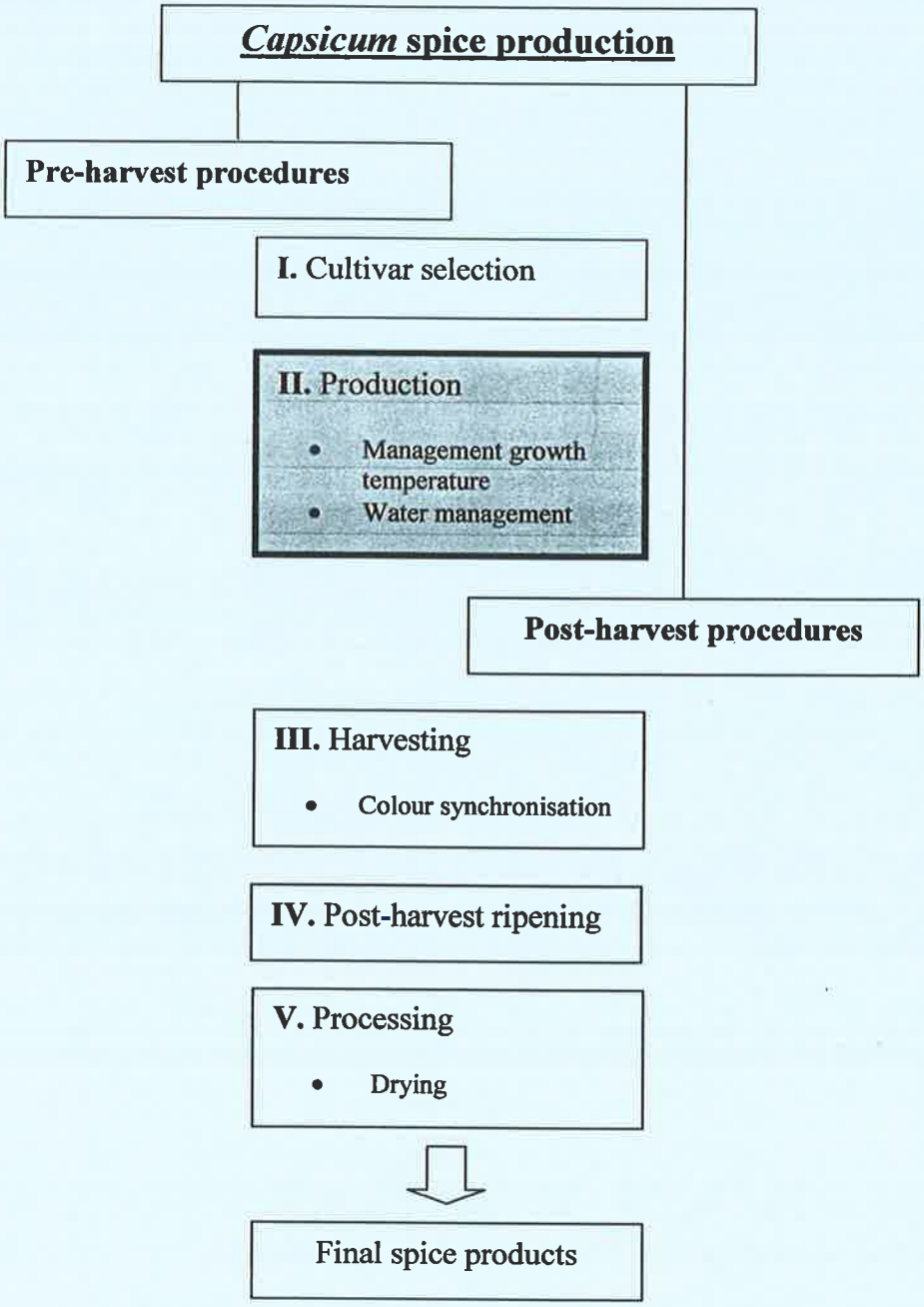
a) Irrigation at a two-day frequency is optimal for pot-grown *Capsicum* plants of both cultivars in order to obtain high yields while maintaining a good quality of the final spice product. For field management, time of irrigation can be scheduled according to the level of soil moisture. According to the average SWC from the 2D treatment, irrigation should be initiated when SWC is at or below 15%, or when Ψ_{soil} drops to – 20 kPa. However, this relationship only holds true in areas with a sandy soil type.

b) Irrigation every 2D did not induce any observable water stress, while some symptoms of water stress were induced by 4D and 6D irrigation treatments. For example, a large reduction in g_S and Ψ_L were observed coincidentally with declining SWC.

c) Vegetative growth was initially suppressed by water stress (4D and 6D treatments), resulting in stunted plants that had a small, compact canopy. Well-watered plants (2D) showed a branched canopy with new shoot and flower development throughout the experiment.

d) Subsequently, water stress showed a direct effect on harvested fruit yield, although it was applied after anthesis at node four. A reduction in yield was totally due to reduced fruit numbers resulting from fruit abscission. However, no significant effect was observed on an individual fruit basis (individual fruit weight, colour and pungency) for either cultivar.

e) The differences in yield reduction between 4D and 6D irrigation treatments compared to the 2D suggested that the response to water stress increased with its severity. For example, 6D treatment induced more severe water stress than 4D leading to total yield reduction by 43% and 23% respectively.



The *Capsicum* spice production system.

Areas covered by this chapter are highlighted.

Chapter Six

Response to partial rootzone drying

6.1 Introduction

The results of the experiments described in the previous chapter confirmed that the *Capsicum* plant is sensitive to drought stress. Prolonged soil drying of pot-grown *Capsicum* significantly reduced plant dry weight and final crop yield relative to fully watered plants without any effect on fruit quality. This response indicates that a hydraulic stress was responsible for significant changes in leaf water status and stomatal conductance. However, appropriate management of water stress at certain stages of plant development can result in a beneficial effect on fruit quality. This technique is known as regulated deficit irrigation (RDI), where irrigation is reduced for specific periods during the growing season in order to manipulate crop water use. Although RDI may result in quality improvements, for example, of flavour of grape berries (McCarthy, 1997) (cited in Dry *et al.*, 1999) or of chilli fruit pungency (Quagliotti, 1971; Levy *et al.*, 1989; Estrada *et al.*, 1999), it is often associated with a reduction of yield in *Capsicum* plants (Techawongstien *et al.*, 1992; Jaimez *et al.*, 1999) or other plant species (Simpson, 1981). Furthermore, the required water deficit may be difficult to determine, and needs careful monitoring of soil moisture (Dry *et al.*, 1996). By comparison, the use of the irrigation technique known as partial rootzone drying (PRD) may induce beneficial effects on fruit quality without any changes in crop yield (Dry *et al.*, 1996).

PRD requires a modified irrigation system in which part of the rootzone can be simultaneously exposed to both wetted and drying soil (Dry *et al.*, 1996). This can be achieved experimentally by splitting roots into two parts and planting them into separate pots or, by separating roots with a soft plate within containers, or in the soil (Dry *et al.*, 1996; Loveys *et al.*, 1997) so that irrigation can be applied to separated roots independently. Then, one side of the separated-root system is allowed to dry out, while the other is kept wet with a soil moisture at or close to field capacity (Dry *et al.*, 1996; Loveys *et al.*, 1997). After a certain period of time, irrigation is changed between the root halves, allowing the wet side to dry while the dry side will be rewatered. This strategy results in a positive effect on fruit composition and water use efficiency, while yield is still maintained

and shoot vigour is reduced. Most recently, PRD has been used for commercial production of winegrapes, without the use of a plastic membrane (Loveys *et al.*, 1997). Using PRD with *Capsicum* plants may allow yields to be maintained while improving fruit quality and decreasing water usage. Nothing is known about the response of *Capsicum* plants to this technique. It also provides an opportunity to study the physiological effect of soil drying on fruit quality in the absence of a hydraulic water stress. This study, therefore, was aimed *to investigate the effects of PRD on Capsicum, yield components and spice quality for chilli cv. 'Caysan SPS705'.*

6.2 Materials and methods

Preparation of split-root plants: Approximately 27 to 43 days after transplanting (details in Appendix I), seedlings with 1 cm diameter stems were prepared for the split root study in the glasshouse. The root system of each seedling was divided into two equal portions, and the base of the stem was cut longitudinally for about 2.5 cm. Plants were then replanted into two adjacent pots (20 cm diameter) (Figure 6.1), with approximately half of the roots located in either pot. Young leaves and new shoots were firstly pruned out to avoid excessive transpiration (Figure 6.2). Full irrigation was applied by hand every day as required until both shoots and roots were well developed after approximately one month (Figure 6.3).

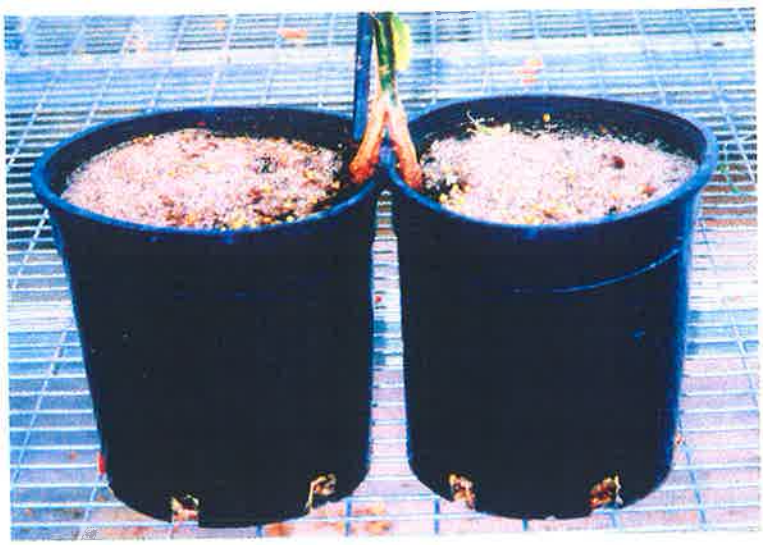


Figure 6.1 Split-root plants.

Figure 6.2 Leaves and shoots of the spilt-root plants were pruned after transplanting.

Figure 6.3 Plants after recovery from the first pruning.

Plant survival of 95% was observed after conversion to split root plants. Unhealthy plants were discarded and replaced by healthy split root plants. Twenty plants were randomly relocated into in the same glasshouse, using two rows with 45 cm × 60 cm spacing. Individual plants were labelled for designated treatments (see Figure A3 in Appendix II for plot layout).

Treatments: Treatments consisted of control (Ct) and treated (T) plants termed partial rootzone drying (PRD). Treatments were applied from December 8, 1998 (designated day 0 (D0)) when plants started setting fruits at the fourth node (Figure 6.4). Ct plants had both pots watered to near the maximum soil water-holding capacity (about 30% volumetric soil water content, SWC), and they were rewatered every one or two days. The pots of both Ct and T plants were termed left (L) and right (R) for convenience. T plants had one pot that was allowed to dry out, while the other pot was watered as for Ct plant. At seven day intervals (termed 'drying period'), when the soil moisture content fell to approximately 10%, the irrigation was switched between pots. Seven drying periods were applied and the experiment ended on D56.

Figure 6.4 Stage of plan growth when treatments were applied.

Soil water content: The volumetric soil water content (SWC, %) was measured daily prior to irrigation between 15:00 and 16:00 h, refer in section on soil water content to Chapter 5.2 as done for section on leaf water potential and stomatal conductance. Measurement was taken from D1 to D49.

Leaf water potential: Measurement of leaf water potential was conducted on D21, D35 and D55 between 10:00 and 11:00 h on one leaflet per plant. The data in Table 6.1 are the means of ten measurements.

Stomatal conductance: Measurement of stomatal conductance was conducted daily on each plant, using three fully expanded leaves per plant. Measurement was taken from D0 to D7 between 10:00 to 12:00 h. On D35, measurements were taken three times between 10:00 to 16:00 h. The data in Table 6.2 and 6.3 are the means of ten plants.

Harvesting: Fruit were hand harvested on February 1, 1999 (D55). All plant materials were dried at 45°C for 7 days, and dry weights were recorded. Samples of dried red fruits from individual plants were ground and kept in plastic bags in the dark at ambient temperature until analysis of spice colour and pungency (details in Chapter 3). The experiment was evaluated with a randomised complete block design with ten replications.

6.3 Results

6.3.1 Soil water content

Changes in SWC over time are shown in Figure 6.5. The SWC of both pots of the Ct plants was maintained between 30 and 35% (Figure 6.5). The averaged SWC from both pots of the Ct plants was maintained at approximately 32%. This was achieved by daily irrigation of the Ct pots in order to restore field capacity. For the T plants, the SWC of the wet side was kept at 30% or above, for example for the L pot from D0 to D7 of the first irrigation cycle, while the SWC of the dry side (R) declined from 30% to about 13% (Figure 6.5). The minimum SWC of different cycles also varied in response to plant transpiration. This was dependent on weather conditions, though air temperature in the glasshouse was controlled between 20 to 30°C during day and night. However, the maximum and the minimum SWC of different cycles averaged 34% and 13% respectively.

According to the soil water release curve in Chapter 5 (Figure 5.3), SWC in both pots of the T plants was maintained throughout the growing season at levels above the critical point at which plants suffer from stress, except on day 21.

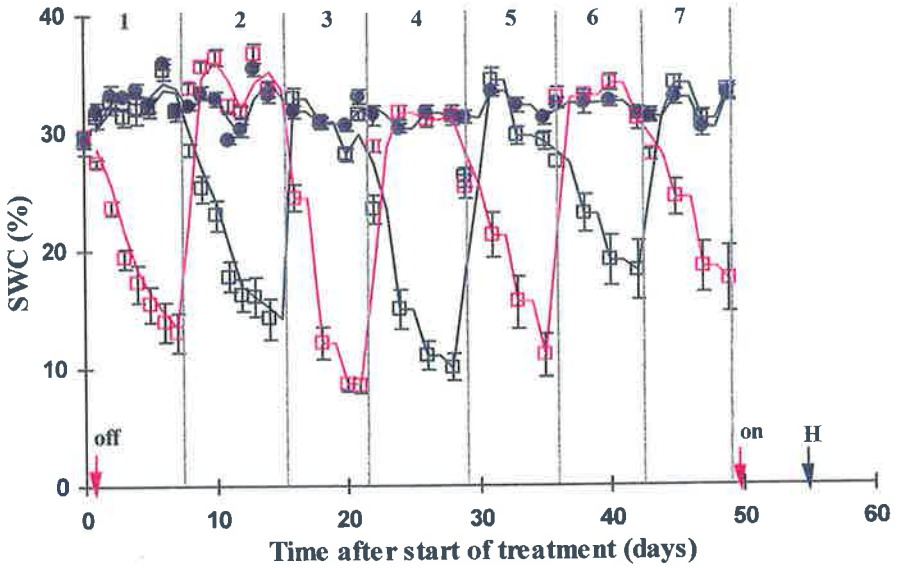


Figure 6.5 Volumetric soil water content (SWC, %) of control (Ct, mean of both containers, ●, black) and treated (T), (left container (L, □, black); right container (R, □, red)) of split-root chilli plants cv. 'Caysan SPS705'. C: both containers irrigated; T: one side not irrigated at any one time from D1 (off) until D50 (on). Vertically lines indicate days when irrigation of T was switched from one pot to the other. Numbers in bold represent drying period, H indicates harvesting date. Each data point is the mean value ± SE (n=10).

6.3.2 Plant response

Leaf water potential: No significant difference in Ψ_L between treatments was observed at any time (Table 6.1).

Table 6.1 Effects of partial drying on leaf water potential of split-root chilli plants cv.'Caysan SPS705'. Measurements were taken at various times as the experiment progressed prior to irrigation. Each data point is the mean of ten plants per treatment, ns = no significant difference.

Time after start of treatment (days)	Irrigation		Leaf water potential (MPa)		Significant difference (P<0.05)
	Cycle	Day	Control	PRD	
21	3	7	-0.72	-0.75	ns
35	5	7	-0.68	-0.65	ns
55	8	7	-0.53	-0.52	ns

Stomatal conductance: The g_S of the T plants remained lower than that of the Ct plants for much of the experimental period. Figure 6.6 shows the typical g_S of the T plants for one drying period. On D5 to D7 g_S of the T plants was significantly lower than of the Ct plants.

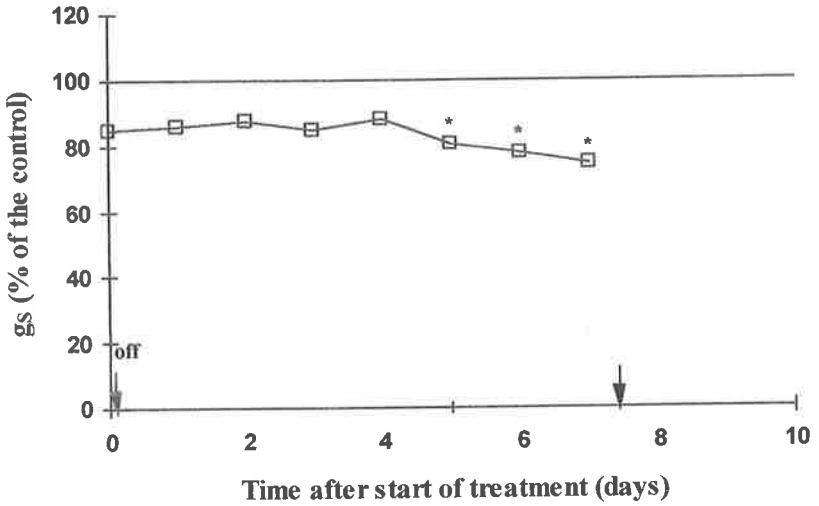


Figure 6.6 Effects of partial drying on stomatal conductance (g_S) of split-root chilli plants cv. 'Caysan SPS705'. g_S (□) of treated (T) plants as % of the control (Ct) plants is shown. Arrows indicate days when irrigation of T switched from one side to the other. * indicates those days when g_S of T was significantly different ($P<0.05$) to the control. Each data point was the mean value of ten plants. Measurement was taken to D7 for only the first drying cycle.

Also, the average g_S measured from D0 to D7of the T plants was 17% lower than Ct (Table 6.2). As the experiment progressed, the g_S of T and Ct plants was not significantly different: for example, on D35 both treatments showed a similar diurnal variation in g_S (Table 6.3). However, overall the g_S of T plants was less than that of the Ct, and this corresponded to a reduction of SWC in the unirrigated T pot.

Table 6.2 Means of stomatal conductance (g_S, $mmol.m^{-2}.s^{-1}$, ± SE) during period from D0 to D7 for both control and PRD plants (refer to Figure 6.6).

	Treatment
Control	228 ± 18
PRD	189 ± 15
% difference	-17
Significance	$P<0.001$

Table 6.3 Effects of partial drying on stomatal conductance (g_s, mmol.m^{-2}.s^{-1}) of split-root chilli plants 'Caysan SPS705'. Measurements were taken on January 12, 1999 (35 days after start of treatment; at D7 of the irrigation cycle 5). Each data point is the mean value ± SE (n=10), ns = no significant difference between treatments (P<0.05).

Time	Stomatal conductance (mmol.m^{-2}.s^{-1}, ± SE)		
(h)	Control	PRD	Significance
10:00-11:00	130 ±12	118 ±8	ns
12:00-13:00	114 ±10	105 ±8	ns
15:00-16:00	99 ±8	95 ±7	ns

Abscission: Some flower abscission was observed with both treatments. However, this was found to occur when the air temperature in the greenhouse rose above 30°C. Overall, continuous flowering was observed in both treatments as the experiment progressed. There was no sign of visible wilting of either Ct or T plants throughout the experiment.

6.3.3 Yield components

Total fruit yield: PRD had no significant effect on yield for any fruit colour category relative to the control (Table 6.4).

Table 6.4 Effect of partial drying on fruit yield of split-root chilli plants cv. 'Caysan SPS705'.

Treatment	Dry weight (g.plant^{-1}) by colour category				
	Red	Breaker	Green	Immature	Total
Control	44.8 a[z]	0.6 a	9.3 a	0.2 a	55.0 a
PRD	37.6 a	0.6 a	11.0 a	0.7 a	50.0 a

[z] Means within the same column for each experiment followed the same letter are not significantly different based on Least Significant Differences (n=10) at P<0.05.

Fruit number and dry weight: Total fruit number was not affected by PRD, but colour development was slightly delayed for T plants (Table 6.5). This was indicated by the lower proportion of red to green fruits on the T plants at harvest (Table 6.5).

Table 6.5 Effects of partial drying on fruit number of split-root chilli plants cv. 'Caysan SPS705'.

Treatment	Number of fruits per plant by colour category				
	Red	Breaker	Green	Immature	Total
Control	23.2 a[z]	0.4 a	9.5 b	1.5 a	34.6 a
PRD	19.1 b	0.3 a	16.2 a	4.6 a	40.2 a

[z] Means within the same column for each experiment followed the same letter are not significantly different based on Least Significant Differences (n=10) at $P<0.05$.

Individual fruit dry weight of the different colour stages, including marketable red fruit, was not affected by PRD (Table 6.6). PRD also had no effect on average fruit dry weight as means across all categories (Table 6.6).

Table 6.6 Effects of partial drying on fruit dry weight of split-root chilli plants cv. 'Caysan SPS705'.

Treatment	Individual fruit dry weight by colour category ($g.fruit^{-1}$)				
	Red	Breaker	Green	Immature	Average
Control	2.0 a[z]	1.6 a	1.1 a	0.1 a	1.2 a
PRD	2.0 a	1.9 a	0.7 a	0.1 a	1.2 a

[z] Means within the same column for each experiment followed the same letter are not significantly different based on Least Significant Differences (n=10) at $P<0.05$.

Plant biomass: PRD did not influence leaf, stem, root or total plant dry weight nor the shoot to root ratio (Table 6.7).

Table 6.7 Effects of partial drying on plant yield components of split-root chilli plants cv. 'Caysan SPS705'.

Treatment	Shoot dry weight ($g.plant^{-1}$)			Root dry weight[y] ($g.plant^{-1}$)	Total plant dry weight[yz] ($g.plant^{-1}$)	Shoot/root (ratio)
	Leaf	Stem	Total			
Control	17.13 a[x]	18.65 a	35.78 a	23.70 a	57.90 a	1.52 a
PRD	16.59 a	18.06 a	34.65 a	27.70 a	64.40 a	1.43 a

[z] Total plant dry weight = Shoot dry weight + Root dry weight.
[y] Data are means for four plants per treatment.
[x] Means within the same column for each experiment followed the same letter are not significantly different based on Least Significant Differences (LSD, n=10) at $P<0.05$.

Spice quality: Spice colour intensity, indicated as ASTA colour values, of the fruits of T plants was not affected by PRD (Table 6.8). Although the colour intensity for chilli spice was low for both treatments, it was sufficient for commercial use. The colour ranged from 103 to 177 ASTA for individual plants.

Table 6.8 Effects of partial drying on spice colour and pungency of split-root chilli plants cv. 'Caysan SPS705'.

Treatment	Marketable fruit	
	Colour (ASTA)[z]	Pungency (SHU)[y]
Control	133 a[x]	8,910 a
PRD	126 a	6,625 a

[z] American Spice Trade Association colour units.
[y] Scoville Heat Units were calculated by multiplying the amount of capsaicin and dihydrocapsaicin per gram dry weight by 16 million heat units for pure capsaicin and dihydrocapsaicin.
[x] Means within the same column for each experiment followed the same letter are not significantly different based on Least Significant Differences (n=10) at $P<0.05$.

Pungency was not significantly affected by PRD, although reduced by 25% relative to that of the Ct. In general, the pungency of chilli spice was relatively low for both treatments according to the minimum acceptance of 10,000 SHU required for commercial production.

6.4 Discussion

6.4.1 Soil water and irrigation scheduling

The irrigation strategy modified in this study was successfully applied to the T plants, allowing half of the root system to dry to a level that would have caused stress in normal plants (if the whole root system of the normal plants had experienced soil drying to this level; as shown in Chapter 5), while maintaining the wet half of T plants and both sides of Ct plants at a relatively high SWC. The amount of water applied during each irrigation period was adequate to refill soil moisture to field capacity on the wet side of T plants, and the amount was also sufficient for the Ct plants.

PRD appeared to induce some symptoms of water stress such as a reduction in g_S, without any change in Ψ_L. Therefore, the experience of PRD plants could not be classified as drought conditions compared to the response of plants to water stress experiments in Chapter 5. The midday Ψ_L of water stressed plants was significantly lower (-1.0 to -1.2 MPa) than that of well-watered plants (~0.5 MPa) (Chapter 5). A difference of more than 0.5 MPa in Ψ_L between well-watered and stressed chilli plants was defined as severe water stress (Aloni *et al.*, 1991). On the other hand, the Ψ_L of PRD plants in this study was never significantly less than that of the control and similar to the well-watered treatment in Chapter 5.

Since there was no measurable change in leaf water status with PRD, it is likely that the water requirement of the whole plants was met by irrigation of just one half. It is possible that the wet half of the root system used relatively more water, in order to compensate for the dried half, and thus maintained a consistent water flow to the shoots. This has been described previously for PRD grapevines (Dry and Loveys, 1999; Dry *et al.*, 2000; Stoll *et al.*, 2000). In addition, Dry *et al.* (1996) also found that a partial reduction in g_S (usually of the order of 15 to 20% and never more than 50%) induced by PRD was beneficial for water-use efficiency (WUE). WUE is defined as the ratio of carbon dioxide assimilation and transpiration rates (Düring *et al.*, 1996). This was supported by the amount of crop produced per unit of water applied for grapevines doubling in response to PRD (Dry *et al.*, 1999). A small reduction of g_S (approximately 17%) for T plants was found in this study,

but assimilation and dry matter production was not detrimentally affected. Although a reduction of g_S was observed on the early period of experiment, it was sufficient to represent for T plants through the experiment in this study due to the reasonable levels of SWC in both pots of the T plants. PRD-treated chilli plants, therefore, potentially showed an increase in WUE only if the water use was reduced, although this was not the aim of this study and actual water use was not measured. More detailed analysis of soil moisture would be useful to provide information on the differential water use of roots of both sides of the T plants.

6.4.2 Root to shoot signalling

It has been hypothesised that root to shoot signals occur in plants that are undergoing soil drying (Gowing *et al.*, 1990; Davies and Zhang, 1991; Turner *et al.*, 1996). Although there was no attempt in this study to examine this relationship between roots and shoots, it is likely that the same phenomenon occurs in *Capsicum* plants (Ismail and Davies, 1997; Ismail and Davies, 1998). It was noted that roots in drying soil are rehydrated during the night by water supplied from the wet roots in grapevines (Stoll *et al.*, 2000). This leads to a sufficient sap flow from the dry roots during the day to allow a significant flux of a chemical signal such as abscisic acid (ABA) to the shoot (Stoll *et al.*, 2000). However, an associated suppression of leaf growth (Gowing *et al.*, 1990; Davies and Zhang, 1991) or shoot growth (Dry *et al.*, 1996) was also found in these cases. In my study, PRD did not affect shoot dry weight, but a small reduction in g_S was observed. This was similar to cucumber plants in which drying half of the root system had no effect on leaf growth (Melkonian and Wolfe, 1995). With another plant species such as sorghum (Ebel *et al.*, 1994), drying of 25% of the root system did not affect leaf elongation, g_S, or Ψ_L compared to the control treatment. However, when sorghum had 50% of the root system dried, it caused a reduction in leaf growth without any change in Ψ_L relative to the control treatment. This suggested that a non-hydraulic signal was responsible (Ebel *et al.*, 1994), similar to the results in the study of Dry *et al.* (1996). Therefore, the response to PRD was different from the response to water stress, and it also appears to vary among plant species.

Previous split root experiments with other species have resulted in reports of differing sensitivity of vegetative growth and stomatal behaviour to non-hydraulic signalling of soil drying. For instance, in passion fruit (*Passiflora* sp.), half drying of the root system reduced leaf expansion (by 26%) without accompanying changes in g_S (Turner *et al.*,

1996), similar to sorghum (*Sorghum bicolor* L. Moench.) (Ebel *et al.*, 1994) and maize (*Zea mays*) (Saab and Sharp, 1989). On the other hand, stomata of cowpea (*Vigna unguiculata* L. Walp.) responded very quickly to the soil drying prior to any change being detected in the rate of leaf expansion (Ebel *et al.*, 1994). However, in some species such as tomato (*Lycopersicon esculentum* Mill.)(Tan *et al.*, 1981) and peach (*Prunus persica* L. Batsch) (Tan and Buttery, 1982), a reduction in shoot development accompanied by changes in stomatal conductance was observed when parts of the root system were exposed to soil drying. In the above cases, there was no alternation of drying from one side to the other. In the PRD experiment on grapevine, both stomatal conductance and shoot growth responded to non-hydraulic signalling (Dry, 1997). Therefore, *Capsicum* plants in my study behaved differently to the grapevine. The first response of *Capsicum* to soil drying may be a reduction in stomatal aperture well before there is any effect on vegetative growth. At this early stage, change in g_S is readily reversible and there may be no long-term detrimental effect on dry weight production. This response, induced by PRD, appears to be under the control of non-hydraulic root-to-shoot signalling. It is only when the soil dried out further that shoot growth is affected in the case of *Capsicum* - this is associated with hydraulic stress (as indicated by a decrease in leaf water potential; Chapter 5). Once this stage is reached, there are long-term implications for dry weight production, as shown in Chapter 5. If this is so, PRD of *Capsicum* may not be useful for control of shoot vigour- however, partial closure of stomata in response to PRD will increase water-use efficiency.

6.4.3 Yield components

There was no significant effect of PRD on any yield component, while the final yield of marketable fruit was reduced when *Capsicum* plants experienced water stress (Chapter 5). This again suggests that the plant response to PRD was different from the response to water stress.

The development of plant growth and the final yield production is partly dependent on the effect of water stress via a reduction of photosynthetic rate. Jaimez *et al.* (1999) found a 40% reduction in fruit yield corresponded to a 40% reduction of CO_2 assimilation, and this was also accompanied by large reduction in both g_S and Ψ_L, when sweet pepper plants were subjected to water stress. A small reduction in g_S is not likely to result in large decrease in CO_2 assimilation (Simpson, 1981). Also, PRD did not affect carboxylation efficiency in grapevines (Dry, 1997). Therefore, it is likely that PRD treatment of

Capsicum caused no significant reduction in CO_2 assimilation particularly because there was no reduction in leaf area. This may explain, in part, the maintenance of yield in this experiment.

Since there was no reduction in vegetative growth in response to PRD, there was no opportunity for increased partitioning of assimilates to reproductive growth as an aid to yield maintenance.

6.4.4 Fruit composition

Time of ripening, indicated by larger proportion of green fruit at the time of harvest, appeared to be delayed by PRD. This disagreed with previous reports in which PRD advanced grapevine berry ripening (indicated by the rate of sugar accumulation) in two out of three seasons (Dry, 1997). Turner *et al.* (1996) also found an earlier flowering of passionfruit in response to half-drying of potted plants. While our results differed from the above findings, it is possible that fruit composition of *Capsicum* may be sensitive to stress at an early stage of fruit development, that is at a stage before PRD was applied in this experiment.

Colour and pungency content are widely accepted as important quality components for spice production. PRD did not alter the concentration of secondary metabolites (carotenoids, capsaicinoids). Again the stage of fruit development exposed to PRD was perhaps not the most sensitive stage of fruit composition in response to PRD. Similarly, water stress imposed at a similar stage had no effect on spice colour and pungency (Chapter 5). However, when water stress was applied at an early stage of plant development (ie. involving the flowering period), pungency of fruit significantly increased in some cultivars of *Capsicum* (Quagliotti, 1971; Levy *et al.*, 1989; Estrada *et al.*, 1999). The response of plants to PRD may vary according to the growth phase at which it is applied. Since *Capsicum* plants continue to flower throughout their growing season, it would be difficult to separate the effect of PRD on the vegetative growth phase from that on the reproductive phase. Therefore, the flowering period should be limited by removal of later flowers that may be useful to concentrate on only the reproductive phase.

There was no reduction in shoot growth in response to PRD in this study; therefore the potential partitioning of secondary metabolites to fruit may not have taken place. An

increase in ABA induced by PRD, as shown in grapevine (Stoll *et al.*, 2000), may have some direct effect on fruit quality. The response of *Capsicum* plants to PRD still needs more investigation, especially for treatments applied at an early stage when fruit is most sensitive to any environmental influence. The effect of PRD on plant growth in relation to changes in chemical signals induced by PRD should be the subject of further study. However, the potential risk to yield of any stress imposed also during flowering period must be considered.

Spice pungency of all treatments was relatively low compared to the previous experiment (Chapter 5). A seasonal change in pungency levels was observed which suggests that other external factors such as air temperature may be at play, and this was investigated as previously described in Chapter 4.

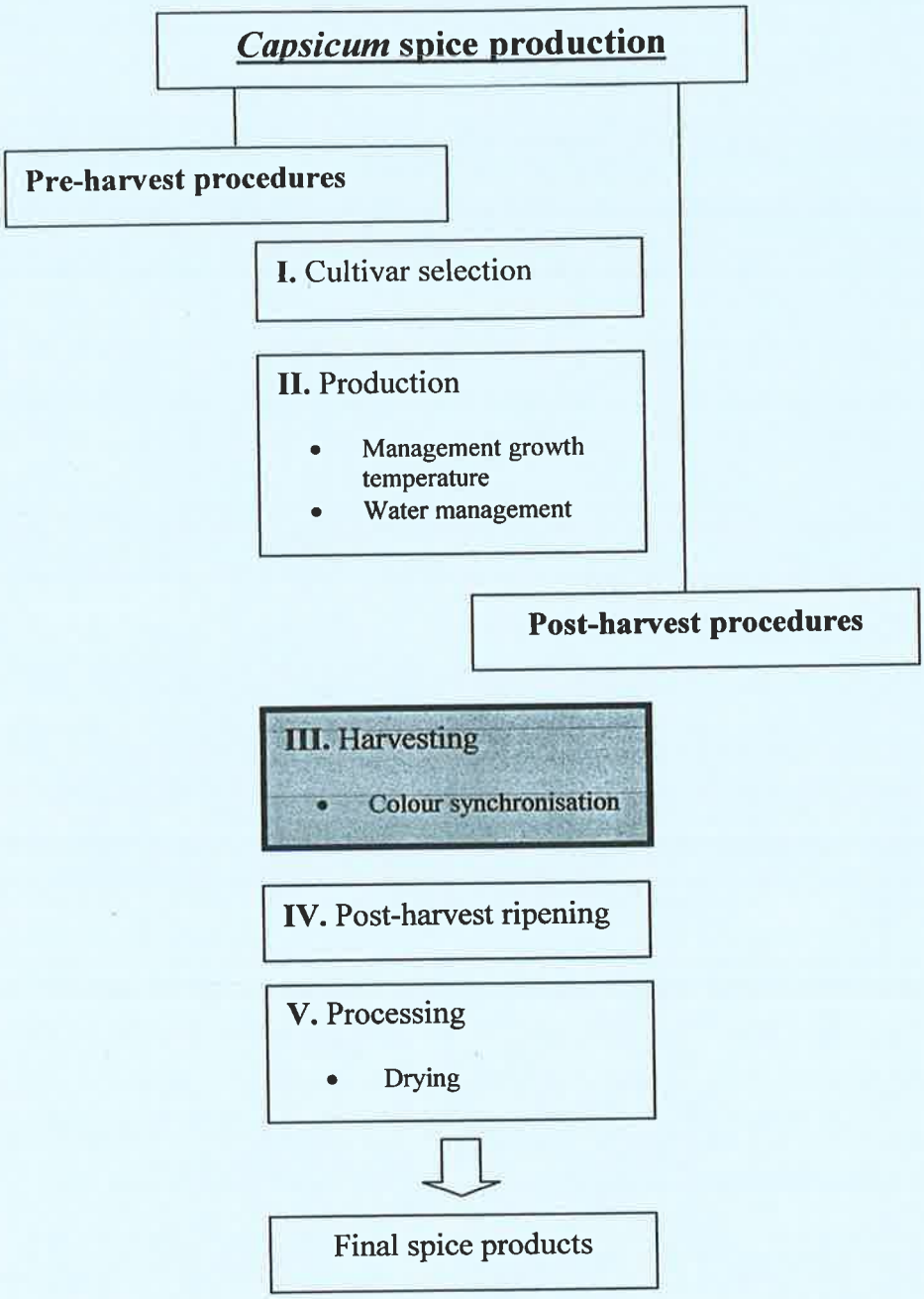
6.5 Conclusion

a) PRD successful maintained marketable fruit yield, but did not substantially alter fruit quality, neither spice colour intensity nor pungency levels.

b) PRD induced some of the symptoms of water stress, such as a reduction in g_S, but there was no concomitant change in Ψ_L. The response to PRD was generally different from the response to water stress. Also, the response to PRD was different to that of some other species.

c) Plants may experience a hydraulic stress if the amount of water from the wet side of the root system cannot be sustained at a sufficiently high level (at least above 15% of SWC measured by TDR for soil conditions in this study). This situation can lead to a large reduction in g_S, Ψ_L and subsequently affect final yield production similarly to the effect of water stress as shown in the previous study (Chapter 5). This suggests that careful monitoring of soil moisture is essential.

d) Alternation of the drying from one half of the root system to the other with the PRD strategy could be beneficial for reducing crop water usage, without any detrimental effect on fruit yield. However, more information on soil moisture and water use for each pot of the split root plant is required. Further investigation in the field may also be useful for commercial use, especially in water limited regions, if PRD can definitely show a beneficial effect.



The *Capsicum* spice production system.

Areas covered by this chapter are highlighted.

Chapter Seven

Ethephon as a harvesting aid for synchronising fruit maturity

7.1 Introduction

Traditional hand harvesting of *Capsicum* fruit is not economically feasible due to high labour costs, especially for a large broad acre production in Australia. In addition, it is difficult work as the *Capsicum* fruit, especially very pungent types, often damage pickers' skins. Fruit are normally harvested repeatedly from the same plants by hand, as different stages of fruit maturity are present on the plants, but completely red fruit are required for processing. Once-over mechanical harvesting would reduce the high production costs, but the lack of uniformity of ripening stages reduces harvesting efficiency. To increase efficiency, ethylene releasing chemicals such as ethephon may possibly aid harvesting, since ethephon has been shown to promote colour development in various capsicum types (Sims *et al.*, 1970; Worku *et al.*, 1975; Batal and Granberry, 1982).

Several factors that influence fruit response to ethephon have been previously noted. Crop maturity is one of the most important factors influencing time of ethephon application. Various responses have also been found in response to differing stage of maturity. Ethephon should not be applied when there are a large number of immature fruit on the plant, since ethephon causes significant abscission of less mature Tabasco fruit (Conrad and Sundstrom, 1987). However, when 20% of capsicum fruit have reached the mature red stage and 30% are turning colour and 50% are green, ethephon application induced red colour development in bell, chilli and pimiento peppers which also lead to an advanced harvest (Sims *et al.*, 1974).

Although ethephon can induce colour development associated with fruit ripening in capsicums, its effectiveness varies with other factors such as rate and number of applications, ambient temperature at application, the *Capsicum* type (Sims *et al.*, 1970; Batal and Granberry, 1982) or even the cultivar (Beaudry and Kays, 1988; Rylski, 1986). Most previous work (Sims *et al.*, 1970; Worku *et al.*, 1975) found that ethephon had the greatest effect at application rates in the concentration range of 1000 to 5000 $\mu L.L^{-1}$. Ethephon within this range (Conrad and Sundstrom, 1987) effectively advanced red colour

development of Tasbasco pepper; however, a similar concentration was ineffective in the study of Knelvel and Kemp (1973). Singh *et al.* (1992) also found that various types of *Capsicum* plants exhibited different responses to ethephon. The response to ethephon of paprika pepper cv. 'PS72285' and cayenne chilli cv. 'Caysan SPS705', preferred cultivars for commercial production in Australia (J. Small, pers. comm. 1996), is not known. The possibility of using ethephon to synchronise fruit maturity in these cultivars therefore needs to be investigated. Furthermore, there is limited information on final quality of dehydrated products following pre-harvest application of ethephon. While ethephon was found to increase red pigment intensity of dehydrated paprika fruit in some studies (Worku *et al.*, 1975; Batal and Granberry, 1982), nothing is known about whether it has any effect on pungency levels. Thus, this chapter aimed *to investigate the effects of ethephon on yield, colour and pungency of dehydrated paprika cv. 'PS72285' and cayenne chilli cv. 'Caysan SPS705', and to evaluate its possible advantages for once-over harvesting.*

The main findings of this chapter were published in Krajayklang *et al.* (1999).

7.2 Materials and methods

Plant materials and experimental design: Approximately 45 days after sowing (details in Appendix I), both paprika and chilli plants were transplanted and randomly assigned to experimental units. For each cultivar, experimental Units consisted of 16 plants with four treatments and four replications in the shadehouse as a randomised complete block design. Plants were arranged into four rows with 40 cm × 40 cm spacing and 0.5 m was allowed between replicates. Four seedlings in each block were randomly labelled for each treatment (see plot layout of Figure A4 in Appendix II).

Treatments: Four different concentrations of ethephon (Ethrel®, 2-chloroethyl phosphonic acid, RHONE-POULENC Rural Australia Pty. Ltd., Baulkham, NSW) were used as treatments. Ethephon solutions of 0 (control), 1000, 3000 or 5000 $\mu L.L^{-1}$ were applied once as foliar applications until run off (250 mL per plant) on January 30, 1997 (designated day 0 (D0)) and February 13, 1997 (designated day 0 (D0)) for paprika and chilli, respectively. The labelled plants from each replicate were removed before spraying to minimise the possibility of spray drift between treatments. Controls were sprayed with plain water in a like manner, and all plants were allowed to dry for approximately 30 min before they were returned to the shadehouse. Plants averaged 20% fully coloured red fruit

at the time of ethephon treatment, as suggested by Sims *et al.* (1974). Average temperatures on the dates of application were 26 and 28°C for paprika and chilli, respectively (more detail of weather conditions in Appendix IV).

Parameters measured: Plant response to the chemical was observed after spraying. Fruit abscission measurements were taken from 48 h after treatment by daily collecting abscised fruit under each plant and bulk weighing each colour category: red; breaker (green fruit turned partially red); green and defective (immature or with fungal damage). Foliage remaining on the plant before harvest was compared visually with that of control plants. The result was then expressed as a percentage of leaf retention. The fruit from each individual plant were harvested by hand on February 5, 1997 (D6) and February 28, 1997 (D15) for paprika and chilli, respectively (more details for time frame in Appendix I), counted and weighed (fresh weight only) separately for different colour categories as marketable or nonmarketable fruit. The red fruit only were prepared for chemical analysis of colour and pungency (chilli only) as previously described in Chapter 3.

7.3 Results

7.3.1 Plant density and growth habit

Both paprika and chilli plants grew normally throughout the experiment with a mean plant density of 6.25 plants.m^2 in this study. Both showed an upright growth habit, and were generally more than 50 cm tall from the soil surface (15 cm high pot). Single fruit developed at each branching node mainly pointing down toward the ground for both cultivars.

7.3.2 Yields of harvested fruit and fruit abscission

There was no significant effect of treatment on percentage of red marketable fruit numbers for paprika plants (Table 7.1). However, breaker fruit as a percentage of total harvested fruit increased with ethephon use, while the percentage of fruit that were green and immature decreased with ethephon use (Table 7.1).

Table 7.1 Harvested fruit maturity of 'PS72285' paprika and 'Caysan SPS705' chilli after ethephon treatments were applied.

Ethephon concentration ($\mu L.L^{-1}$)	Total harvested fruit (abscised excluded) (% on a number basis)			
	Marketable	Breaker	Green	Immature or defective
	'PS72285' paprika			
0	21 a[z]	28 b	37 a	13 a
1000	19 a	59 a	18 b	4 b
3000	23 a	59 a	16 b	3 b
5000	24 a	63 a	12 b	1 b
	'Caysan SPS705' chilli			
0	59 b[z]	22 a	16 a	1 b
1000	74 a	26 a	0 b	0 b
3000	88 a	10 b	0 b	2 b
5000	79 a	5 b	0 b	16 a

[z] Values within columns for each cultivar followed by the same letter are not significantly different at $P<0.05$.

The yield of harvested red marketable fruit was affected very little by ethephon treatment, but there was a significant increase in the yield of breaker fruit and a decrease in the yield of green fruit compared to the controls (Table 7.2). The reduction of total yield of harvested fruit in response to ethephon treatment (Table 7.2) could be attributed to the considerable loss of fruit by abscission (Table 7.3).

Table 7.2 Effects of ethephon treatments on yield, colour and pungency of 'PS72285' paprika and 'Caysan SPS705' chilli.

Ethephon	Fresh weight of harvested fruit (g.plant^{-1})					Marketable fruit (abscised excluded)		
						DW	Colour	Pungency
concentration (µL.L^{-1})	Marketable	Breaker	Green	Defective	Total	(g.plant^{-1})	(ASTA)[y]	(SHU)[z]
	'PS72285' paprika							
0	82 a[x]	134 b	150 a	21 a	387 a	14 a	288 a	nd
1000	46 a	197 a	54 b	5 b	302 b	8 a	278 a	nd
3000	53 a	185 a	38 bc	6 b	282 b	9 a	279 a	nd
5000	57 a	188 a	28 c	3 b	276 b	10 a	272 a	nd
	'Caysan SPS705' chilli							
0	199 a[x]	76 a	41 a	2 a	318 a	38 a	141 b	13418 b
1000	103 b	43 b	0 b	0 a	146 b	20 b	164 a	19547 a
3000	98 b	14 c	0 b	0 a	112 c	22 b	136 b	19814 a
5000	91 b	8 c	0 b	0 a	99 c	20 b	139 b	11145 b

[z] Scoville Heat Units were calculated by multiplying the amount of capsaicin and dihydrocapsaicin per gram dry weight by 16 million Scoville units for pure capsaicin and dihydrocapsaicin; nd = no pungency was detectable.

[y] American Spice Trade Association colour units; calyxes were removed before analysis.

[x] Means within columns for each cultivar followed by the same letter are not significantly different at $P<0.05$.

Table 7.3 Abscission response of 'PS72285' paprika and 'Caysan SPS705' chilli to ethephon treatments.

Ethephon concentration ($\mu L.L^{-1}$)	Abscised fruit FW ($g.plant^{-1}$)			Leaf retention on the bush (%)
	Marketable	Unmarketable	Total	
	'PS72285' paprika			
0	0 b[z]	8 a	8 b	100 a
1000	9 a	17 a	26 a	29 b
3000	14 a	21 a	35 a	14 c
5000	11 a	24 a	35 a	6 c
	'Caysan SPS705' chilli			
0	7 b[z]	11 c	18 b	99 a
1000	63 a	82 ab	145 a	48 b
3000	48 a	75 b	123 a	20 c
5000	47 a	110 a	157 a	12 c

[z] Means within columns for each cultivar followed by the same letter are not significantly different at $P<0.05$.

For the chillies, marketable fruit as a percentage of total harvested fruit increased with ethephon use, while the percentage of fruit that were breaker and green decreased (Table 7.1). However, the yield of harvested red marketable fruit decreased significantly for all ethephon treatments (Table 7.2). Total yield of harvested fruit was also significantly decreased (Table 7.2); this was due to a large amount of fruit abscission (Figure 7.1) caused by ethephon (Table 7.3).

7.3.3 Intensity of extractable red colour

Ethephon had no effect on extractable red colour of dehydrated paprika fruit (Table 7.2), but for dehydrated chilli fruit the intensity of extractable red colour was improved by the 1000 $\mu L.L^{-1}$ ethephon treatment (Table 7.2). No significant effect was obtained with chilli at higher ethephon rates.

7.3.4 Intensity of pungency

No pungency was detectable in dehydrated paprika fruit. As shown in Table 7.2, ethephon at 1000 and 3000 $\mu L.L^{-1}$ increased pungency levels in dehydrated chilli fruit compared to the control. However, fruit treated with 5000 $\mu L.L^{-1}$ ethephon were not significantly different to the control (Table 7.2).

7.3.5 Defoliation and fruit skin damage

Leaf damage was first observed for both cultivars 5 min after spraying with ethephon, and continue developing. Its severity was also dependent on chemical concentration (Figure 7.1).

Figure 7.1 Leaf injury at 24 h following ethephon application at the highest level of 5000 $\mu L.L^{-1}$.

This was followed by defoliation, firstly of the youngest leaves. Approximately 24 hours after spraying, severe leaf damage and defoliation of mature leaves occurred. At about one week after spraying, defoliation had occurred on all treated plants, but was most severe for the 5000 $\mu L.L^{-1}$ treatment rate. The differences in defoliation were very obvious. There was only 6% leaf retention on the bush compared to nearly 100% for the control paprika plants (Table 7.2). A similar response was obtained for chilli plants with 12, 20, 48 and 99% leaf retention on the bush for 5000, 3000, 1000 $\mu L.L^{-1}$ and control treatments, respectively (Figure 7.2). No leaf damage caused by ethephon was observed on the control plants.

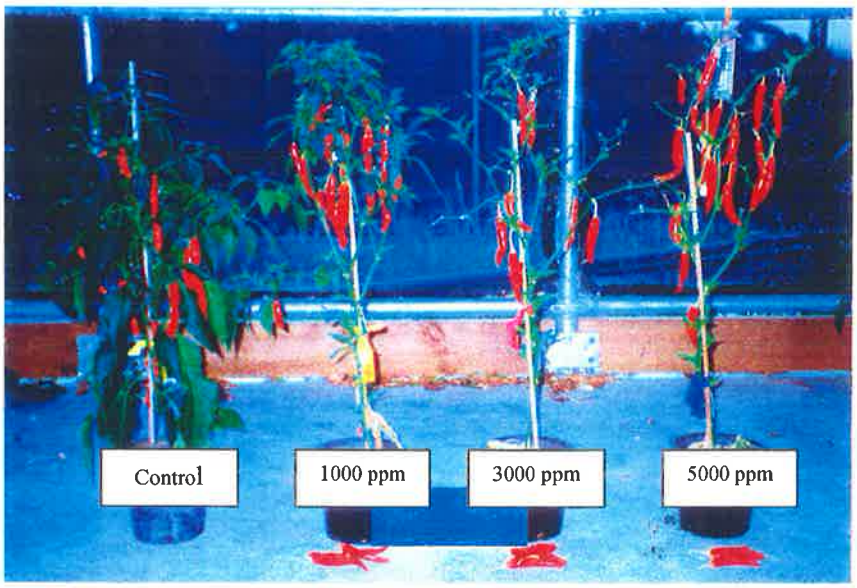


Figure 7.2 Plant response to ethephon application of 0, 1000, 3000 or 5000 $\mu L.L^{-1}$ (ppm) concentrations of chilli cv. 'Caysan SPS705'.

Ethephon also caused fruit skin damage (Figure 7.3), especially at the highest concentration. This was first observed a few minutes following chemical application. Symptoms were similar to the damaged leaves with brown and pitted areas scattered over the fruit surface. There appeared to be more damage on treated paprika fruit than on treated chilli fruit, but no damage was observed on the control fruit.

Figure 7.3 Fruit skin damage from ethephon-treated chilli plants.

7.4 Discussion

Although the amount of red marketable fruit (either including red fruit in the abscised group) was not significantly increased by ethephon treatments, there were significantly fewer green fruit at harvest for both cultivars. This suggests an acceleration of colour development of both 'PS72285' paprika pepper and 'Caysan SPS705' chilli fruit due to ethephon. Similar responses have also been found in various cultivars of *Capsicum annuum.* Ethephon reduced the percentage of green fruit, increased the percentage of breaker fruit and thus gradually increased the percentage of red fruit (Sims *et al.*, 1970; Knavel and Kemp, 1973; Sims *et al.*, 1974; Batal and Granberry, 1982).

The yield of red marketable fruit was not significantly increased by ethephon. Instead the yield was significantly reduced for chilli, but not altered for paprika. This was a result of red fruit drop in all ethephon treatments for the chilli. The paprika fruit were harvested at an earlier stage (one week earlier than the estimated harvesting time which would be about two weeks after spraying), before excessive abscission had occurred. Abscission had, however, commenced for the ethephon-treated fruit and would have led to a reduced yield similar to that of the chilli plants, had the harvest been delayed further. None of the ethephon treatments gave a significant increase in the dry weight of harvested red fruit compared to the control, a result similar to that of the study of Cooksey *et al.* (1994). In other studies, however, ethephon did increase the yield of red marketable fruit (Batal and Granberry, 1982), although total fruit number or weight of harvested fruit from the treated plants was significantly less than that from the control.

The effectiveness of ethephon to increase yield of red fruit by inducing fruit colour development has been reported to be primarily dependent on several factors. Ethephon, applied when 1 to 25% of the fruit are red to pink and harvested 14 to 21 days later, has been effective with most cultivars. Environmental conditions have shown an interaction with the chemical to accelerate both ripening and senescence. Under low temperature conditions (less than 21°C), the chemical is less effective, even at a concentration rate of 3000 $\mu L.L^{-1}$ ethephon (Knavel and Kemp, 1973). Higher temperatures after treatment, as under our growing conditions, have not only accelerated fruit ripening but also increased the potential of senescence such as defoliation, abscission and sun scald (de Wilde, 1971). Similar responses were observed in this study. Cantliffe and Goodwin (1975) also

suggested that in more sensitive cultivars, perhaps at lower concentration of ethephon yet leaving longer on the plants, benefit may accrue in terms of uniformity of colouring as less fruit drop, less sensitive cultivars may require higher concentrations of ethephon. However, another report (Sims *et al.*, 1970) suggested that higher concentrations are required for field experiments than for greenhouse experiments, and for cooler growing areas than for warmer areas. Therefore, evaluation of ethephon concentrations below 1000 $\mu L.L^{-1}$ was not considered in this study. Abscission may be reduced, but differences between the control and 1000 $\mu L.L^{-1}$ ethephon in colour pigment and overall red fruit yield were minimal or not significant, and therefore lower concentrations of ethephon would have even less effect on improving these factors.

Worku *et al.* (1975) and Batal and Granberry (1982) previously reported that ethephon increased the total extractable colour of dehydrated paprika fruit. However, overall there was little or no effect of ethephon on the intensity of extracted pigment in this study, similar to that of Cooksey *et al.* (1994) and Kahn *et al.* (1997). While the 1000 $\mu L.L^{-1}$ ethephon rate increased the intensity of red pigment extracted from dehydrated chilli fruit, the increase was on average less than 24 ASTA units. High light penetration into the canopy as less leaf obtained on the treated plants may possibly affect fruit colouring, however there was no sign of improved extractable colour change for dehydrated paprika in response to increased rates of ethephon. Skin damage caused by high ethephon treatments reduced the quality of fruit appearance, and this could possibly dilute the ASTA colour reading through lower colour values for higher chemical rates.

Ethephon increased the intensity of chilli pungency at 1000 and 3000 $\mu L.L^{-1}$ by about 50%, but not at the highest ethephon level. While this was a significant effect, yield losses would prevent the use of these treatments commercially. The expression of pungency has previously been noted to vary according to environmental conditions (Ahmed *et al.*, 1987; Lindsey and Bosland, 1995), as also found in the temperature study (Chapter 4).

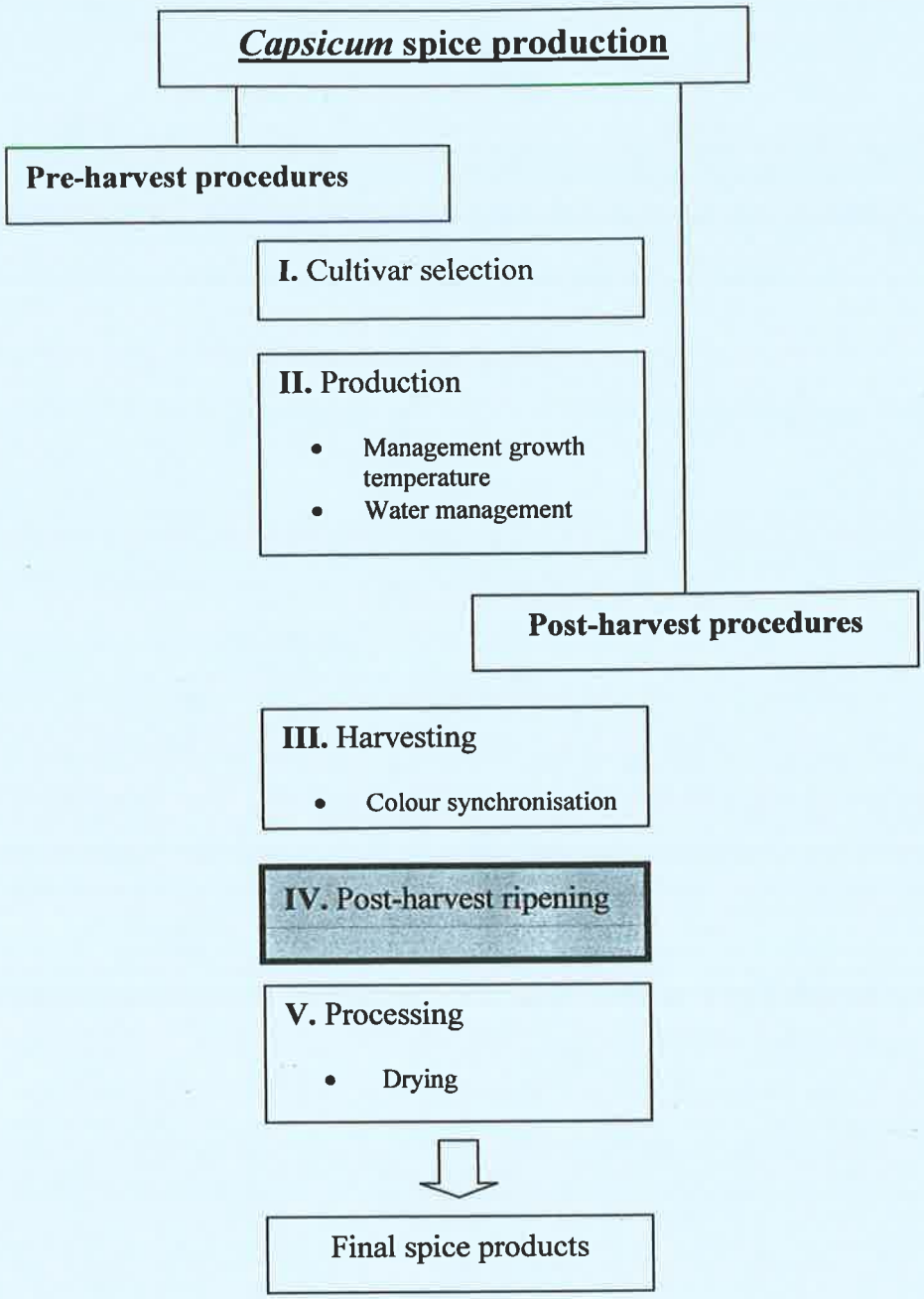
7.5 Conclusion

Ethephon significantly accelerated fruit colour development, as indicated by an increased percentage of red chilli fruit, and a decreased percentage of green paprika fruit. The colour change of *Capsicum* fruit was therefore associated with fruit ripening as it was accelerated by the plant hormone ethylene.

Ethephon had a negative effect on yield, reducing it up to 70% (for total yield) and 50% (for red fruit). It also promoted defoliation and fruit skin damage. A reduction of yield was mainly due to fruit abscission caused by ethephon. Plant damage and abscission, in part, may be enhanced due to the high temperature (at or above 30°C observed in the chilli experiment compared to 28°C in the paprika experiment) at the time of ethephon application. Therefore, ethephon should not be applied when the air temperature is above 30°C, and this would be the case in normal growing seasons in Australia.

For chilli, ethephon at 1000 $\mu L.L^{-1}$ increased extractable colour pigment of dehydrated fruit; pungency was also increased by both 1000 and 3000 $\mu L.L^{-1}$ ethephon, but increased abscission at these rates made ethephon treatments commercially unviable. Also, paprika fruit quality, as shown by extractable red colour of spice powder, was consistent without any marked effect of ethephon.

Application of ethephon was not economically useful for promoting once-over harvesting of either 'PS72285' paprika or 'Caysan SPS705' chilli under the conditions of this study, according to yield loss. It is more practical to leave the fruit on the bush until the maximum number of red fruit is obtained naturally, as control plants showed no tendency to abscise fully developed fruit.



The *Capsicum* spice production system.

Areas covered by this chapter are highlighted.

Chapter Eight

Colour at harvest and post-harvest ripening behaviour

8.1 Introduction

Colour stage at harvest is one of the important factors influencing colour (Isidoro *et al.*, 1990) and pungency levels (Ahmed *et al.*, 1987) of dehydrated capsicum products. It is common to harvest only completely red fruit since they have the higher level of red pigments (Deli *et al.*, 1996) that is necessary to achieve the best price in the trade. A traditional practice of post-harvest ripening termed 'curing' has been shown to increase the red pigment content of harvested red paprika (Gooindarajan, 1985b). Fruit are stored for 20-30 days in open areas (approximately 25 to 30°C) in a warehouse after harvesting, and this method is now routinely followed in Hungary prior to dehydration (Gooindarajan, 1985b). However, the benefit of curing to increase the red pigment content could be a varietal characteristic similar to variation between cultivars in the stability and formation of colour during fruit ripening on the plant prior to harvesting. Little information on this practice is available.

Since fruit in different positions on the *Capsicum* plants ripen at different times in the growing season, cost-effective once-over harvesting yields a mixture of fruit of different ripeness stages. Pre- or post-harvest treatments with a chemical ripening agent may be an alternative method to increase the number of red ripe fruit by inducing fruit colour development (Saltveit and Dilley, 1977; Biles *et al.*, 1993; Gómez *et al.*, 1998). However, the results from these treatments are often unsatisfactory. Detached mature green pimiento or sweet capsicum (bell pepper) failed to develop a red colour after treatment with ethephon or ethylene (Lockwood and Vines, 1972; Knavel and Kemp, 1973). In contrast, foliar application of ethylene-releasing chemicals to the plant of chilli, pimiento (Sims *et al.*, 1970; Lockwood and Vines, 1972), sweet capsicum (bell pepper) (Osterli *et al.*, 1975) and 'Tabasco' (Conrad and Sundstrom, 1987) successfully increased the colour development of their fruit. This was also found for paprika and cayenne chilli on the plant in Chapter 7. From these results, ethylene seems to have control over *Capsicum* fruit colour changes associated with fruit ripening, but overall information on this is limited.

The ripening of fruit has been classified into two categories, climacteric or non-climacteric (McGlasson, 1978). Fruit in the genus *Capsicum* are generally not considered to be climacteric (Lurie *et al.*, 1986; Biles *et al.*, 1993), and this has been specifically reported for some cultivars of *Capsicum annuum* (Saltveit and Dilley, 1977; Wall and Biles, 1994) and *C. frutescens* (Lu *et al.*, 1990). This is due to the lack of the typical increase in respiration (carbon dioxide) production and ethylene production during ripening (Biale, 1964; McGlasson, 1978). However, the hot chilli cv. 'Chooraehong' (*C. frutescens*) was reported to be climacteric (Gross *et al.*, 1986), with a respiratory climacteric peak observed, but very low ethylene levels were produced in association with fruit ripening. Many types of the climacteric fruit, for example banana (Biale, 1964), can be induced to change colour more rapidly during ripening when treated with exogenous ethylene. The exogenous ethylene induces autocatalytic biosynthesis of ethylene, and this accelerates fruit ripening (Biale, 1964). However, ethylene synthesis does not occur spontaneously in some non-climacteric fruit after treatment with exogenous ethylene (Biale, 1948). Given the often conflicting information, it is not clear whether red colour of unripe harvested capsicum fruit can be induced with exogenous ethylene, and whether quality changes of the processed products would occur.

Therefore, this chapter aimed *to investigate the effects of ripeness stage at harvest and ethylene treatment on harvested paprika (cv. 'PS72285') and chilli (cv. 'Caysan SPS705') fruit ripening to improve colour quality, pungency, and yield of red fruit after simulated once-over harvesting.*

The main findings of this chapter were published in Krajayklang *et al.* (2000).

8.2 Materials and methods

Plant material: Healthy fruit of 'PS72285' paprika and 'Caysan SPS705' chilli were hand harvested on the same day at different stages of ripeness. Seven ripeness stages were harvested; light green (G), deep green (DG), breaker (B, slight colouration), breaker red (BR, some red colour), bright red (R1, 100% red), deep red and succulent (R2), and deep red and partially dried (R3). Ten fruit of each colour category were randomly selected and separated into two groups, one as a control and one for ethylene treatment. The experiment was replicated three and four times for paprika and chilli, respectively.

Ethylene treatment and storage conditions: Within one hour after harvest, five fruits from each stage of ripeness were weighed in bulk and enclosed in 2.2 L plastic containers with 5 g of calcium hydroxide ($Ca(OH)_2$) to absorb evolved carbon dioxide (CO_2). Ethylene was injected through a septum port into the sealed containers, to obtain 100 parts per million ($\mu L.L^{-1}$). Ethylene was re-injected every 12 h for 48 h after flushing with air for 5 min. Containers were then opened, and fruit were stored in the same container without sealing at room temperature (20 ± 2°C) under cool-white fluorescent light for 7 to 10 days depending on rates of degradation. The lights had a photon flux of 70 $\mu mol.m^{-2}.s^{-1}$ at the level of the containers as measured with a quantum radiometer-photometer (Model LI-185A, John Morris Scientific Instruments, Sydney). Lights were on for about 12 hours out of every 24 hours. Visual colour, weight loss, visual appearance, carbon dioxide (CO_2) and ethylene (C_2H_4) evolution were determined during storage. Control fruit were enclosed in containers as above, but no ethylene was injected.

Visually external and internal quality assessments:

Fresh fruit colour

During storage, the development of surface fruit colour was daily scored using a subjective scale of 0 to 11 in which light green = 0, mature deep green = 1, breaker (chocolate colouration) = 2, breaker red (some red) = 3 to 7, light red (100% of surface red) = 9, deep red (fully red) = 10, and red-ripe (deep red and partially dried) = 11 (Figure 8.1). This scale was modified from a scale previously developed by Lownds *et al.* (1994) and Saltveit (1993) for fruit colour measurement in capsicum and tomato fruit, respectively.

Visual quality appearance

External quality was judged daily on a scale of 0 to 5 based on a degree of both fruit skin shrivelling (water loss) and calyx yellowing: 0 = extremely severe (calyx totally yellowing and severe shrivelling), 1 = severe (>50% of calyx yellow and much shrivelling), 2 = moderate (half calyx yellowing and some shrivelling), 3 = slight (slight yellowing and slight shrivelling), 4 = trace (no yellowing but little shrivelling) and 5 = excellent (no yellowing and no shrivelling).

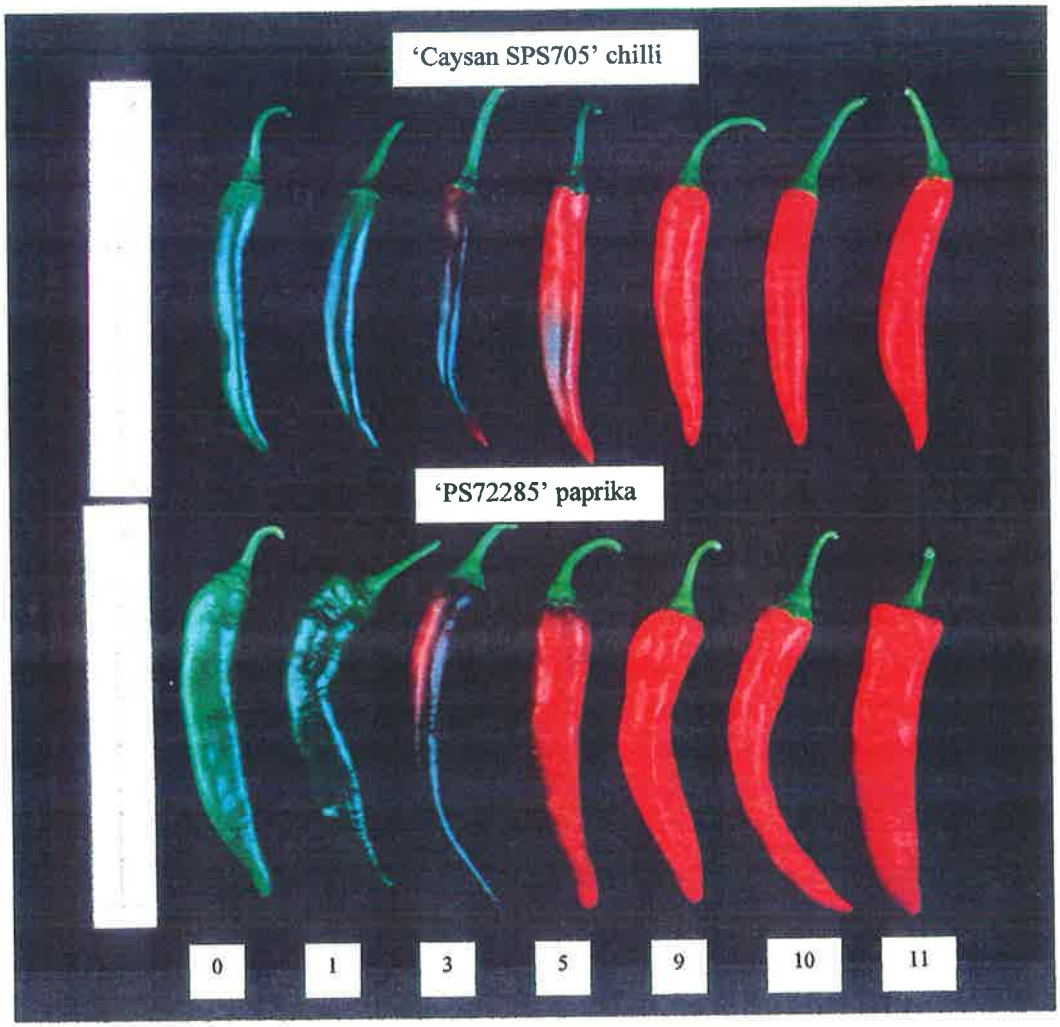


Figure 8.1 Ripening stages of 'Caysan SPS705' chilli (top) and 'PS72285' paprika (bottom). Each plate shows differences in surface colour of a single capsicum fruit at harvest. Seven stages are scaled from 0 to 11 based on the amount of red colour development;

0 = light (totally green but with low intensity),
1 = deep green (completely green and intense colour),
2 = breaker (chocolate colouration but no reddening),
3, 5, 7 = breaker red (25%, 50% to 75% red surface colouration),
9 = light red (100% red with little intensity),
10 = deep red (completely red with intense colour and succulent appearance),
11 = deep red and ripe (completely red with intense colour and partially dried appearance).

Decay evolution

Decay incidence in fruit was evaluated at the end of storage, both externally and internally. Numbers of fruit manifesting stem-end rots or other rots (mycelium present) were expressed as a percentage of total fruit for external decay incidence. Fruit were then cut horizontally into two parts, and evaluated for internal decay on a scale of 0 to 4. The scale ranged from 0 = no rot, 1= no rot, but slight discolouration on seed, 2 = slight rotting of fruit (a few spots), 3 = medium rotting on fruit (some spots scattered over <50% of pericarp surface), 4 = severe rotting on fruit (>50% rotting of pericarp surface).

Gas measurements: Rates of C_2H_4 and CO_2 production were measured daily by gas chromatography, using the same fruit throughout, after treatment with or without ethylene. A static, closed system was employed, whereby the storage container was sealed for a set period before taking gas samples for assessment.

C_2H_4 measurement

To measure C_2H_4, a 1 mL gas sample was collected from the container after 4 h of sealing the containers. C_2H_4 was quantified using a Varian chromatograph model 3400 equipped with a flame ionisation detector (Varian Australia, Mulgrave, Victoria) and a Porapak Q stainless steel column (60 cm × 3.1 mm i.d.) of 80/100 mesh. Temperature conditions were 50°C for the column, 135°C for the injector and 150°C for the detector. Flow rates of the carrier gas nitrogen, air and hydrogen were 50, 300 and 40 $mL.min^{-1}$, respectively. Results were expressed as µL of ethylene produced per kg of fresh weight and per hour ($\mu L.kg^{-1}.h^{-1}$). A gas standard containing 2.2 $\mu L.L^{-1}$ C_2H_4 standard (BOC Gases, Torrensville, South Australia) was used for calibration.

CO_2 measurement

CO_2 was measured at hourly intervals for 4 h by injecting 1 mL gas samples into a Varian 3300 chromatograph equipped with a thermal conductivity detector (Varian Australia, Mulgrave, Victoria) and a silica column (35 cm × 3.1 nm i.d.) of 80/100 mesh. Temperature conditions were 28°C for the column and 90°C for the injector and detector, and the flow rate of the carrier gas helium was 5 $ml.min^{-1}$. A calibration was performed using a 0.5% CO_2 standard (BOC Gases, Torrensville, South Australia). Results were expressed as mL of CO_2 produced per kg of fresh weight and per hour ($mL.kg^{-1}.h^{-1}$).

Fruit cavity ethylene measurement

The internal C_2H_4 concentration in the fruit cavity was measured in comparison with the external concentration surrounding the fruit inside containers, in order to determine ethylene uptake by fruit. Two groups of five fruit were enclosed in separate containers; one was used for a control (no C_2H_4 injection) and the other for a 100 $\mu L.L^{-1}$ C_2H_4 treatment. C_2H_4 was injected into the sealed container, and it was left at room temperature for 12 h. Two 1 mL gas samples were taken from each container, and used for determining the external C_2H_4 concentration. The container was then opened and flushed with air for 5 min. To measure the C_2H_4 concentration in the internal fruit cavity, samples were taken by inserting the needle of a 1 mL syringe through the ovary wall. The measurement was repeated every 12 h for 48 h after reapplication of ethylene. Where appropriate, the same fruit were used throughout the experiment. Results were expressed as $\mu L.L^{-1}$ of C_2H_4 concentration at that time of measurement. A Varian gas chromatograph was used for C_2H_4 analysis as previously described.

Colour determination and chemical analysis of spice: After quality evaluation and gas measurements, all fruit were dried in a hot air oven at 45°C to constant weight and ground with an electric mill. Ground samples were kept in airtight plastic bags in the dark at room temperature, and used for final quality measurements. Surface colour of powder samples was determined as the reflected L*/a*/b*/C* and h° colour values as previously described in section 3.6.2. For final chemical analysis, both extractable red colour (see section 3.6.3) and pungency (see section 3.6.4) were measured following procedures as previously described.

Statistical analysis: The experiment was conducted as a completely randomised factorial design, with seven colours at harvest × two ethylene applications. Each cultivar was evaluated as a separate experiment with replications as different times of harvest, approximately within a month of the total experiment (see time frame in Appendix I).

8.3 Results

8.3.1 Fruit appearance and decay

External quality of the green and the deep green harvested fruit at the end of storage was very poor for both, primarily due to significant shrivelling and calyx yellowing. However, breaker to the red ripe harvested fruit were acceptable after ten days of storage with slight calyx yellowing and little shrivelling (Table 8.1).

Table 8.1 Internal and external quality of *Capsicum* during ripening at room temperature after or without ethylene application.

Treatment factor		External appearance	Decay incidence[y]	
			External decay	Internal decay
		(0-5)[z]	(%)	(0-5)
		'PS72285' paprika		
Ripeness at harvest	Green	1.2 c[x]	0 a	0 a
	Deep green	1.2 c	0 a	0 a
	Breaker	2.7 b	0 a	0 a
	Breaker red	3.0 ab	0 a	0 a
	Bright red	2.9 ab	0 a	0 a
	Deep red	3.2 a	0 a	0 a
	Deep red + dried	3.0 ab	0 a	0 a
Ethylene	Without (-)	2.4 a[x]	0 a	0 a
	With (+)	2.5 a	0 a	0 a
Interaction	Ripeness * Ethylene	ns	ns	ns
		'Caysan SPS705' chilli		
Ripeness at harvest	Green	1.5 c[x]	0 a	0.1 a
	Deep green	1.7 bc	0 a	0.3 a
	Breaker	2.3 ab	0 a	0.3 a
	Breaker red	2.4 ab	0 a	0.3 a
	Bright red	2.8 a	0 a	0.4 a
	Deep red	3.0 a	0 a	0.5 a
	Deep red + dried	3.0 a	0 a	0.5 a
Ethylene	Without (-)	2.4 a[x]	0 a	0.3 a
	With (+)	2.4 a	0 a	0.3 a
Interaction	Ripeness * Ethylene	ns	ns	ns

[z] External appearance was scored on a scale of 0 to 5 based on fruit skin shrivelling (wt. loss) and calyx discolouration: 0 = extremely severe, 1 = severe, 2 = moderate, 3 = slight, 4 = trace and 5 = zero (excellent).
[y] Decay incidence in fruit was evaluated at the end of storage. Numbers of fruit manifesting stem-end rots or other rots (mycelium present) were expressed as the percentage of total fruit for external decay incidence. Fruit were then cut horizontally into two parts, and evaluated for internal decay on a scale of 0 to 4: 0 = no rot, 1= slight discolouration on seed, 2 = slight rotting of fruit, 3 = medium rotting on fruit, 4 = severe rotting on fruit.
[x] Means within columns for each cultivar and each treatment factor followed by the same letter are not significantly different at $P < 0.05$; ns = not significantly different.

Ethylene had no effect on external quality or decay incidence in paprika or chilli (Table 8.1). Harvested chilli fruit of all ripeness stages developed a slight discolouration on their seeds after storage, indicating possible internal fungal development (Table 8.1), while harvested paprika fruit showed no sign of any fungal development throughout the experiment. There was no visible major disease or other rot development for any fruit after storage for both cultivars (Table 8.1).

8.3.2 Fruit colour development

Ethylene application did not influence the final colour of fresh paprika and chilli fruit (Table 8.2). However, final colour development of paprika was slightly delayed by ethylene application, but responds differed for each colour stage as indicated by the significant interaction (Table 8.2). Furthermore, time taken for either cultivar to complete red colouration was delayed by ethylene application, and the response to ethylene was different for each colour stage based on a significant interaction (Table 8.2).

Green and deep green harvested fruit achieved less than 50% red colouration after storage for both cultivars (Table 8.2). Up to eight days additional exposure to ethylene did not further promote colour development of these fruit (data not shown). Fruit harvested at breaker stage or later developed a full red colouration. Within six to seven days, fruit harvested at breaker stage reached a 100% red colour (stage 9), and the breaker red stage took four to five days for both cultivars (Table 8.2).

Table 8.2 Colour development of *Capsicum* fruit harvested at different colour stages during storage at room temperature with or without ethylene application.

Treatment factor		Colour development		
		Final colour (0-11)[z]	Time to final colour (days)	Time to 100% red (days)[y]
		'PS72285' paprika		
Ripeness at harvest	Green	4.4b[x]	8.2abc	-
	Deep green	4.7b	9.3a	-
	Breaker	10.2a	8.7ab	6.8a
	Breaker red	10.2a	7.7bc	4.7a
	Bright red	10.4a	7.0c	0.4b
	Deep red	11.0a	3.5d	0.0b
	Deep red + dried	11.0a	0.0e	0.0b
Ethylene	Without (-)	8.9a[x]	5.9b	2.2b
	With (+)	8.8a	6.7a	2.6a
Interaction	Ripeness * Ethylene	ns	*	***
		'Caysan SPS705' chilli		
Ripeness at harvest	Green	2.1e[x]	9.5a	-
	Deep green	4.3d	9.0a	-
	Breaker	9.4c	8.9a	5.7a
	Breaker red	9.8bc	8.8a	4.3a
	Bright red	10.8ab	8.5a	0.0b
	Deep red	11.0a	4.9b	0.0b
	Deep red + dried	11.0a	0.0c	0.0b
Ethylene	Without (-)	8.3a[x]	6.8a	2.1a
	With (+)	8.4a	7.3a	1.9a
Interaction	Ripeness * Ethylene	ns	ns	*

[z] Skin colour was scored on a scale from 0 to 11: 0=green, 1=deep green, 2=slight colouration, 3=25% red, 5=50% red, 7=75% red, 9=100% red (light red), 10=deep red and succulent, 11=deep red and partially dried.
[y] Time to 100% red colour development during storage; 0 = at red harvest day, - = did not achieve 100% red colour.
[x] Means within columns for each cultivar and each treatment factor followed by the same letter are not significantly different at $P< 0.05$; ns = not significantly different, *, ** or *** = significantly different at $P<0.05$, 0.01 or 0.001, respectively.

8.3.3 Colour of spice powder

Reflected colour of the powder was not affected by ethylene treatment for any cultivar (Table 8.3). No interaction was found between the treatment factors for all reflected colour values (Table 8.3).

Table 8.3 Reflected colours of *Capsicum* powder made from fruit that were harvested at different colour stages and ripened with or without ethylene.

Treatment factor		Colour characteristics [z]				
		L*	a*	b*	C*	h°
		'PS72285' paprika				
Ripeness at harvest	Green	52a[y]	19c	48d	52b	69a
	Deep green	52a	24c	51cd	57b	65b
	Breaker	45b	36b	55ab	66a	58c
	Breaker red	43b	42a	56a	70a	54d
	Bright red	42bc	42a	58a	71a	54cd
	Deep red	41bc	42a	54abc	68a	53d
	Deep red + dried	38c	42a	52bc	67a	51d
Ethylene	Without (-)	45a[y]	35a	53a	64a	58a
	With (+)	45a	36a	54a	65a	57a
Interaction	Ripeness * Ethylene	ns	ns	ns	ns	ns
		'Caysan SPS705' chilli				
Ripeness at harvest	Green	52a[y]	10c	42c	44c	79a
	Deep green	52a	19b	49b	53b	70b
	Breaker	47b	40a	59a	71a	56c
	Breaker red	46b	40a	58a	71a	56c
	Bright red	45b	41a	58a	72a	55c
	Deep red	42c	42a	57a	71a	54c
	Deep red + dried	42c	42a	56a	70a	53c
Ethylene	Without (-)	47a[y]	33a	54a	64a	60a
	With (+)	46a	34a	46a	65a	60a
Interaction	Ripeness * Ethylene	ns	ns	ns	ns	ns

[z] Colour values measuring in CIELAB; L* = lightness (0=black, 100=white), a* = green (negative) to red (positive), b* = blue (negative) to yellow (positive), C* = chroma (0=least intense), h° = hue angle (0°=red-purple, 90°=yellow).

[y] Means within columns for each cultivar and each treatment factor followed by the same letter are not significantly different at $P< 0.05$; ns = non significant difference.

As colour stage at harvest increased, paprika powder from redder fruit obtained a darker colour as indicated by a reduction of L* value, from 52 to 38, for the green and red-ripe harvested fruit, respectively (Table 8.3). Both a* (red component) and b* (yellow component) values increased with greater ripeness stages (Table 8.3). Colour saturation (C*) increased for green to breaker-harvested fruit, and then remained constant (Table 8.3). The higher C* value of partially to fully red harvested fruit represents a more vivid colour. The hue angle of green harvested fruit after storage describes a more yellow colour with the highest angle of 69, while an increase in ripeness stage at harvest resulted in an increase in red colour with the lowest angle of 51 in red-ripe harvested fruit (Table 8.3).

The colour of chilli powder showed the same characteristics. With increasing ripeness stage at harvest, powder of stored fruit was more intense in red colour. The L* value ranged from 52 to 42, C* ranged from 44 to 70, a* ranged from 10 to 42, b* ranged from 42 to 56, and hue angle was about 70 to 53 for green harvested fruit to red-ripe harvested fruit, respectively (Table 8.3). However, it was clear that most reflected colour values (except the L* value) showed a significant change for green to breaker stage, and then remained constant (Table 8.3).

Deep-red coloured fruit (stage 10 or 11) achieved the maximum extractable red colour, 194 and 120 ASTA units for partially dry paprika and chilli fruit, respectively (Table 8.4). Partially dry chilli fruit provided a more intense extractable red colour of spice powder than the succulent ones. There was no difference for paprika powders between these stages, as ASTA values were more variable. Green and deep green fruit at harvest obtained a very low colour intensity of 50 to 60 ASTA units for paprika and about 25 to 40 ASTA units for chilli (Table 8.4). Breaker to bright red fruit at harvest, while appearing visually as red or deep red when ripe, had reduced extractable colour of the spice powder.

Table 8.4 Extractable red colour and pungency of *Capsicum* powder made from fruit that were harvested at different colour stages and ripened with or without ethylene.

Treatment factor		Colour (ASTA)[y]	Pungency (SHU)[z]
		'PS72285' paprika	
Ripeness at harvest	Green	50d[x]	-
	Deep green	63d	-
	Breaker	104c	-
	Breaker red	139b	-
	Bright red	139b	-
	Deep red	169a	-
	Deep red + dried	194a	-
Ethylene	Without (-)	123a[x]	-
	With (+)	123a	-
Interaction	Maturity * Ethylene	ns	-
		'Caysan SPS705' chilli	
Ripeness at harvest	Green	25f[x]	16,000a
	Deep green	38e	16,000a
	Breaker	74d	17,000a
	Breaker red	89c	17,000a
	Bright red	90c	18,000a
	Deep red	106b	17,000a
	Deep red + dried	120a	22,000a
Ethylene	Without (-)	76b[x]	18,000a
	With (+)	79a	18,000a
Interaction	Maturity * Ethylene	ns	ns

[z] Scoville heat units were calculated by multiplying the amount of capsaicin and dihydrocapsaicin per gram dry weight by 16 million Scoville units for pure capsaicin and dihydrocapsaicin; - = no measurement was taken due to negligible pungency of the fruit.
[y] Extractable red colour was measured in the American Spice Trade Association, colour units (ASTA) per gram dry weight; calyxes were removed before analysis.
[x] Means within columns for each cultivar and each treatment factor followed by the same letter are not significantly different at $P< 0.05$; ns = non significant difference.

8.3.4 Pungency

Pungency or hotness was absent in paprika fruit. Pungency of chilli powder did not increase significantly with ripeness (Table 8.4). Ethylene had no effect on pungency (Table 8.4).

8.3.5 CO_2 and C_2H_4 production

During post-harvest ripening: Neither respiration nor ethylene production of any harvested fruit was affected by exogenous ethylene treatment for both paprika (Figure 8.2) and chilli (Figure 8.3).

Typically, respiration declined directly after harvest for all coloured fruit without a significant respiratory peak throughout the experiment for both paprika (Figure 8.2) and chilli (Figure 8.3). Although, respiration increased slightly during storage in some detached paprika fruit, this was not associated with any particular surface colour changes or increased ethylene production (Figure 8.2, A, B, C, D). C_2H_4 production was very low for all fruit with or without ethylene treatment and for both cultivars. The C_2H_4 concentration was very high only at the first measurement (on day 2) in all treated fruit. This was entirely due to contamination from previous ethylene treatment since C_2H_4 concentration dropped significantly after day 2 which became similar to the control fruit until at the end of storage in both cultivars (Figure 8.2, 8.3).

At harvest: A distinct climacteric pattern was apparent although it was a broad peak, when comparing the respiration and ethylene production rates of different ripeness stages of paprika fruit immediately after harvest (Figure 8.4A). These climacteric increases were associated with fruit surface colour change. The C_2H_4 peak occurred at the breaker red stage when the fruit surface was 50% red (Figure 8.4A). The C_2H_4 production of the peak was 0.4 $\mu L.kg^{-1}.h^{-1}$, about three-fold of that for the green fruit at 0.1 $\mu L.kg^{-1}.h^{-1}$ (Figure 8.4A). CO_2 peaked at about 81 $mL.kg^{-1}.h^{-1}$ at the same time as C_2H_4 (Figure 8.4A). After this stage, both C_2H_4 and CO_2 production declined gradually with an increase in ripeness.

The respiratory climacteric was absent for just harvested chilli fruit. However, CO_2 production was high from green to breaker red fruit, and markedly declined with ripeness thereafter (Figure 8.4B). In contrast, C_2H_4 production increased until fruit was turning colour (breaker), and declined after it peaked at 0.33 $\mu L.kg^{-1}.h^{-1}$ when the fruit were completely red (R1) (Figure 8.4B).

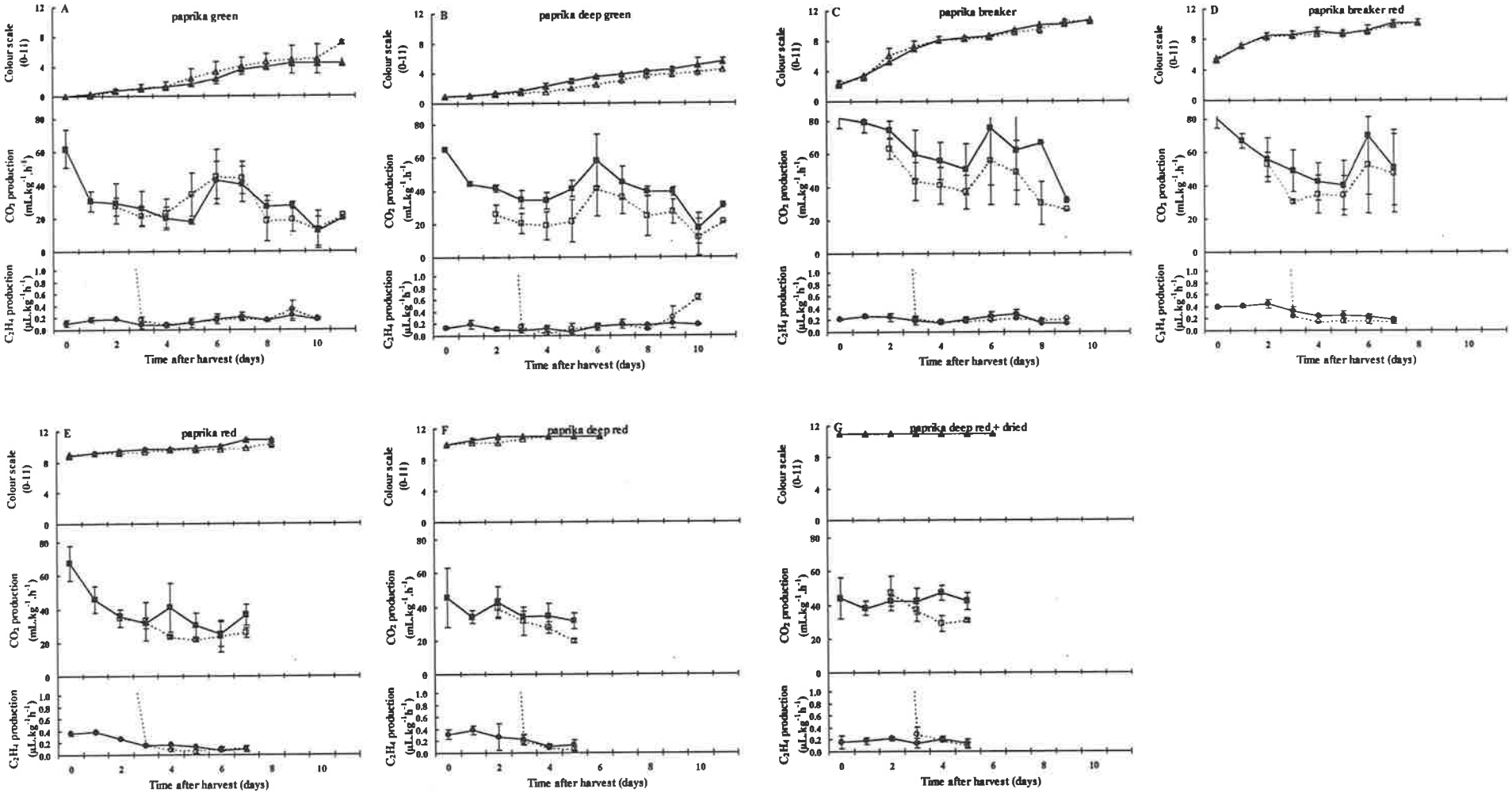


Figure 8.2 Colour development (▼, ▽), ethylene (●, O) and carbon dioxide (■, □) production of paprika fruit harvested at different coloured stages; (A) green, (B) deep green, (C) breaker, (D) breaker red, (E) red, (F) deep red, (G) deep red and dried. Fruits exposed to air (closed symbols and solid lines) or 100 $\mu L.L^{-1}$ ethylene (open symbols and broken lines) for 48 h. Three replications of five fruits were used for each measurement. Error bars represent the SE of the mean.

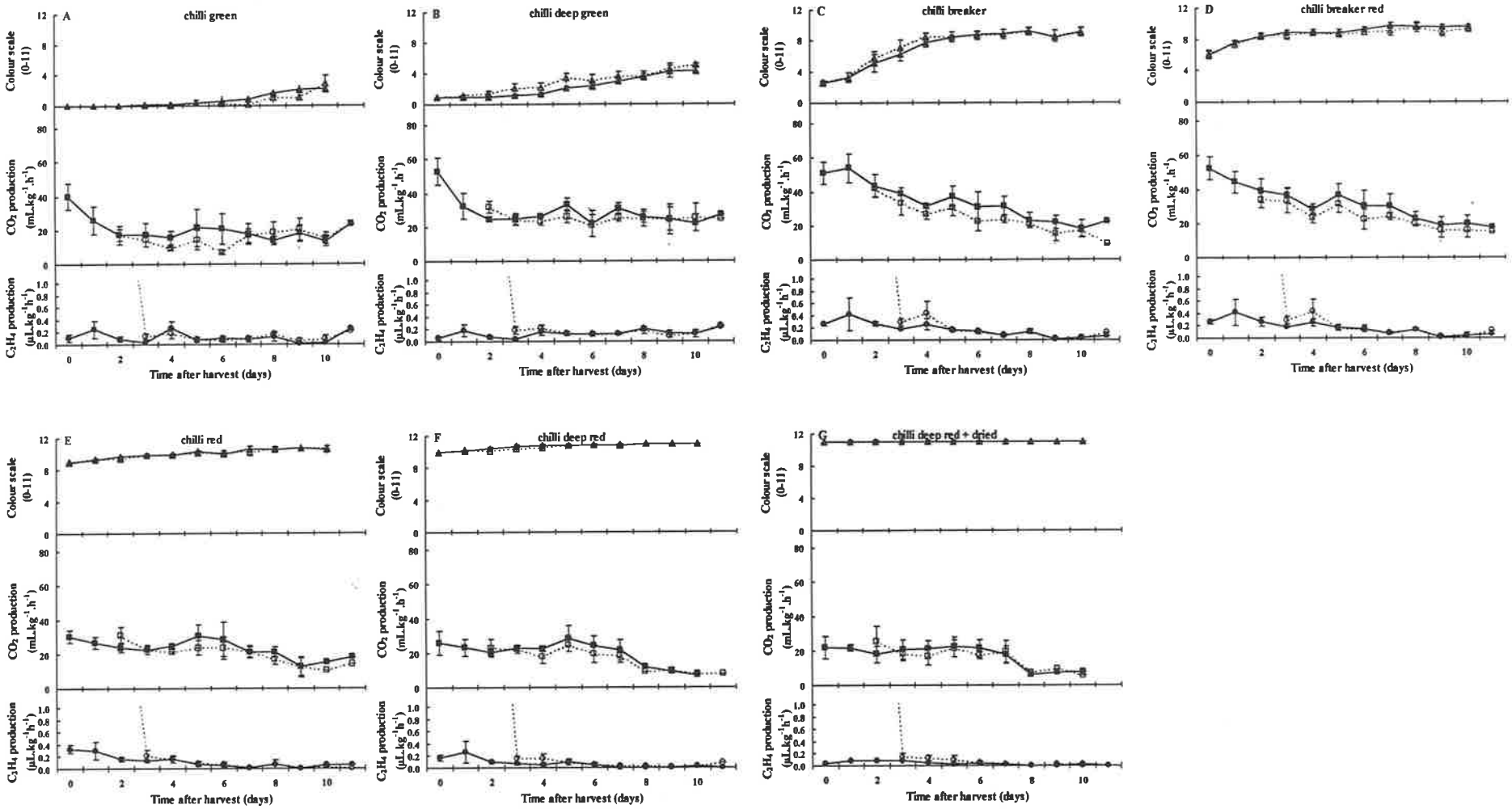


Figure 8.3 Colour development (▼, ▽), ethylene (●, O) and carbon dioxide (■, □) production of chilli fruits harvested at different coloured stages; (A) green, (B) deep green, (C) breaker, (D) breaker red, (E) red, (F) deep red, (G) deep red and dried. Fruit were exposed to air (closed symbols and solid lines) or 100µL.L^{-1} ethylene (open symbols and broken lines) for 48 h. Four replications of five fruits were used for each measurement. Error bars represent the SE of the mean.

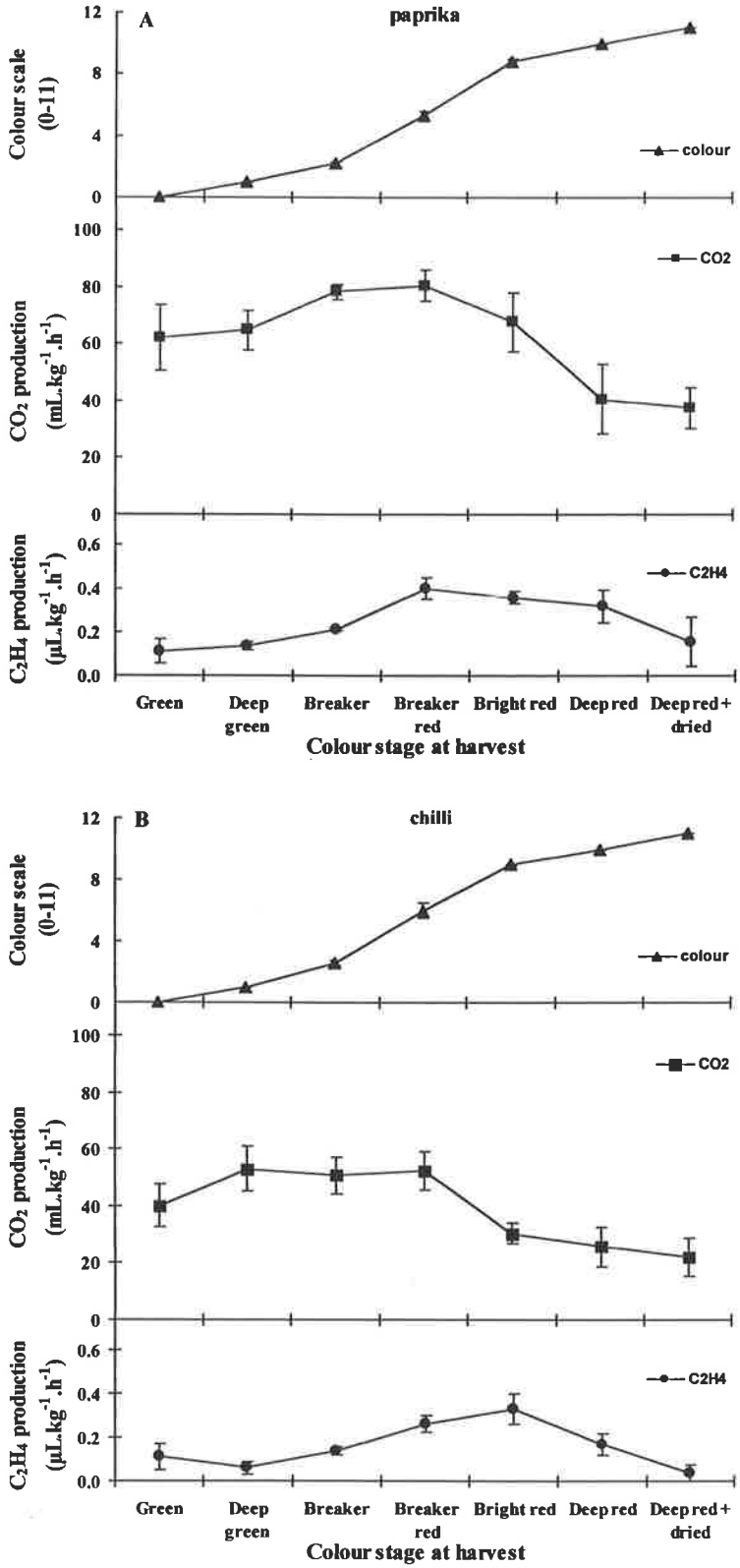


Figure 8.4 Changes in colour (▼), ethylene (●) and carbon dioxide (■) production in different ripeness stages of just harvested paprika (A) and chilli (B) fruits. Stage of ripeness is based on skin surface colour: green (G), deep green (DG), breaker (B, slight colouration), breaker red (BR, some red), bright red (R1), deep red but succulent (R2), deep red and partially dry (R3). Three (for paprika) or four (for chilli) replications of five fruits were used for each measurement. Error bars represent the SE of the mean.

Internal ethylene atmosphere: At harvest, both control and ethylene treated fruit had a similar internal ethylene atmosphere concentration (Table 8.5). After ethylene was injected into the container, internal and external ethylene atmospheres were markedly different for treated and untreated fruit (Table 8.5). About 0.02-0.06 and 18.2-22.8 $\mu L.L^{-1}$ ethylene were detected in the internal atmosphere inside the cavity of control and treated fruit, respectively. The external atmosphere surrounding control fruit contained 0.04-0.05 $\mu L.L^{-1}$ ethylene, while that of treated fruit was about double that of the treated-fruit cavity at about 40.2-43.9 $\mu L.L^{-1}$ (Table 8.5). The difference may have been caused by air leakage into the syringe during the sampling and after retracting the needle due to reduced pressure within the syringe. The syringe may also cause damage to fruit and therefore affect ethylene production, but the average increase of internal ethylene atmosphere of control fruit from 0 to 48 h after harvest was very small at 0.03 $\mu L.L^{-1}$ (Table 8.5). A similar minimal effect of syringe wounds is to be expected in ethylene treated fruit.

Table 8.5 Internal and external ethylene atmosphere of harvested paprika fruits with or without ethylene application. Measurement was obtained from five readings after ethylene was applied at 12 hours intervals for 48 hours.

Treatment	Time after harvest (h)	Ethylene concentration ($\mu L.L^{-1}$)	
		Internal	External
Control	0	0.02 ±0.02[z]	-
	12	0.04 ±0.01	0.04 ±0.00
	24	0.04 ±0.01	0.04 ±0.00
	36	0.06 ±0.02	0.05 ±0.00
	48	0.05 ±0.02	0.05 ±0.00
Treated	0	0.01 ±0.01	-
	12	22.8 ±2.34	40.2 ±0.19
	24	18.2 ±2.40	43.9 ±0.03
	36	20.4 ±3.78	42.7 ±0.02
	48	19.1 ±3.37	40.6 ±2.10

[z] Mean ± SE; - = no measurement.

8.4 Discussion

Fruit appearance: Green harvested fruit appeared to be more susceptible to water loss than red-ripe harvested fruit after ten days of storage. It has been noted that during the growing season green chilli fruit are highly sensitive to water loss and disorders influenced by environmental conditions such as heat damage (Wall and Biles, 1994). Therefore, a control of water loss, especially for green fruit, is essential if fruit are to be stored for the fresh market. From our results, it also appears that harvested paprika and chilli fruit can be stored at room temperature for at least ten days without any severe fungal development prior to dehydration. Successful post-harvest storage in an open area of a warehouse (approximately 25-30°C) for a maximum of 30 days after harvest has been recently used in Hungary in order to improve fruit colour content in dehydrated paprika production (Gooindarajan, 1985b).

Fruit colour development: Exogenous ethylene treatment was not effective for inducing red colour development of detached paprika and chilli fruit under the conditions in this study. Green harvested fruit failed to fully colour even when treated with C_2H_4. Similar results were found in detached green pimiento (Knavel and Kemp, 1973) or bell pepper fruit (Lockwood and Vines, 1972) after they were treated with either ethylene (500 $\mu L.L^{-1}$) or ethephon (1000 $\mu L.L^{-1}$). While C_2H_4 could diffuse through the fruit cuticle, it was not able to promote post-harvest colour development in this study. However, C_2H_4 slightly delayed the colour development of paprika fruit, but not chilli. Lockwood and Vines (1972) also found that C_2H_4 had an effect on delaying pimiento fruit colour development. The reason for this action is not known. This may be related to variation among pepper species, since differences in carotenoid profiles among species have been observed during fruit colour development that was associated with fruit ripening (de Guevara and Pardo-Gonzalez, 1996).

Once fruit had been harvested at or near the breaker ripeness stage, they coloured normally for both cultivars. While on the plant, fruit were able to develop their colour satisfactorily with the occurrence of respiration and ethylene climacteric peaks. Surface colour change in capsicum fruit normally involves both the degradation of chlorophyll (green component) and the *de novo* production of ketocarotenoids, principally capsanthin and capsorubin (red components) (Mínguez-Mosquera and Hornero-Méndez, 1994). With the onset of ripeness,

there was an accumulation of red pigments that were not found in green paprika fruit (Márkus *et al.*, 1999). This suggested that red colouring of fruit attached to the plant appears to involve some additional factors, such as other hormones or presence of sufficient pigment precursors, that interacted with ethylene to induce full colour changes from green to deep red. In addition the chlorophyll degradation process is impaired in harvested fruit.

Ripening pattern: This study found no response of detached chilli or paprika fruit to applied external ethylene. It has been reported that an application of ethylene or propylene to climacteric fruit can stimulate both respiration and autocatalytic ethylene production, while in non-climacteric fruit exogenous ethylene stimulates respiration only (McGlasson, 1978; McGlasson, 1985). According to the first assertion, it was found that harvested paprika and chilli fruit behaved in a non-climacteric fashion because no respiratory climacteric rise was observed. The second assertion also did not hold in this study, as exogenous ethylene appeared to not stimulate the respiration rate in paprika nor chilli fruit. On the other hand, it seemed to suppress the respiration rate in paprika fruit that were harvested close to the breaker stage (Figure 8.2B, C, D) compared to the untreated fruit during the first four days after harvesting. This may be a reason for the delayed colour development of treated paprika fruit. However, the difference in response to applied exogenous ethylene suggests that it varied between species and between cultivars within the same species (McGlasson, 1985; Wills, 1998).

When comparing the respiration and ethylene production rates of just harvested control fruit from green to red-ripe stages, a climacteric rise was present when fruit changed colour that was not observed during storage. This suggests that the process of ripening associated with respiration, ethylene production and surface colour change in capsicum fruit proceeds differently on and off the plant. Differences in respiratory pattern of various fruit during their ripening on and off the plant have been previously recorded (Rhodes, 1970). Some climacteric fruit such as avocado only showed a respiration climacteric rise after detachment from the tree. Pineapple, as an example of a non-climacteric fruit, shows a rise that could be interpreted as a respiration climacteric only on the plant, but in no case was a peak evident after detachment from the plant (Dull *et al.*, 1967) (cited in Rhodes, 1970). This was similar to the paprika fruit in this study. It is therefore difficult to decide whether

there is a distinct difference in the mechanism of fruit ripening between climacteric and non-climacteric types of fruit on and off the plant, and what that difference arises from.

A comparison to the levels and patterns of C_2H_4 and CO_2 production in other studies, supports that paprika and chilli fruit in this study could be classified as non-climacteric. The highest C_2H_4 production level for paprika and chilli fruit at 22°C was 0.40 and 0.33 $\mu L.kg^{-1}.h^{-1}$ in this study. This was similar to the New Mexico type chilli (Wall and Biles, 1994) and 'Messilla' chilli (Villavicencio *et al.*, 1999), with 0.27 and 0.50 $\mu L.kg^{-1}.h^{-1}$, respectively. This was lower than the 0.7 $\mu L.kg^{-1}.h^{-1}$ reported for the only climacteric type, 'Chooreahong' hot chilli (Gross *et al.*, 1986). Maximum CO_2 production in paprika fruit under the conditions in this study was about 80 $mL.kg^{-1}.h^{-1}$ and in chilli fruit 53 $mL.kg^{-1}.h^{-1}$ which was also comparable to previous studies of capsicum fruit (Gross *et al.*, 1986; Biles *et al.*, 1993).

Colour of spice powder: The concentration of colour pigment in dried paprika and chilli powder was not affected by ethylene application, mirroring the lack of effect on visual fruit colour. Extractable colour on a dry weight basis was highest in fruit allowed to dry on the plant (pre-harvest dehydration). Similar findings were reported for red-ripe harvested paprika fruit (Kanner *et al.*, 1977) and field-dried cayenne chilli (Lease and Lease, 1956). In addition, there was a similar high level of colour for paprika fruit that were succulent but deep red. These stages of ripeness have been previously mentioned as being optimal for harvest of spice paprika in Hungary (Márkus *et al.*, 1999) and in USA for both paprika and chilli spices (Lease and Lease, 1956). In addition to achieve better spice colour quality, it is necessary to allow fruit partially dry (at the red-ripe stage), as β-carotene is completely converted to more stable red coloured xanthophylls by the red-ripe stage (Márkus *et al.*, 1999). This improved the colour stability during processing (Márkus *et al.*, 1999) and storage life of spice powder (Lease and Lease, 1956).

Although reflected colour, measured as L*, a*, b*, C* or h° values, indicated changes in spice surface colour at different colour stages, these could not be used for characterising colour pigment content. For example, two cultivars of capsicum fruit with significant differences in total colour pigment concentration showed the same visual colour which was measured as L*a*b* values (Gooindarajan, 1986). While the L* value was able to better

differentiate between powders from different colour stages at harvest, this measurement did not always mirror changes in ASTA colour values. Therefore, L* values can not be used to replace the industry standard of measuring extractable red colour as described by the ASTA method.

Pungency: Pungency of chilli powder in this study did not vary with different ripeness stages after storage. This agreed with previous reports (Somos, 1984), in which the degree of hotness remained constant during paprika fruit maturation. However, Balbaa *et al.* (1968) found that the level of pungency increased as fruit matured to a red stage for *C. frutescens* and *C. minimum*, and slightly declined as *Capsicum* fruit (no species mentioned) reached a fully red colour (Mathew *et al.*, 1971) (cited in Cotter, 1980). As only red fruit are processed into spice powder, always the maximum possible pungency will be achieved for the spice if harvest is based on colour stage.

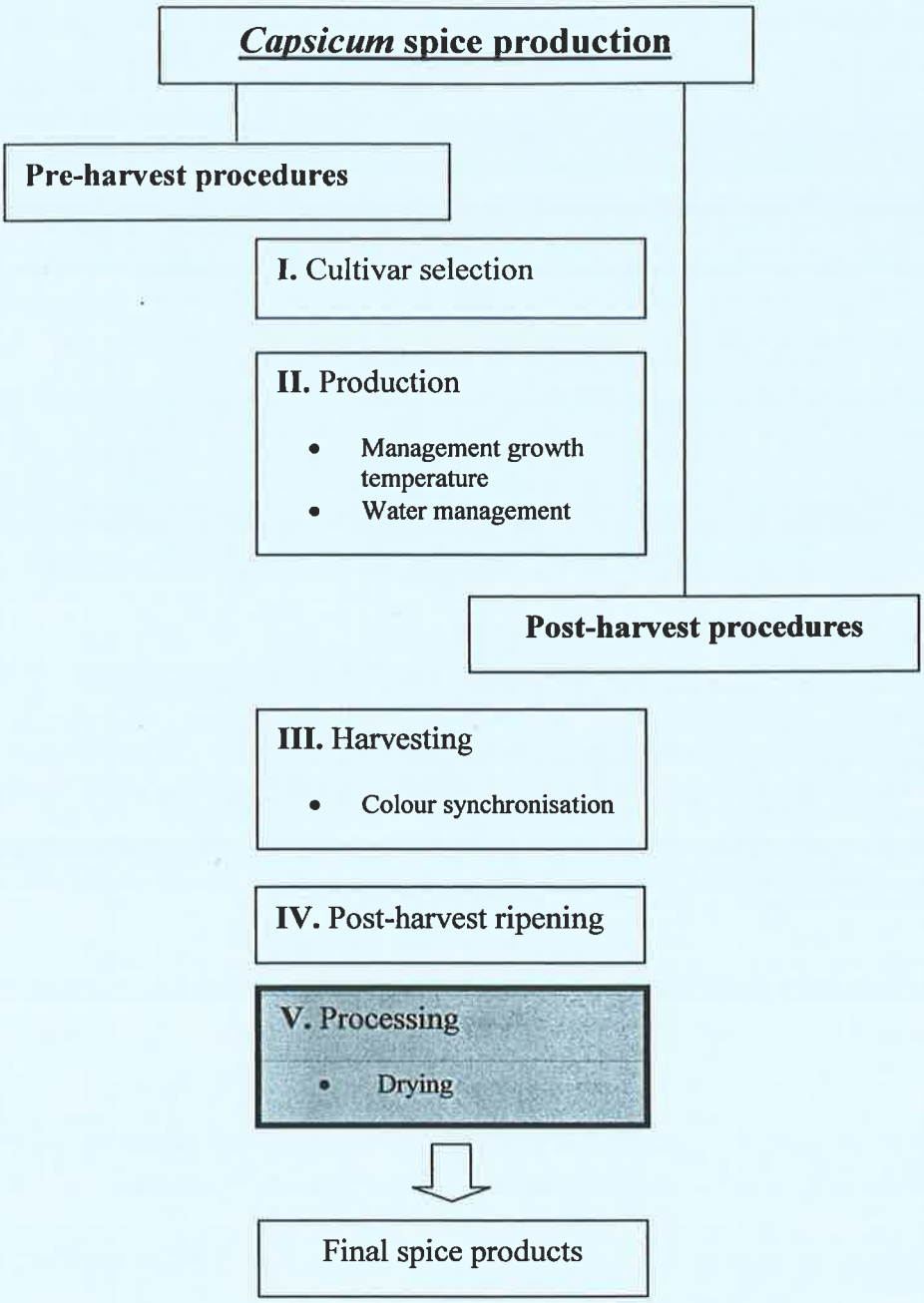
8.5 Conclusion

a) Harvesting of paprika and chilli fruit at different colour stages significantly affected final colour of spice powder, but not pungency levels. Green or deep green harvested fruit failed to fully colour red after harvest, while fruit that were harvested at or after the breaker stage completed their red colour development. However, allowing fruit of both cultivars to fully ripen and/or partially dry on the bush resulted in the maximum colour content after processing.

b) Measurement of extractable red colour with the ASTA colour units was the most suitable method for spice colour assessment, compared to the reflected colour values.

c) Exogenous ethylene application neither affected red colour developments nor pungency of any colour stage at harvest. A distinct climacteric pattern associated with surface colour change was found when paprika fruit were attached to the mother plant. However, once fruit were harvested, a respiratory climacteric was not observed. Both paprika and chilli fruit in this study can be classified as non-climacteric.

d) Therefore, fruit that are fully red at harvest, either succulent or partially dried, are needed to produce the best spice quality. After once-over machine harvesting, fruit that have not achieved this stage should be culled before processing.



The *Capsicum* spice production system.

Areas covered by this chapter are highlighted.

Chapter Nine

Drying process

9.1 Introduction

With industrial drying processes, harvested capsicum fruit are normally washed, roughly cut into pieces of unequal size and placed on drying racks (J. Small, pers. comm., 1998). At this stage colour degradation is significant; therefore it is considered advisable to dry fruit as fast as possible in order to reduce colour loss.

Several factors have been found to affect the drying rates; drying conditions (ie. temperature, relative humidity) and properties of the product (ie. rate of moisture loss, relative area of cut surface) (Sigge *et al.*, 1998), with increasing drying temperature and decreasing relative humidity increasing drying rate. However, overheating at temperatures such as 80°C results in poor colour quality (Lease and Lease, 1962). The optimum drying temperature is recommended to be below 65°C (Li *et al.*, 1994). However, with lower temperatures ranging from 50 to 60°C colour quality may also suffer due to prolonged drying (≥20 h) in industrial driers (Zapata *et al.*, 1992) (cited in Ibrahim *et al.*, 1997). Under such conditions, the drying process could take up to 36 h to achieve an 8% final moisture content when using a heat-pump drier running at a low temperature (45°C) (J. Small, per. comm., 1998). Considering the prolonged periods, and due to the initial high moisture content of the fruit, fungal development may occur rapidly inducing colour loss. More effort is required to improve this process in order to increase the drying rate, especially under conditions of lower drying temperature.

A combination of modified drying conditions and a conventional cutting method to increase rates of moisture loss shows promise, as has been practiced with spice *Capsicums* in USA (Purseglove *et al.*, 1981). For example, for a 65°C drying temperature, slicing reduced drying times to 8 h to dry fruit to a moisture content (MC) of 7-8%, while 12 h was required for whole fruit of a Cayenne type chilli with 80% MC prior to drying (Lease and Lease, 1962). Therefore, one approach to achieve faster drying and to reduce fungal development is to cut fruit, but this also gives fungi access to fruit tissues. Also, no information is available on the effects of this practice on final spice colour quality.

The *Capsicum* fruit surface is naturally covered with a cuticle composed of the bio-polymer cutin and embedded wax, with epicuticular waxes on the outer surface. This cuticle is the major barrier to water loss in *Capsicum* fruit (Schönherr, 1976; Lownds *et al.*, 1993). An alternative approach to slicing is for fruit to be dipped in a drying oil that increases the water permeability of the cuticle while denying fungi access to the tissues. Application of a drying oil greatly accelerates the drying process of grapes, and results in a good quality product; it is therefore widely used in that industry (Fogerty and Burton, 1981). This drying oil is normally applied by spraying or dipping. It is made up of an oil (2% w/v) containing mostly ethyl esters of $C_{14}/C_{16}/C_{18}$ fatty acids as the active ingredient, which is emulsified in aqueous potassium carbonate (K_2CO_3, 2.5% v/v) (Fogerty and Burton, 1981). The change in drying rate is suggested to be due to the rearrangement of waxes on the grape surface resulting in an increase in their permeability to water (Grncarevic, 1963). However, there has been no report on the effects of drying oil on *Capsicum* fruit.

This study therefore *investigated the effects of various drying oil conditions in combination with different cutting methods on the drying rate and resultant colour quality and aroma on paprika spice powder.* This also included preliminary experiments *examining drier types and drying temperatures and their effects on drying rate and final spice colour.*

The main findings of this chapter are in press (Krajayklang *et al.*, 2001).

9.2 Specific materials and methods

9.2.1 Preliminary studies

Preliminary studies determined some of the drying parameters that were used in later studies.

General procedure:

Fruit were collected at a commercial mature stage (deep red ripe stage) from different pot-grown paprika plants during the growing season of 1996/97 at the Waite Campus, Adelaide University (details in Appendix I). Harvested fruit were randomly grouped into a sample of five to six fruit, weighed, chopped into 3 pieces and used for each drying treatment. The

drying process was ended when samples had reached constant weight. This was done by frequently measuring weight, and the final weight was recorded.

Initial dry matter for the following experiments was simply determined by cutting sample fresh fruit into 1 cm × 1 cm sections, and drying 10 g at 100°C in a laboratory hot air oven for 24 h. Differences between initial and final weight was expressed as moisture loss, and calculated as % of dry matter. Fruit generally had a 35-40% dry matter content. Samples were all ground after drying, and used for determining extractable red colour as previously described in section 3.6.3 (Chapter 3). No pungency measurements for paprika samples were carried out.

Treatments:

Experiment 1: Drying temperatures

Fruit samples were tested under different drying temperatures in a laboratory hot air oven, with two replications per treatment;
at 70°C,
at 60°C for 6 h followed by 40°C.

The drying process was ended when samples had reached constant weight.

Experiment 2: Types of drier

Fruit samples were tested with three types of driers (Figure 9.1), with four replications for each drier;

a Greenhalgh heat pump drier (MR-2 Model with a package unit, Dry Air Systems Pty. Ltd., Victoria) at 40°C (≤40% RH),

a laboratory hot air oven (Qualtex Solidstat, Watson Victor Ltd., Australia) at 60°C for 6 h, followed by 40°C to constant weight,

a Dynavac freeze drier (Model FD-5, Australia) at a temperature of -45°C and a vacuum of 5.3×10^{-3} kPa.

The drying process was ended when samples had reached constant weight.

Figure 9.1 Driers: (a) heat pump, (b) hot air oven and (c) freeze drier.

9.2.2 Dipping and cutting treatments

This experiment was aimed at investigating the effects of dipping oil and cutting on drying rate and colour quality, and to examine their effect on subsequent colour loss during storage.

Experiment 3: Combination of dips with cutting methods

Experimental design and treatments:

The experiments were conducted during late summer in 1999 at the Waite Campus, Adelaide University (details in Appendix I). Fruit of uniform size and free from any damage were hand harvested at a commercial mature stage (deep red ripe stage) and randomly divided into 10 groups of five fruits. The first group was used to determine dry weight as described above for each harvest, and the rest were randomly assigned to different treatments. Approximately 30-35% of dry matter was recorded.

The experiment was based on a randomised complete block with factorial design. Treatment factors consisted of three different dips (control, cold oil or hot oil) and three different cutting methods (uncut, cut into halves or cut into 2.5 cm × 2.5 cm sections). Dips were prepared from a solution of Voullaire's 'EE-muls-oyle' (10%v/v) from Victorian Chemical Pty Ltd. (Richmond, Victoria) and K_2CO_3 (0.2% w/v) in distilled water. The solution was adjusted to pH 11 with potassium hydroxide (KOH) and hydrochloric acid (HCl) as required, and emulsified in an ultrasonic bath for 20 min. This solution was modified after initial observations (data not shown) that showed that drying rates were increased by increasing oil concentration from 2%, as used for commercial Sultana drying, to 10%. Furthermore, an additive effect was also observed when the oil was heated to 65°C (data not shown). The dip treatments thereafter were based on a 10% oil concentration. The dips were heated in a water bath to 65°C as hot-oil dips or left at room temperature of 20-22°C as cold-oil dips. Distilled water at 22°C was used as a control dip.

Fruits were fully immersed in the dips for 3 min, and left to air dry at room temperature until fruit weights matched their pre-dipping weight (~30 min). Fruits were cut, using a scalpel, into longitudinal halves or 2.5 cm × 2.5 cm sections. Fruits were placed into drying trays in single layers and were placed in the middle shelf of an industrial hot air oven (Figure 9.2) at 45°C until constant weight was achieved. The weight of individual fruits was recorded every 6 h during drying. Fitted curves were calculated for the % moisture content (MC) over time; where: % MC = $(w_t - w_f)/w_t \times 100$, w_t = weight during drying, and w_f = final weight. The time taken to reach 10% MC was used for comparing the drying rate, as this MC results in the best quality (Wall and Bosland, 1993).

Figure 9.2 An industrial hot air oven.

Spice colour measurements:

Dried fruit were ground into powder using a Culatti electric mill. The powder samples were kept in plastic bags and stored in the dark at 37°C for eight weeks for accelerated testing of colour loss (Klieber and Bagnato, 1999). Surface colour (see section 3.6.2) and extractable red colour (see section 3.6.3) were measured after grinding and again after storage, as previously described in Chapter 3.

Aroma determination:

The aroma was initially examined according to a previous report by Luning *et al.* (1995), who described the aroma of dried paprika as having fresh 'hay-like' characteristic. Prior to storage, the aroma was scored by one researcher on a modified scale of 0 to 4 where 0 = no aroma, 1 = slight, 2 = medium, 3 = strong, 4 = extreme. This score was based on only one characteristic of hay-like aroma. However, at the end of six-weeks storage, a triangle test was performed to determine if there was a detectable difference in aroma (Larmond, 1977; Stone and Sidel, 1993) between paprika made from powders which were pre-dipped in water (A) as a control, or in hot oil (B). A set of 36 assessments was carried out, using 36 different panellists (one assessment per panellist). A set of samples was presented in a balanced design, using either two samples of A and one sample of B, or two samples of B and one of A. Each panellist was asked to sniff three coded samples from left to right and to select the different sample. The numbers of correct responses were added together to form the total number of correct responses. For this experiment the minimum number of correct responses to show a significant difference ($P<0.05$) between samples was 18 referring to the total of 36 assessments (Stone and Sidel, 1993).

Data analysis:

This experiment was replicated with three harvests within one month. Regression analysis was used to model changes in MC during drying (exponential curve). This was then used to determine the time of drying to a final MC of 10%.

Experiment 4: Action of dips on fruit surface waxes

To understand the structure of the fruit cuticle and possible effects of experimental dipping treatments upon this layer, various treatments were applied and the cuticle examined using electron microscopy.

Segments (5 mm × 5 mm) of fresh skin surface were removed from the treated paprika fruit (2-3 segments per treatment), using the red succulent stage for all treatments (except for the control treatment using both red succulent and red ripe stages). Treatments consisted of:
control (untreated, no dip),
hot water dip (65°C),
hot oil (10%) dip (65°C),
hot oil (10%) dip plus 1 h heated in hot air oven at 45°C.

Scanning electron microscopy:
The segments were then placed on aluminium stubs connected to a holder. Samples were then rapidly frozen in liquid nitrogen slush (-156°C) and cryotransferred under vacuum to a cold stage in the prechamber of the cryosystem. The frozen specimens were etched in the prechamber by raising the temperature of the stage to -96°C for 3 min. This process sublimed some of the surface ice. The specimens were then sputter-coated with a thin film of gold/palladium alloy (Au 80%, Pd 20%) in the prechamber and transferred to the cryostage in the Scanning Electron Microscopy (SEM) for observation. An Oxford CT 1500 HF Cryotransfer System (Oxford Instruments, Pennant Hills, NSW) interfaced to a Philips XL30 Field emission SEM (Philips Electron Optics B.V., Eindhoven, The Netherlands) was used. Observation was maintained for 5 min for each specimen under a 3-5 kV electron beam and 5.5-10.0 mm working distance. Transverse and surface images were taken.

9.2.3 Statistical analysis

Means separation was based on LSD at $P<0.05$ for all experiments using GENSTAT 5 (Genstat Software, Lawes Agricultural Trust, England), except for Experiment 4. Specific analyses were previously described for each relevant experiment.

9.3 Results

Experiment 1

Maximum moisture loss occurred during the first 6 h of drying for both treatments. Under a high temperature of 60-70°C, treated fruit required about 24 h to reach constant weight, while 48 h was required for a modified drying temperature regime of 60°C for 6 h followed by 40°C for 42 h. Although there was no significant effect of temperature regime on extractable red colour of the spice samples (Table 9.1), slight browning was observed for the 70°C dried samples.

Table 9.1 Drying temperatures affecting drying time and final colour.

Treatments	Drying time (h)[y]	Colour (ASTA)[z]
Hot air oven at 70°C	24 b[x]	401 a
Hot air oven at 60°C for 6 h then 40°C	48 a	394 a

[z] American Spice Trade Association, colour units.
[y] Drying time was recorded when samples reached constant weight.
[x] Means followed by the same letter within columns are not significantly different at $P< 0.05$ by LSD.

Experiment 2

Heat pump drying did not accelerate the drying process compared to the hot air oven, both requiring about 48 h to reach a stable weight, while 60 h of drying was required with the freeze drier (Table 9.2).

Table 9.2 Types of drier affecting drying time and final spice colour.

Treatments	Drying time (h)[y]	Colour (ASTA)[z]
Heat pump drier (45°C, RH ≤40%)	48 b[x]	467 a[x]
Hot air oven (60°C for 6 h then 40°C)	48 b	394 a
Freeze drier (-45°C, 5.3×10^{-3} kPa)	60 a	357 a

[z] American Spice Trade Association, colour units.
[y] Drying time was recorded when samples reached constant weight.
[x] Means followed by the same letter within columns are not significantly different at $P< 0.05$ by LSD.

Although extractable red colour was not significantly different among treatments (Table 9.2), treated fruit from the heat pump drier and hot air oven visually obtained a brown-red colour, while fruit developed a dull colour in the freeze drier (Figure 9.3).

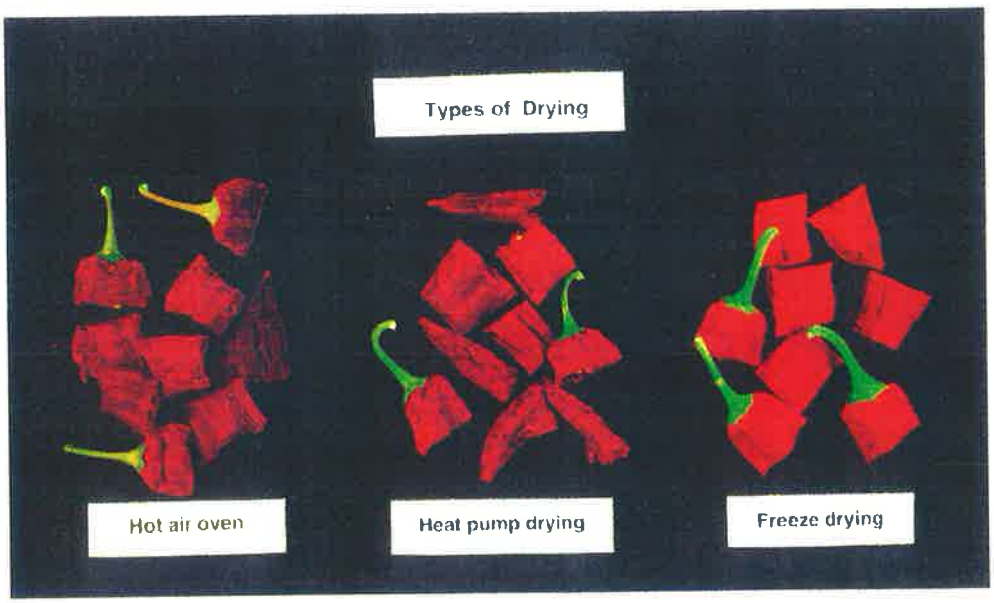


Figure 9.3 Final surface colour after drying in three different driers.

Experiment 3

Drying rate:

Application of cold oil (uncut sample) significantly reduced drying time to 41 h, while uncut samples that were dried without the application of oil (control) required at least 56 h to reach a commercial dryness of 10% MC (Table 9.3). An additive effect was also observed when oil was heated to 65°C. Fruit then took only 30 h to reach 10% MC without cutting (Table 9.3).

Table 9.3 Effect of dipping oil and cutting on drying time of paprika cv.'PS72285'.

Treatment		Drying time to 10% MC
Oil	Cutting method	(h)
No oil	Uncut	56 a[z]
	Cut into half	25 c
	Cut into 2.5 cm × 2.5cm sections	11 e
Cold oil	Uncut	41 b
	Cut into half	17 d
	Cut into 2.5 cm × 2.5 cm sections	10 e
Hot oil	Uncut	30 c
	Cut into half	15 de
	Cut into 2.5 cm × 2.5 cm sections	7 e

[z] Means followed by different letters are significantly different at $P< 0.05$.

Cutting fruit into halves or small sections without oil treatment also accelerated drying of non-dipped fruit to 25 and 11 h respectively (Table 9.3). Combining oil and cutting methods significantly reduced drying time by more than 50%. However, the drying rate of fruit that were cut into small sections following oil applications was not significantly different compared to no oil application (Table 9.3).

Spice Colour:

Surface colour recorded as L*, C* and h° values was found not to vary between oil treatments, and there was no interaction between oil and cutting methods. However, cutting method had an effect on surface colour (Table 9.4). When samples were cut, the colour was slightly lighter (higher L* value) and more intense in red colour component (higher a* value). However, there was no significant difference between the cutting methods for the Chroma (C*) or the hue angle (h°).

Table 9.4 Quality measurements of paprika cv.'PS72285' after drying and before storage.

Two way analysis		Colour characteristics[z]				
Treatment factor	Treatment	L*	a*	b*	C*	h°
Dipping oil	No oil	35 a[y]	41 a	44 a	60 a	47 a
	Cold oil	36 a	42 a	47 a	63 a	48 a
	Hot oil	35 a	42 a	46 a	62 a	48 a
Cutting	Uncut	33 b[y]	40 b	44 a	60 a	47 a
	Cut into half	36 a	42 a	46 a	62 a	47 a
	Cut into 2.5 cm × 2.5 cm sections	36 a	43 a	48 a	64 a	48 a
Oil*Cutting	Interaction	ns	ns	ns	ns	ns

[z]Colour values measured in CIELAB colour space. L* = lightness (black to white; 0 to 100), a* = bluish-green(-)/red-purple(+), b* = yellow(-)/blue(+), $C^* = \sqrt{a^2 + b^2}$ = chroma (dull to vivid; low to high value), h° = $\tan^{-1}$ (b/a) = hue angle.
[y] Means followed by different letters within a column and treatment factor are significantly different at $P < 0.05$, ns = non significant.

Neither cold nor hot oil dips altered colour quality of dried paprika fruit (Table 9.5). With cutting, the ASTA colour value significantly increased by 8% for the fruit cut into small pieces. Under accelerated shelf life testing conditions, colour loss was clearly found for all treatments after storage (Table 9.5). Oils had no effect on the level of colour loss indicated as % change of ASTA colour units/week, but colour loss was 5% less for cut samples (Table 9.5). No interaction between oil and cutting factors was found for the initial, the final or the % change in extractable colour.

Table 9.5 Colour measurements before and after storage in the dark at 37°C for 2 months.

Treatment		Extractable red colour[z]		
Oil	Cutting	Initial (ASTA)	Final (ASTA)	% Change (ASTA decrease/week)
Dipping oil	No oil	234.9 a[y]	127.6 a	0.9 a
	Cold oil	235.7 a	129.7 a	0.7 a
	Hot oil	244.7 a	137.7 a	0.8 a
Cutting	Uncut	229.5 b[y]	119.3 b	1.1 a
	Cut into half	238.2 ab	135.3 a	0.7 b
	Cut into 2.5 cm × 2.5 cm sections	247.6 a	140.3 a	0.6 b
Oil*Cutting	Interaction	ns	ns	ns

[z] Extractable red colour expressed as American Spice Trade Association (ASTA) extractable colour units.
[y] Means followed by different letters within a column and treatment factor are significantly different at $P < 0.05$, ns = non significant.

Aroma:

Prior to storage; treatments vs control samples

Dried paprika had a 'hay-like' or a fresh grassy aroma at the beginning of storage. Slight differences in this aroma ($P<0.05$) were found due to oil applications reducing the intensity of fresh hay-like aroma from a score of 2.0 to 1.5, while cutting had no effect (Table 9.6).

Table 9.6 The characteristic of 'hay-like' aroma prior to storage.

Two way analysis		Aroma
Treatment factor	Treatment	(0-4)[z]
Dipping oil	No oil	2.0 a[y]
	Cold oil	1.4 b
	Hot oil	1.5 b
Cutting	Uncut	1.7 a[y]
	Cut into half	1.6 a
	Cut into 2.5 cm × 2.5 cm sections	1.7 a
Oil*Cutting	Interaction	ns

[z] 'Hay like' aroma was scored by a scale from 0 to 4; 0 = no aroma, 1 = slight, 2 = medium, 3 = much, 4 = extreme.
[y] Means followed by different letters within a column and treatment factor are significantly different at $P<0.05$, ns = non significant.

After storage; heated oil vs control samples

After storage for eight weeks at 37°C, the aroma of hot oil-treated samples was significantly different to water-dipped samples (>18 correct responses out of 36, $P<0.05$), according to a triangle test (Table 9.7). No aroma testing of cut samples after storage was carried out.

Table 9.7 Sensory analysis for paprika powder after 2 month storage in the dark at 37°C. The triangle test was performed to determine if there was a detectable difference between paprika powders which were pre-dipped in water as a control and in oil, using thirty-six panellists.

Correct responses when hot oil was presented		Pooled result
Once	Twice	
11/18[y]	11/18	22/36[z]

[y] Entries are the ratios of correct to total decisions.
[z] Minimum numbers of correct judgements to establish a significant difference at probability level 0.05 for the Triangle test was 18 (Larmond, 1977).

While it was difficult to describe differences between samples, panellists noted that control samples had a 'fresher', 'deeper', 'stronger' or 'more defined' aroma than oil-treated samples. Control samples produced an 'earthy', 'hay-like', 'straw-like', 'grassy' or 'green vegetable-like' aroma, whereas oil-treated samples were described as having a 'nutty' or rancid odour. This aroma was also found in a few control samples after storage, but at a lower intensity than for oil-treated samples.

Experiment 4

Cuticle structure of untreated fruit:

The skin surface of segments from the untreated fruit (paprika cv. 'PS72285', *Capsicum annuum* L.) showed no stomata in their epidermis (Figure 9.4a, d). The outer surface of the fruit cuticle carried a continuous coverage with an amorphous wax layer. This consisted of dense clusters of small granular- and/or small rod-shaped platelets surrounded by zones of smooth wax coating on top of the surface (Figure 9.4b-f). This characteristic varied slightly with maturity due to differences in crystalline wax distribution. A more uniform wax layer and a finer granular structure blooming on top of the surface were observed from segments of red succulent fruit (Figure 9.4b, c). However, at a later stage clusters of granular-shaped waxes were absent, but the fruit surface was still covered by a continuous amorphous layer covered with some thin plates and/or rod-shaped waxes (Figure 9.4e, f).

Transverse sections from untreated red ripe fruit (partially dried) (Figure 9.5a, b) revealed a similar cuticle structure to the one of red succulent fruit (image not shown). The typical cuticle consisted of only one layer of reticulate cuticle as shown between the arrows in Figure 9.5b, and the epicuticular wax consisted of a smooth thin film covering the cuticle (Figure 9.5a). The thickness of the cuticle averaged 15.4 ± 0.5 μm including the wax layer.

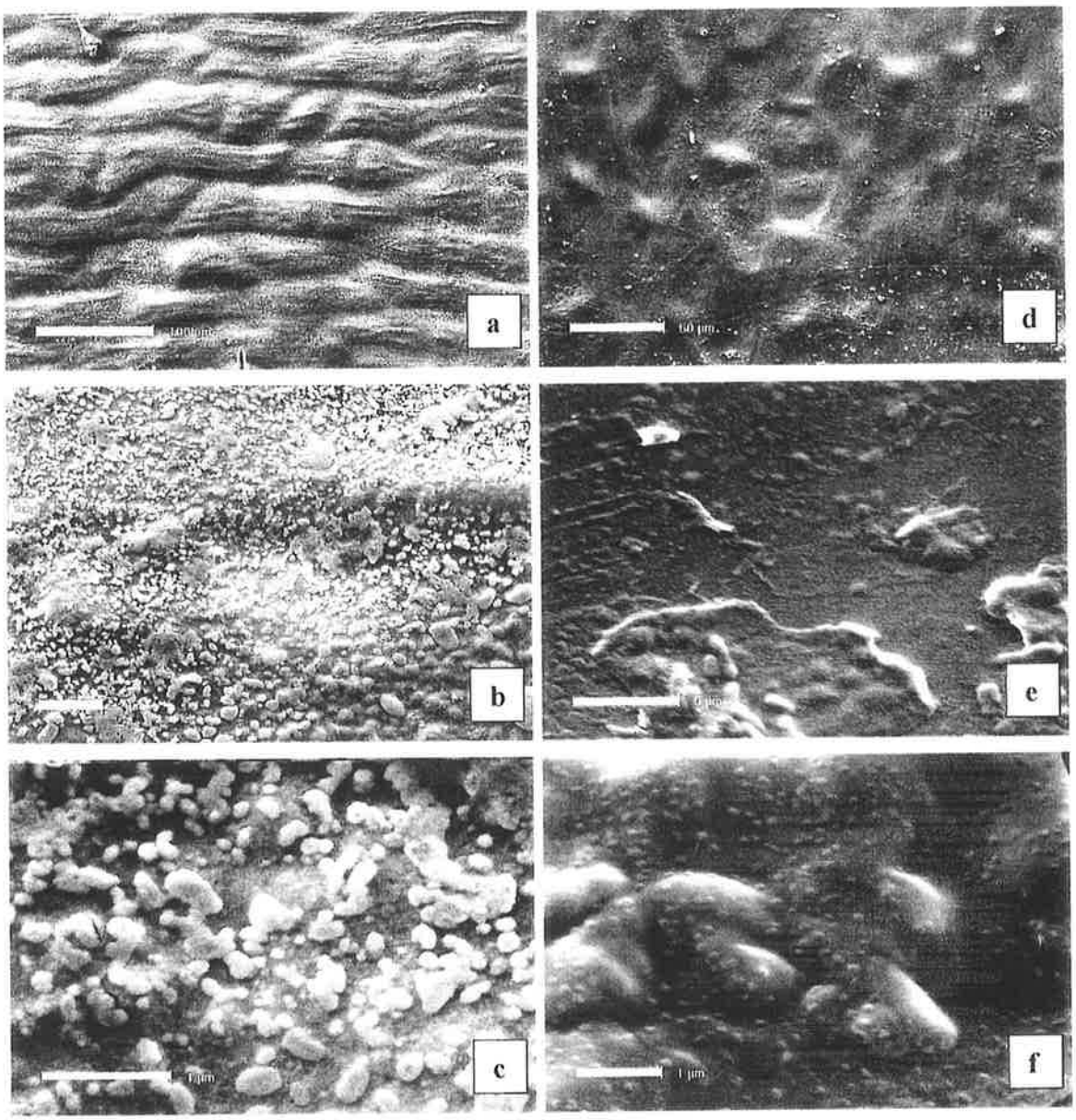


Figure 9.4 Scanning electron micrograph of cuticular wax sections from untreated red paprika fruit at two maturity stages.

Red succulent stage: (a) view of cuticle surface showing a uniform cell structure without stomata (× 546, bar = 100 μm), (b) granular layer of wax covering the entire surface (× 15,000, bar = 2 μm), and (c) close-up of wax crystals on the surface (× 60,000, bar = 1 μm).

Red ripe stage (partially dry): (d) view of cuticle surface without stomata (× 1,000, bar = 50 μm), (e) cuticle surface revealing a continuous structure with some wax clusters (× 10,000, bar = 5 μm), and (f) close-up of surface wax clusters consisting of small cylindrical rodlets (× 40,000, bar = 1 μm).

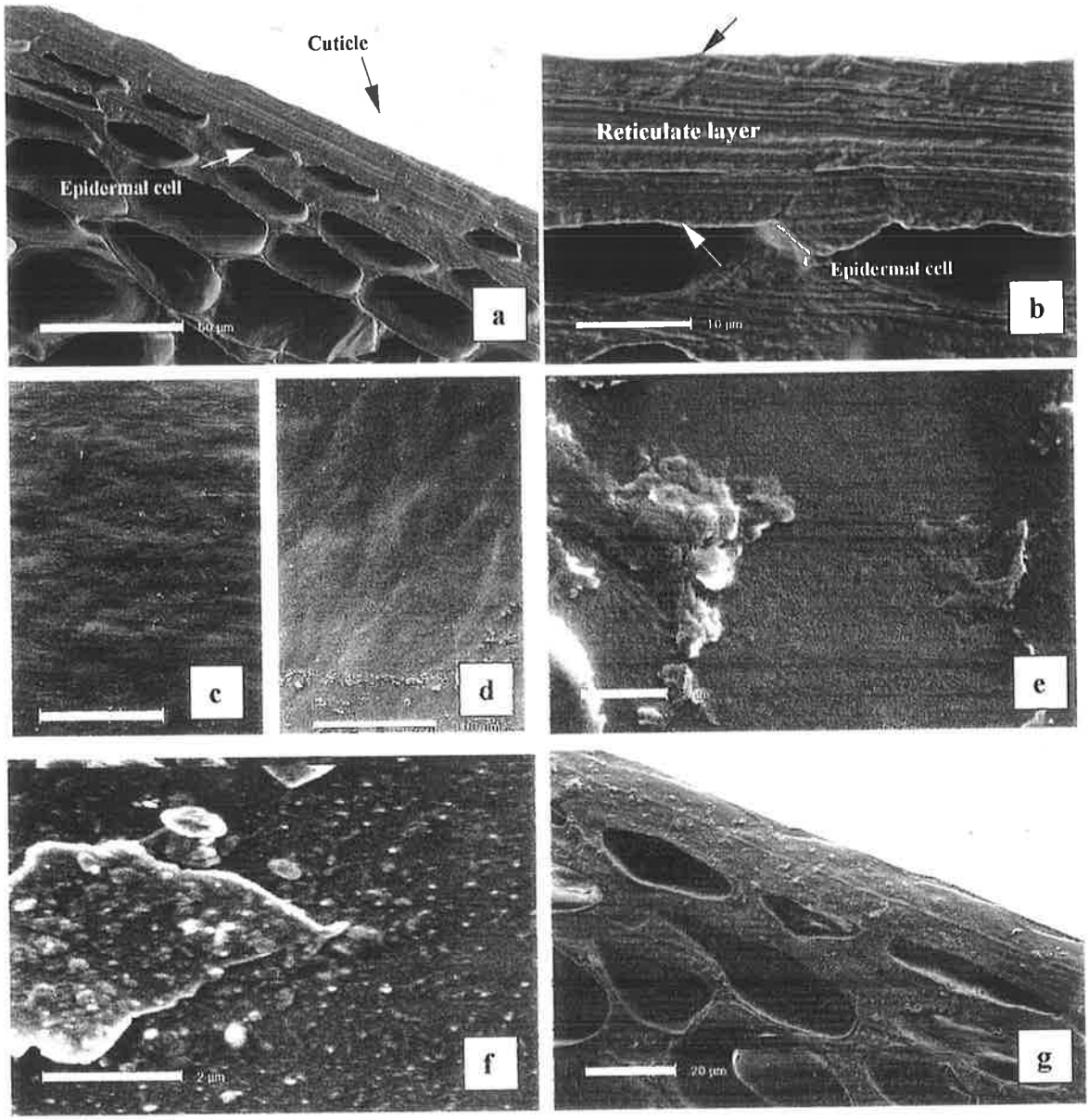


Figure 9.5 Scanning electron micrograph of sections from paprika fruit.

Untreated segments: transverse sections revealed entirely reticulate layer (a) at red succulent stage (× 1,334, bar = 50 μm), and (b) red succulent stage at higher magnification (× 5,335, bar = 10 μm).

Treated segments: cuticular wax treated with (c) double-distilled water (× 250, bar = 200 μm), (d) hot water at 65°C (× 510, bar = 100 μm), (e) double-distilled water (× 20,000, bar = 2 μm), (f) hot water at 65°C (× 30,000, bar = 2 μm), and transverse section treated with (g) hot water at 65°C (× 1,600, bar = 20 μm).

Cuticle structure of treated fruit:

Water vs control (untreated)

With lower magnification, segments treated with double-distilled water and hot water revealed a similar surface structure (Figure 9.5c, d) relative to the control. Although less fine granular waxes were observed under higher magnification from sections treated with double-distilled water (Figure 9.5e), smooth layer of wax covering the entire surface was still observed from both sections treated with double-distilled water (Figure 9.5e) and hot water (Figure 9.5f). Furthermore, there was no effect of hot water on the fruit cuticle as shown in the transverse section (Figure 9.5g) compared to that of the control.

Hot oil vs control

Transverse sections from hot oil treated fruit (Figure 9.6b, d) revealed the same structure as for untreated ones (Figure 9.5a), even when followed by hot air drying. The cuticle thickness was approximately 15.3 ± 0.8 μm for hot oil-treated segments including surface waxes, although this waxy layer was hard to distinguish in the transverse sections. However, slight changes in surface waxes were observed due to hot oil compared to the control surface (Figure 9.6a, c). Small flakes of wax platelets were observed vertically to the surface (Figure 9.6a) that could not be seen in control sections. The oven drying following hot oil dipping caused the surface of the fruit to appear more amorphous (Figure 9.6c).

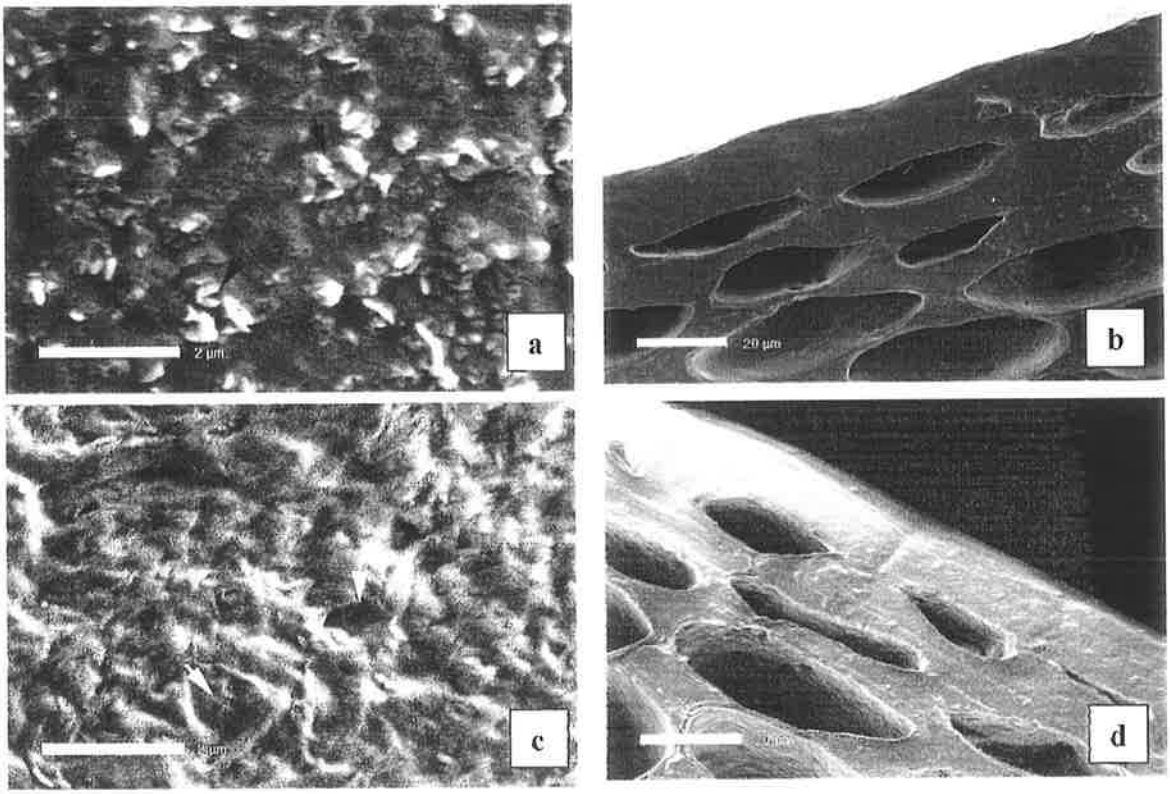


Figure 9.6 Scanning electron micrograph of segments from red succulent mature stage of paprika fruit.

Surface sections: (a) treated with hot oil at 65°C (× 30,000, bar = 2 µm), (c) treated with hot oil at 65°C and heated in oven at 45°C for 1 h (× 30,000, bar = 2 µm).

Transverse sections: (b) treated with hot oil at 65°C (× 2,000, bar = 20 µm), (d) treated with hot oil at 65°C and heated in oven at 45°C for 1 h (× 1,000, bar = 20 µm).

9.4 Discussion

9.4.1 Drier type and drying conditions

A high drying temperature of 70°C reduced the drying time of dehydrated *Capsicums* by half (from 48 to 24 h relative to 60°C) with the initial extractable colour being unaffected. A maximum drying temperature of about 77°C was also used in the study of Lease and Lease (1962), however these elevated temperatures may lead to a lower initial colour and lower colour stability. In addition, browning was observed in this study and that reduced the visual quality of spice powder dried at 70°C. Therefore, it is safer to reduce the maximum drying temperature to about 60 to 65°C in order to preserve colour quality (Lease and Lease, 1962).

Alternative approaches of the two stage drying at 60°C for a few hours followed by 40°C in hot air drier was also useful for dehydrating *Capsicums*, for example during peak season. Drying at a lower temperature of 45°C at low RH in a heat pump drier also preserved *Capsicum* spice colour, and the drying time was the same as for hot air drying. Both of these driers were superior to the freeze drier, which required a longer drying period, and had a higher cost of energy consumption and of the drier itself. Heat pump driers have previously shown potential for preserving colour quality (Chou *et al.*, 1994; Dahlenburg and Tugwell, 1995), as also shown in this study. Also, energy costs are reduced compared to hot air driers (Chou *et al.*, 1994). Therefore, it may be more economical for commercial production to use a heat pump drier, with the added benefit of a lower drying temperature compared to a hot air drier.

9.4.2 Mechanisms of water loss due to different treatments

Plant cuticles are known to be efficient barriers to the movement of water between fruit tissue and the atmosphere. They are heterogenous structures consisting of a polymer matrix of cutin acids and non-cutin constituents, mainly cellulose, and methanol- and chloroform-soluble cuticular lipids that are embedded within the cutin and also cover the outer surface of the plant cuticle (Holloway, 1982).

A typical plant cuticle is shown in Figure 9.7 as determined by electron microscopy (Holloway, 1982). While cuticle structures vary between plants, two main ultrastructure types are distinguished (Gouret *et al.*, 1993), cuticles characterised by an almost entirely reticulate structure, and cuticles characterised by an outer lamellate region with an inner reticulate region (Figure 9.7).

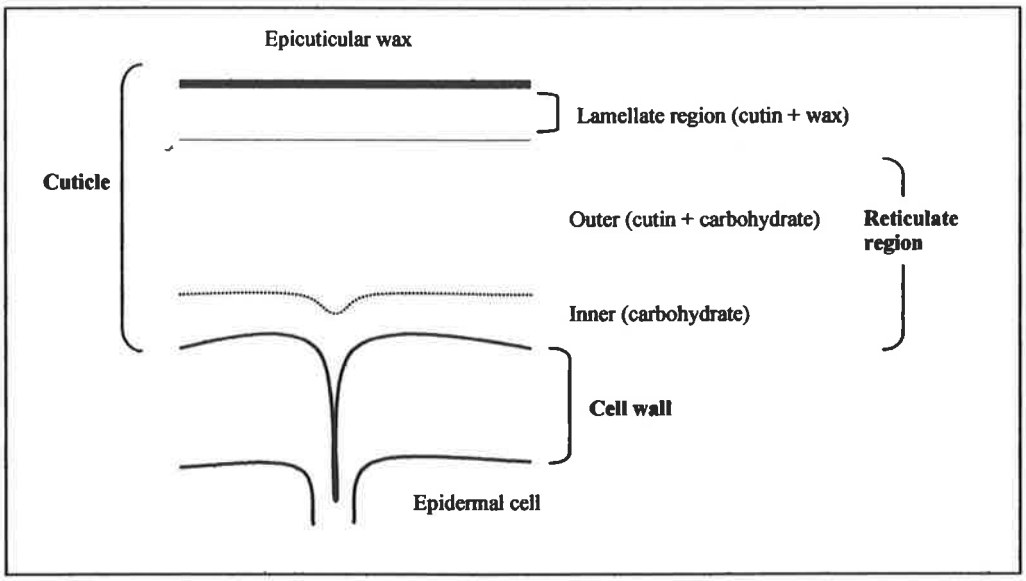


Figure 9.7 Diagrammatic representation of a transverse section of a typical plant cuticle.
Source: Jeffree *et al.* (1976, p. 124).

Control fruit:

The rate of drying is basically limited by the rate of water loss which was entirely through the cuticle, since fruit in the genus *Capsicum annuum* L. do not have stomata on their skin surface. The diffusion of water through the waxy cuticle is inversely proportional to the amount of cuticle (Martin and Stott, 1957). The cuticle layer of *Capsicum* fruit in this study had only one thick layer which was previously described as a wholly reticulate layer (Gouret *et al.*, 1993). This structure was similar to other plant cuticles in the species of *Lycopersicon* (fruit), *Galium* (leaf) and *Prunus* (leaf) (Gouret *et al.*, 1993). Therefore, the thickness of this cuticle is an important determinant of water movement out of *Capsicum* fruit.

Cold oil:

The drying oil significantly enhanced the rate of paprika drying. Successful application of drying oil has been previously noted to reduce the drying time of grapes for raisin production (Grncarevic, 1963; Fogerty and Burton, 1981; Uhlig and Walker, 1996; Uhlig *et al.*, 1996). However, the concentration of the oil of 2% for commercial raisin drying and dip temperatures were different from this study, as grapes have a less resilient cuticle than paprika fruit. While the drying oil accelerated paprika drying, much higher levels of 10% of the drying oil were needed for *Capsicum* fruit, possibly due to the more substantial cuticle and epicuticular waxes of the paprika fruit.

Overall, the outer surface of the paprika fruit cuticle was covered with dense clusters of granular- and /or rod-shaped platelets present both upon (epicuticular waxes) and underlying (embedded waxes) the surface. This waxy surface was hydrophobic, since treatment with double-distilled water did not remove the waxy coating off the fruit surface. As evident from microscopy, the action of the drying oil was not through the removal of the wax platelets, since the waxy layer still remained on the oil-treated surface. However, its action to increase the drying rate may be through a physical rearrangement of the wax structure, so that its permeability to water is increased. This action of drying oil has been previously described for dried grape berries (Chambers and Possingham, 1963; Possingham, 1972; Grncarevic and Lewis, 1976). While the actual process is unknown, the action of each component in the drying solution (ethyl esters and K_2CO_3) has been reported recently (Uhlig *et al.*, 1996). The alkali cations cause a swelling of the cuticle (Schreiber and Schönherr, 1990) which was suggested to have an important role in the regulation of the water permeability of the grape berry skin (Uhlig and Walker, 1996; Uhlig *et al.*, 1996). The ethyl esters, which are unsaturated, are suggested to change the arrangement of the wax components, and this may influence membrane permeability (Uhlig *et al.*, 1996).

Hot oil:

Increasing the temperature of the drying oil to 65°C increased the drying rate compared to the cold oil treatment. This suggests that the higher temperature increased cuticle permeability to water due to recrystallising or melting of the wax platelets. Roy *et al.* (1994) found that epicuticular wax of 'Golden Delicious' apple fruits was restructured after heat treatment at 38°C for 4 days. Eckl and Gruler (1980) found that elevated temperature increased water permeability by reorientating soluble cuticular lipids to open hydrophilic

holes in the cuticular barrier. Schreiber and Schönherr (1990) also found structural changes on the cuticle leading to an increase in the volume of the fruit cuticle (*Lycopersicon*, *Cucumis*, *Capsicum*, *Solanum* and *Malus* species) at temperatures ranging from 40 to 50°C, and this led to an increase in water permeability. When the temperature was higher than 45°C this change was irreversible. Therefore, hot drying oil increased water-loss through altering the superficial wax structure as well as permanently changing the physical state of the fruit cuticle.

Cutting:
Cutting increased the rate of drying by providing an alternative pathway for water movement, and principally increased the surface area for water movement out of the fruit. Therefore the effects of cutting and drying oil were different.

9.4.3 Spice quality

Reflected colour

Poor colour quality is commonly observed under high drying temperatures: this results in a darkening which is thought to be due to the action of the enzyme polyphenol oxidase that is present in the fruit skin (Barnett, 1980). While in this study low drying temperatures were used, prolonged drying of uncut fruit may result in a similar outcome with a darker colour, indicated by a lower L* value, relatively to the control. Ramakrishnan and Francis (1973) have suggested that a decreased L* value is a function of the logarithm of the increase in polyphenols. The darkening appeared to be positively correlated with drying times, since it was previously noted that polyphenol oxidase activity was reduced through accelerated drying after drying oil application (Radler, 1964; Barnett, 1980). No effect of drying oils on reflected colour was found in this study, in contrast to cutting, that reduced darkening. Also the average drying times of oil treated fruit or cut fruit were reduced, therefore darkening due to polyphenol oxidase was potentially reduced.

Extractable red colour

Fruit colour pigments may be biosynthesised as well as degraded during drying (Ibrahim *et al.*, 1997). For example, in paprika fruit, capsolutein increased by 19% during drying (Mínguez-Mosquera and Hornero-Méndez, 1994). While an increase in colour pigments

was not observed in this study, it seems likely that colour degradation occurred. A dull colour appearance was observed in response to freeze drying, although extractable red colour was not significantly different among types of driers. This could be due to the colour loss occurring with prolonged drying in the freeze drier. However, the difference was not significant possibly due to sample variation. Overall, colour degradation appeared to be less in relation to the decrease in their drying time due to cutting and oil treatments.

Colour stability was also improved with faster drying due to cutting treatment, possibly as a result of a less destruction of antioxidants such as ascorbic acid (vitamin C) and tocopherol (vitamin E) under accelerated drying. These antioxidants may protect against colour loss in cut fruit, although the actual levels of antioxidants were not recorded in this study. Initial higher levels of antioxidants in ground paprika have been reported to reduce the rate of colour loss during storage (Carvajal *et al.*, 1998; Klieber and Bagnato, 1999).

Aroma

Drying of fresh capsicums greatly changes their volatile composition; many volatiles vaporise and new volatile compounds are formed by chemical reactions (Luning *et al.*, 1994). This affects the aroma of *Capsicum* fruits after drying, resulting in characteristic hay-like aroma that is not found in fresh samples (Luning *et al.*, 1995). Wilkins (1994) reported similar odour attributes of rubber, tomato, and hay in dried Hungarian and Spanish *Capsicum* cultivars. As also shown in this study, all dried paprika samples produced fresh grassy or hay-like characteristics, while a nutty off-odour appeared to develop during storage. This characteristic became distinct after eight weeks of storage, especially with hot oil-treated samples. This odour is commonly referred to as 'rancidity', and is caused by lipid oxidation (Maté *et al.*, 1996). The untreated samples may develop rancidity itself during storage. While this was not detectable for untreated samples, the use of fatty acid esters at high concentrations as an active ingredient in drying oils, potentially introduced rancidity for dipped samples. The residue of commercial dip is not normally a problem for raisins, as consumers cannot detect the very low level on the fruit skin (Barnett, 1980). While higher levels need to be used for *Capsicum* drying, the determination of the detection threshold of the ester residue following the application of drying oil is essential.

According to factors affecting lipid oxidation, oxygen concentration is one of the most important environmental factors affecting this process (Vercelloti *et al.*, 1992). Under the storage conditions in this study, sufficient oxygen was present in sealed plastic bags to allow oxidation processes to proceed at the high temperature of 37°C. However, a rancid odour also developed in paprika powders kept in normal air at room temperature (~22°C) (Bagnato, 1998). Isidoro *et al.* (1990) previously showed that reducing oxygen content during storage reduced colour loss of ground chilli, and this extended the shelf life of paprika powder by four to five times (Klieber and Bagnato, 1999). Therefore, modified atmosphere packaging of *Capsicum* powders should be further investigated to extend the shelf life and to reduce rancidity problems.

9.5 Conclusion

a) The maximum drying temperature for dehydrated *Capsicums* was confirmed not to be above 70°C to ensure a good colour quality of final products.

b) Drying at 45°C and at low relative humidity in a heat pump drier was suitable for preserving colour quality of dehydrated *Capsicum*. Although the drying time was not significantly different from the hot air oven, the energy cost could be reduced with heat pump drying.

c) Application of a drying oil significantly reduced drying time, by 26% for cold oil and up to 50% for hot oil compared to the control, without any detrimental effect on final spice colour. However, an off odour or rancidity was produced due to the high concentration of this drying oil, and this was detectable after prolonged storage. From these results and considering the additional cost of oil application, drying oil is not recommended for *Capsicum*.

d) The best method to increase drying rate was cutting fruit into small pieces, since cutting alone accelerated drying more than did the drying oil, and combining both did not further accelerate drying for finely cut fruit. Cutting fruit either into halves or into small sections resulted in significantly reduced drying times (more than 55% or 80%, respectively). This method is easy to apply, incurs only an initial capital cost and does preserve the final colour and aroma of spice products. Therefore, cutting fruit into small, regular pieces before drying is recommended for commercial spice production.

e) In entire fruit the drying rate is controlled by the diffusion of water through the *Capsicum* fruit cuticle. The increase in drying rate due to cold oil application was due to structural changes of the fruit surface waxes, increasing water permeability of the cuticle, while cutting reduced the proportional surface area of the cuticle and provided a free pathway for water movement. Recrystallization or melting of the wax due to hot oil application further increased cuticle permeability to water compared to cold oil, therefore further increasing the drying rate.

Chapter Ten

General discussion

10.1 The background to this study

A lack of consistent supply of *Capsicum* spice in regard to quantity and quality provides an opportunity to develop a new spice industry in Australia. This product is defined as the dried red fruit of any species of *Capsicum* that is processed into either crushed fruit or powdered. Its quality is valued commercially based on colour and/or hotness, depending on fruit type. The Australian climate and cultural practices are in general suitable for *Capsicum* production. The project, therefore, was aimed at improving the production system for *Capsicum* spices for suitable cultivars that are available in Australia. This involves reducing overall production costs by maximising yield and quality throughout the production system, with five main aspects under consideration: cultivar selection, growing conditions, harvesting, post-harvest handling, and processing.

10.2 Desirable plant characteristics for spice production

Within the *Capsicum* genus, there is a large variability in plant characteristics such as stems, branches, flowers, fruit colour, fruit size, fruit shape and fruit pungency. It is essential to select suitable cultivars that provide superior characteristics for dehydrated capsicums.

Cultivars that have a high yield as well as high fruit colour content and/or pungency are the first priority for economical spice production. *Capsicum* cultivars with multiple stems showing an upright growth habit and producing single large fruit borne on each branching node are the second criteria determining suitability for machine harvesting (Somos, 1984), which reduces the labour cost for spice production considerably (Miles, 1994).

Two types of capsicums belonging to *Capsicum annuum* L. were selected for this study. The first type was the semi-pungent paprika cv. 'SPS72285' which produces deep red carrot-shaped fruit, and secondly the cayenne chilli cv. 'Caysan PS705' which produces pungent, smooth and long red fruit. Both cultivars demonstrated the characteristics required for dehydrated capsicum production, with high yield characteristics as well as

high colour content and/or pungency. However, environmental and processing factors influence yield and/or spice quality, and these were therefore investigated further.

10.3 Responses to environmental growing conditions

Apart from general cultural practices, such as the careful management of nutrition, pests and diseases, the yield and final quality of dehydrated capsicum responds to specific growing conditions; in particular temperature and water stress were investigated.

10.3.1 Response to growth temperature

Air temperature regimes had major effects on internode elongation, shoot dry weight and fruit pungency, while neither final crop yield nor fruit colour content was affected. The response to temperature varied with cultivar. Also, the stage of plant development appeared to be critical for temperature effects on fruit pungency. Although this was not investigated in this study, it has been reported that fruit pungency is sensitive to temperature effects at an early stage of fruit development in the study of Levy *et al.* (1989) and Somos (1984). This may be possibly related to the fact that capsaicinoids start to accumulate approximately 20 days post anthesis, continuing through fruit development (Somos, 1984). Recently, the expression of some enzymes in the placenta tissue of *Capsicum* fruit (as the site of synthesis and accumulation of capsaicinoids (Iwai *et al.*, 1979; Suzuki *et al.*, 1980) has been found to be possibly involved in the capsaicinoid biosynthesis pathway (Curry *et al.*, 1999). Since temperature is known to have a great effect on enzyme activity, it was also possible that temperature in this study could influence enzyme activity and/or its expression at some levels, and changes could occur through the biosynthesis of pungent compounds during *Capsicum* fruit development. The effect of temperature on the relationship between the evolution of pungent compounds and expression of possible enzymes involved in capsaicinoid biosynthesis needs more investigation.

10.3.2 Response to water stress

The substantial reduction in yield in response to water stress was directly associated with other symptoms of *Capsicum* water stress, as described by Somos (1984) and Aloni *et al.* (1991). For example, fruit number, shoot elongation, Ψ_L or g_S, were greatly affected, and these are known to be sensitive to water deficit. Some visible wilting of leaves, abscission

of leaves and fruits, some necrosis and change in leaf colour were also found. On the other hand, quality factors of spice powder such as fruit colour content and pungency were not affected. However, fruit colour pigment (Trudel and Ozbun, 1970; Príbela *et al.*, 1992) and pungency (Levy *et al.*, 1989; Estrada *et al.*, 1999) were increased in some cultivars of *Capsicum* when plants were subjected to water stress at or before anthesis. Early water stress causes significant yield decline, therefore post-anthesis water stress was examined in this study. Some crops benefit from a short water deficit approximately a few weeks prior to harvest (Simpson, 1981), but *Capsicum* did not in this case.

Therefore, water stress appears to have a negative effect on final yield at any stage of *Capsicum* plant development. The positive effects of water stress on the quality of spice powder relating to fruit colour content and fruit pungency, however, may be greatest at an early stage of fruit development. The degree of response to water stress depends on cultivar and species (Quagliotti, 1971; Levy *et al.*, 1989). Other cultivars therefore may need investigation of water deficit stress, especially if there is a desire to improve spice quality with less irrigation.

10.3.3 Response to partial rootzone drying

The response of yield and fruit quality of *Capsicum* plants to a new irrigation strategy was investigated. This was based on a modified irrigation technique known as 'partial rootzone drying' (PRD), previously studied in grapevine by Dry (1997). He found that alternation of irrigation from one half of the root system to the other of split root plants successfully maintained water needs of the whole plants. This significantly increased water use efficiency because yield was not changed in response to a large decrease in irrigation amount. The main reason why PRD was used in my study is because PRD potentially induces some of the 'positive' effects of water stress (e.g. increased berry fruit flavour and colour in grapevines) (Dry, 1997) without the 'negative' effects (e.g. decreased yield).

The same strategy was investigated for potted chilli plants that had their roots equally divided in two pots. Alternating irrigation from one half of the root system to the other every 7 days could maintain yield for dehydrated chilli production. However, fruit colour and pungency were not improved by PRD in this study, unlike PRD-treated tomato (M. Bacon, pers. comm., 2000) and PRD-treated grapevine (Dry, 1997), where fruit colour

pigment content increased due to an increase in the amount of anthocyanins per fruit (Dry, 1997; Dry *et al.*, 1999). The possible mechanism of PRD has previously been suggested to be through physiological responses (Dry, 1997). For example, a reduction in vegetative growth caused by PRD increased bunch exposure of grapevine. Secondly, due to the reduction in vegetative growth, competition for assimilates would be less, resulting in a greater supply of assimilates to fruit. Thirdly, an increase in ABA induced by PRD may have some direct effects on fruit quality (Dry, 1997). While fruit quality in this study was not improved by PRD, the response of plants to PRD may vary with species, and may also vary according to particular phases of plant growth. Since *Capsicum* plants continue to flower throughout their growing season, it would be difficult to separate the effect of PRD on the vegetative growth phase from that on the reproductive phase. Therefore, the flowering period should be limited by removal of later flowers in order to determine exclusively effects on fruit.

PRD in this study induced some of the symptoms of water stress as shown by a small reduction in g_S, but without any concomitant change in Ψ_L. The use of Ψ_L for determining the degree of water stress, therefore, should be reconsidered. The response of plant growth in response to soil drying was not found to be associated with any significant changes in shoot water status as indicated by Ψ_L (Dry *et al.*, 1996; Dry and Loveys, 1997). On the other hand, the reduction in g_S in response to soil drying may be associated with changes in some chemical signals such as abscisic acid (ABA) (Leskovar and Cantliffe, 1992). It has been suggested that ABA induced by partial drying of the root system serves as a signal controlling stomatal closure, and therefore may also affect plant growth and development (Dry *et al.*, 1996; Dry and Loveys, 1997). The use of foliar application of ABA as an antitranspirant for transplanting seedlings into the field may be useful (Leskovar and Cantliffe, 1992). While, this was not the main objective of this study, further determination of the nature of the signals produced in response to PRD and the mechanism of any chemical signal controlling vegetative growth and/or stomatal behaviour should be considered.

The small reduction in g_S in response to PRD of the chilli plants may be beneficial for water use efficiency for spice production, although the actual water use was not recorded in this study. Water-use efficiency of PRD-treated grapevines was increased as a result of partial closure of stomata induced by PRD (Dry *et al.*, 1996). More detailed analysis of soil

moisture associated with change in g_S will provide information on the differential water use of roots of both sides.

10.4 Harvesting aids and post-harvest handling

From this study, the use of ethephon was not found to be economically feasible for spice production. Although it synchronised fruit maturity to some degree by inducing green mature fruit to complete their red colour development faster after application, it caused large numbers of fruit to drop, reducing total fruit yield by up to 70%. Also, no consistent changes of fruit colour and pungency of the spice powder were observed. Therefore, the time when most fruit had turned red on the bush was the optimum period for once-over harvesting at this stage.

Environmental conditions such as air temperature have shown to have an influence on the effectiveness of this chemical (de Wilde, 1971; Knavel and Kemp, 1973). Higher temperatures after application, as under our growing conditions, not only accelerated fruit colour development, but also increased the potential of senescence such as defoliation and fruit abscission (de Wilde, 1971). Ethephon application may be useful in some cooler areas at the same concentrations used in this study or, as previously noted, by using multiple applications with lower concentrations of ethephon (Sims *et al.*, 1970). Foliar application of ethephon has been commercially used in USA for chilli production at a rate of 200-380 $mL.L^{-1}$ (Wall, 1994). However, the response to this chemical still varies with cultivar (Cantliffe and Goodwin, 1975). Further investigation for other locations and cultivars would be useful to clarify its effectiveness under those specific conditions.

After once-over harvesting, approximately 20-30% of the total fruit number is discarded due to a mixture of unripe fruit. An alternative method to increase red fruit yield after harvesting may be the use of ethylene. However, ethylene at 100 $\mu L.L^{-1}$ for 48 hours did not induce red colour development of unripe fruit stored for a maximum of 10 days at 20-22°C. Also, fruit colour content and pungency of spice powder were not affected by ethylene.

It is not known why fruit ripening progressed differently on and off the bush, ripening fully on the bush and showing accelerated ripening in response to ethylene, while this did not

happen off the bush. Investigating the plant physiology during fruit ripening may be useful for a better understanding of *Capsicum* fruit behaviour. The harvested fruit in this study behaved in a non-climacteric fashion because no respiratory climacteric rise was observed. This may be one possibility why the ethylene had no effect on colour development of harvested fruit. The ethylene gas normally serves as a chemical ripening agent in climacteric horticultural crops both before or after harvest (Biale, 1964), but in non-climacteric fruit it shows less effect (McGlasson, 1978), and ripening appears to involve other factors (e.g. ABA) for ripening in non-climacteric fruit (Coombe and Hale, 1973). Differences in respiratory pattern of *Capsicum* fruit such as paprika during their ripening on and off the plant was observed. When comparing the respiration and ethylene production rates of just-harvested paprika fruit from green to red-ripe stages, a climacteric rise was shown when fruit changed colour, but this was not observed during storage. This has also been previously recorded in other fruits (Rhodes, 1970). For example, some climacteric fruits such as avocado, only showed a respiration climacteric rise after detachment from the tree (Rhodes, 1970). Pineapple, as an example of a non-climacteric fruit, shows this rise only before harvest which could be interpreted as a respiration climacteric on the plant, but in no case was a peak reached after detachment from the plant (Dull *et al.*, 1967) (cited in Rhodes, 1970). It is, therefore, difficult to decide whether there is a distinct difference in the mechanism of fruit ripening between those types of fruit on and off the plant. More information is required for understanding the mechanism of fruit ripening, especially for non-climacteric behaviour. This will be useful knowledge for improving post-harvest handling of harvested *Capsicum* fruit in terms of providing a colour index for optimum harvest timing, developing a new technique to induce colour of unripe fruit, or extending storage life.

10.5 Drying process

While the use of ethephon or ethylene is aimed at producing fruit with the maximum colour content, the drying process often degrades red colour pigments of the final spice products. Optimum drying conditions safeguard final colour quality of the dehydrated spice. Three driers were evaluated to optimise drying, as well as drying temperatures and techniques to increase the drying rate. The response of colour content, rather than pungency was tested in this study, since pungency of the chilli fruit is considered stable during drying process. The maximum drying temperature for dehydrated capsicums was at

or below 70°C, otherwise spice colour became brown (darkening reaction) due to the action of the enzyme polyphenol oxidase (Barnett, 1980). While a slow drying process of 45°C in a heat pump drier was suitable for drying, two stage drying with an initial temperature of 60°C for 6 hours followed by 40-45°C until reaching the final drying stage, was equally effective. However, drying was still relatively slow (taking about 36 h to 48 h in commercial and laboratory heat pump driers), and this may result in colour degradation during the prolonged drying period (Zapata *et al.*, 1992) (cited in Ibrahim *et al.*, 1997).

In order to induce faster drying under low drying temperatures, a new technique of dipping fruit in a drying oil prior to dehydration, as for Sultana grapes, was investigated. The action of the drying oil on drying rate was compared to different cutting methods. A concentration of 10% oil plus 2.5% of K_2CO_3 emulsified in water reduce the drying time somewhat. Also, the drying rate was further increased by raising the dip temperature to 65°C. However, cutting fruit into sections was the most effective method to increase drying rate. Combining the drying oil with cutting of fruit into small sections did not provide any further advantage over cutting alone. The cost of the dip, especially given the high concentration needed, is therefore not warranted, since spice colour was better after cutting. However, hygienic operation should be considered for commercial production in order to minimise microbial problems at this stage, as the cut fruit provide rapid access to fungal spores. Cutting also requires a higher initial capital outlay.

The mode of action of the drying oil and cutting were different. The drying oil acted by changing cuticular wax structure and resulting in increased water permeability through the cuticle, as previously noted for grape berries (Chambers and Possingham, 1963; Possingham, 1972; Grncarevic and Lewis, 1976). Cutting did not actually have any effect on the fruit cuticle, but increased free channels for water to escape from the fruit parenchyma, thereby increasing drying rate.

10.6 Practical application of this research

The outcome of this study is a set of recommendations that can help to improve *Capsicum* spice production (Figure 10.1). For immediate use, paprika cv. 'PS72285' and cayenne cv. 'Caysan SPS705' chilli are suitable for dehydrated spice production in conjunction with the appropriate cultural practices and machine harvesting.

Capsicum can be directly seeded in the field, but transplanting of pre-planted seedlings is the most common and convenient method for the growers. This provides an opportunity to discard unhealthy seedlings. *Capsicum* seedlings are now available from local nurseries, and are normally sold in multi-celled trays unless growers produce their own seedlings. Time of transplanting, therefore, is important to ensure that fruit develop under optimal environmental conditions. Avoiding drought stress throughout the growing season which can be observed through plant responses and soil moisture measurement (i.e. maintain above 15% SWC or 20 kPa soil water potential for sandy soils), will ensure that yields are maintained. In conjunction, optimal fertilisation and careful control of pests and diseases are essential practices to maximise final crop yield.

Production of consistently pungent spices requires sufficiently warm regions, with most regions of Australia being suitable, mainly during warm parts of the year, approximately form September to January, depending on climate zone (Table 10.1). Application of pre- and post-harvest treatments of ethylene, by using either ethephon or ethylene gas to induce fruit colour development was not useful under the conditions in this study. Once-over harvesting either by hand or machine can be effectively performed when a maximum number of red fruit are present on the plants. This will avoid the additional cost of repeat harvesting for annual spice production.

Once fruit have been collected, fruit should be graded into two or three (optionally) categories based on their colour stages. Fruit that are damaged or are green should be culled. Slightly red or breaker fruit can be stored for further colour development prior to processing. However, the storage conditions must be considered: that is, temperature, air circulation and humidity of the storage room. For example, storage conditions of 20 to 30°C in an open area (in shade) with good air circulation and a relative humidity of approximately 50% RH are suitable for 20-30 days (Gooindarajan, 1985b).

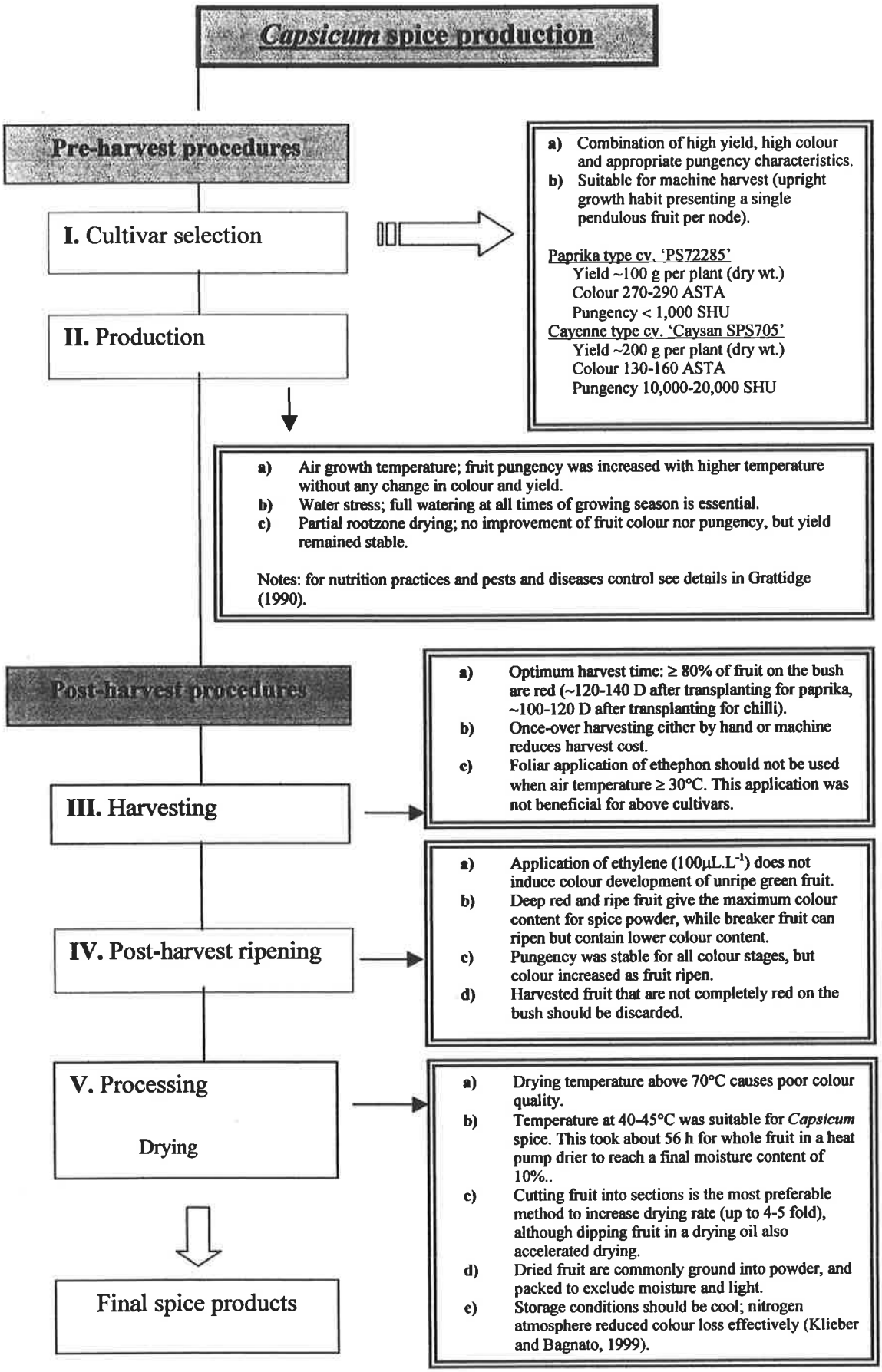


Figure 10.1 Diagram shows summarised practical applications of this research.

Table 10.1 Summarised suitable season for *Capsicum* spice production based on climate zones as shown in Figure 10.2.

Climate zone	Suitable season	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
1	Winter, spring, autumn												
2	Winter, spring, autumn												
3	Spring, Summer, Autumn												
4	Spring, early summer												
5	Spring to summer												
6	Summer												
Season		Winter			Spring			Summer			Autumn		

[a] Climate zones.
Zone 1: Equatorial and Zone 2: Tropical; covering the Queensland's Cape York Peninsula and the far north of the Northern Territory,

Zone 3: Subtropical = much of southeast Queensland, some elevated areas further north and some parts in southwest of Western Australia,

Zone 4: Desert = a part of central Australia,

Zone 5: Grassland = the southern half of the country and the northern half of the country and

Zone 6: Temperate = far south of Western Australia and much of southeast of the country.

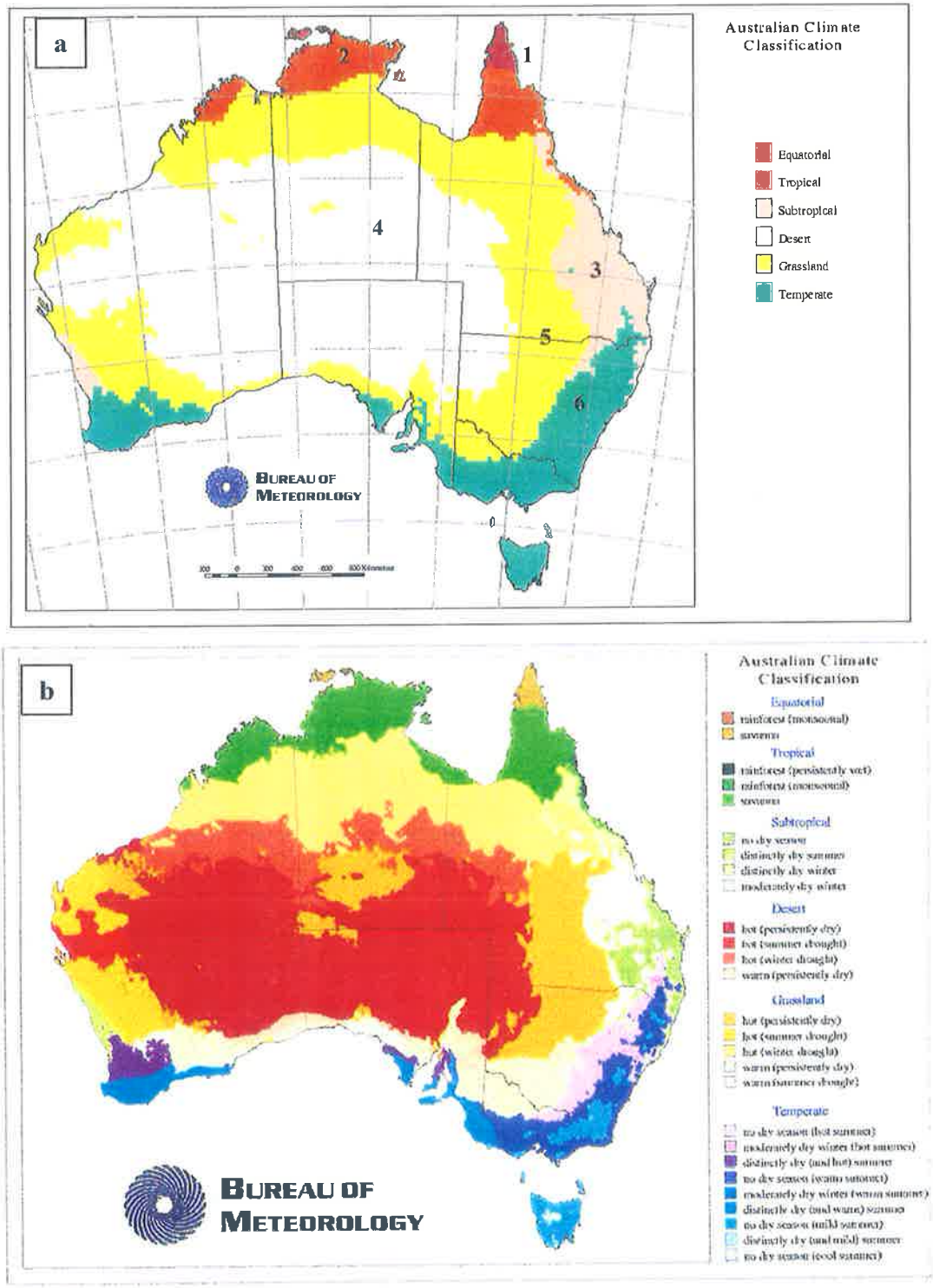


Figure 10.2 Map of climate zones of Australia.

Maps are from the Bureau of Meteorology and can be downloaded from:
http://www.bom.gov.au/climate/how/newproducts/images/zones.shtml

(a) Main climate zones based on temperature and rainfall as indicated by native vegetation; Equatorial (Zone 1), Tropical (Zone 2), Subtropical (Zone 3), Desert (Zone 4), Grassland (Zone 5) and Temperate (Zone 6). (b) Subdivisions within the main climate groups.

Also, hygienic operation is important for reducing any contamination such as fungal development during storage. Storage of only undamaged fruit (free from pests and diseases) is recommended. These procedures would increase production cost, and the final product from this category (slightly red or breaker fruit) can be classified only for second grade spice products due to a low colour content (at least by 20- 40%). Therefore, the economics of this process requires careful consideration. Fruit that are completely red and/or ripe can be used for first grade spice production. This category is most preferable for dehydrated capsicums, and may be the only economically viable one.

Prior to dehydration, dipping in a drying oil should not be used, as this incurs additional costs without providing a benefit over cutting of fruit. However, fruit can be cut to obtain a faster drying rate at 45°C to achieve a final moisture content of 8-12%, depending on buyer specification, since this moisture content is optimal for colour retention in storage while preventing fungal development (Wall, 1994). Dehydration can be initially performed at a higher drying temperature of 60°C for 6 hours, followed by a lower temperature of 40-45°C until reaching the final moisture content. Heat-pump driers are economically suitable for commercial spice production provided that the capacity is not exceeded, therefore it reduces cost of energy consumption over other types of driers. Dried products should be kept in air tighten containers at all times under cool temperatures to avoid colour degradation. The grinding process can be carried out with any mill, depending on specific requirements for size of powder, but a large grinder will generate less heat during grinding, and preserves colour best.

10.7 Possibilities for future research

For maximising production efficiency, further research should be considered in the following areas:

Investigation of new cultivars in different growing seasons and growing regions in order to identify the most suitable *Capsicum* type for each region and to provide spice products all year. This will lead to an increase in spice production, and possibly be helpful for future import replacement.

Further investigation to understand the effects of temperature on the expression of specific enzymes involved in the synthesis of capsaicinoids may be useful. This will require some protocols at molecular level to detect the expression of capsaicinoid biosynthetic genes in response to temperature for each stage of fruit development. Therefore, the relationship between temperature and these changes can be analysed. However, management of stress temperature during the reproductive phase needs to be done carefully. This may harm plant development, and may interfere with the results.

Field trials with broad acre production for each superior cultivar in conjunction with machine harvesting should be investigated to reduce production costs further. Field investigation of ethephon application for that location may not be useful, and is not recommended from this study since most regions are too warm for its application. While ethephon to a limited degree induced fruit ripening, it also caused senescence (abscission of fruit) and therefore reduced yield dramatically. One way of using ethephon is to use it to cause abscission of unwanted plant parts, such as immature fruit, new flowers, buds and leaves prior to harvest. This approach is now being examined in other countries in order to reduce the mixing of unwanted parts with fruit before harvest (Kahn *et al.*, 1997). However, this requires more investigation to clarify the most appropriate concentration of ethephon use and time of application.

The effect of PRD on *Capsicum* spice quality, especially at an early stage of plant growth, would be an interesting issue for further research. The determination of the nature of the signals produced in response to PRD and the mechanism of any chemical signal that controls vegetative growth and/or stomatal behaviour is also valuable information to better understand plant responses. The effect of PRD on water-use efficiency may also be useful for commercial spice production, especially in the areas that have limited water supply. However, this application may not be possible unless PRD can be applied in the field; this would need to be further investigated. Further investigation with a more detailed analysis of soil moisture in relation to changes in g_S and on the differential water use of both parts of the root systems is also needed.

The ripening patterns of *Capsicum* fruit on and off the plant should be further investigated in order to better understand their behaviour. Secondly, the mechanism of fruit ripening, especially the non-climacteric behaviour can be examined further by using a new ethylene inhibitor known as 1-methylcyclopropene (1-MCP). This chemical binds directly to the ethylene receptor and delays further ripening for climacteric fruit such as banana (Golding *et al.*, 1998); this process, however, is reversible as new ethylene receptors form (Golding *et al.*, 1998; Golding *et al.*, 1999). If breaker harvested *Capsicum* fruit do not respond to this chemical by ceasing further ripening, the observation that *Capsicum* fruit behave as non-climacteric fruit is further strengthened, and ethylene is not a major factor in their ripening. Some other factors must be considered such as ABA (Coombe and Hale, 1973), since foliar application of ABA induced colour development associated with ripening in non-climacteric grape berry fruit. Also, a precise concentration for 1-MCP or ABA and appropriate time for 1-MCP exposure would need to be investigated before this hypothesis can be tested.

Further research is required on the effect of drying oil components on *Capsicum* fruit cuticle. Although the drying oil is not an economical technique from this study, it will provide some fundamental information to better understand the *Capsicum* fruit cuticle, possibly leading to the use of alternative chemicals. However, this will require the use of microscopy techniques and cuticle extraction protocols.

Storage and packaging development for maximum storage life of the Australian *Capsicum* spices in relation to high colour, pungency and microbial safety is required. This will facilitate the quality preservation of the final product until it reaches the consumers.

In order to develop *Capsicum* spice industry, the suitable procedures for growing, harvesting and post-harvest handling of selected cayenne chilli and paprika cultivars available in Australia were investigated in this study. However, further research will be necessary, with increased competition in the market requiring producers to look for the cheaper production options and to provide products of international standard. These investigations include new cultivars for different growing seasons and growing regions increased yield and quality of spice for particular regions. Field trials with broad acre production for each superior cultivar in conjunction with machine harvesting are also important for further development to reduce production cost, and therefore maximise production efficiency. Furthermore, the effect of PRD on *Capsicum* spice quality, irrigation levels needed for the PRD capsicum plants, the ripening patterns of *Capsicum* fruit on and off the plant including the effect of 1-MCP and *Capsicum* fruit cuticle changes in response to drying oil are also interesting areas for further study. These will provide further understanding of *Capsicum* fruit responses to these factors, possibly leading to the use of alternative techniques that may be useful for commercial spice production. Australian can produce competitive *Capsicum* spice products throughout the year with consistent quality, and this should lead to import replacement, valued at A$ 2.2 million in 1994.

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Appendices

I. List of experiments

Table A1: Summary

Year	Season	Location	Detail	Experimental chapter
1996/97	Summer	Shadehouse	Harvesting aid	7
1997/98	Summer	Glasshouse	Water stress	5
"	Late summer	Growth chamber	Temperature	4
"	Late summer	Glasshouse	PRD	6
1998/99	Summer	Laboratory	Ripening	8
1999/00	Late summer	Laboratory	Drying	9
"	Late summer	Laboratory	Dips and cutting	9

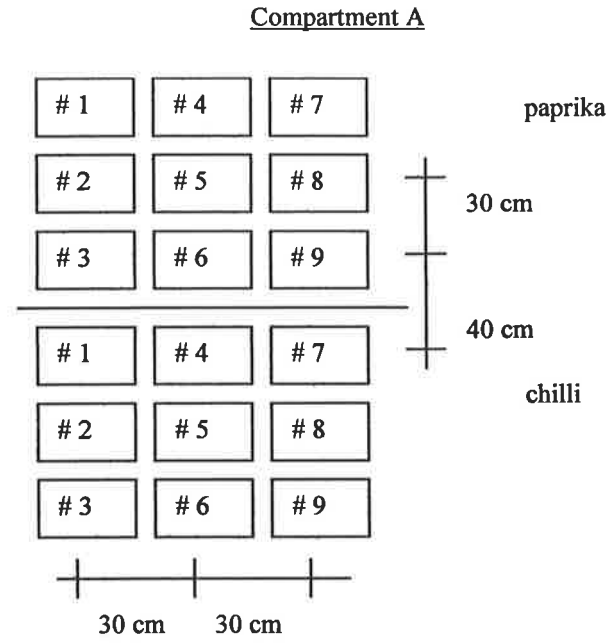
Table A2: Time frame

Chapter No.	Types	Sowing	Transplanting		Treatment applied		Harvesting	
		Date	Date	DAS	Date	DAT	Date	D
7, harvesting aid	paprika	07/09/96	22/10/96	45	30/01/97	100	05/02/97	6
	chilli	07/09/96	22/10/96	45	13/02/97	114	28/02/97	15
5, water stress	paprika	16/12/97	23/01/98	38	24/03/98	60	02/06/98	70
	chilli	16/12/97	23/01/98	38	31/03/98	67	20/07/98	111
4, high temp.	paprika	16/12/97	23/01/98	38	16/02/98	24	09/05/98	82
	chilli	16/12/97	23/01/98	38	16/02/98	24	01/07/98	135
4, low temp.	paprika	16/12/97	23/01/98	38	16/02/98	24	27/06/98	131
	chilli	16/12/97	23/01/98	38	16/02/98	24	21/07/98	155
6, PRD	chilli	24/02/98	02/04/98	37	08/12/98	-	01/02/99	55
8, ripening	paprika	31/08/98	13/10/98	43	-	*	21/04/99	-
		29/11/98	06/01/99	31	-	*	03/05/99	-
		29/11/98	06/01/99	31	-	*	04/05/99	-
8, ripening	chilli	31/08/98	13/10/98	43	-	*	02/04/99	-
		26/10/98	01/12/98	36	-	*	20/04/99	-
		29/11/98	06/01/99	31	-	*	13/05/99	-
		29/11/98	06/01/99	31	-	*	17/05/99	-
9, types of drier	paprika	-	-	-	-	*	04/08/97	-
9, drying temp.	paprika	-	-	-	-	*	04/08/97	-
9, dips	paprika	26/10/98	01/12/98	-	-	*	03-05/06/99	-
9, dips	paprika	-/08/99	-/09/99	~30	-	*	11-12/01/00	-

Key: DAS = days after sowing, DAT = days after transplanting, D = days after treatments applied, - = there was no record, * = Treatments were applied after harvesting.

II. Experimental plot layout

Figure A1 - Chapter 4
Figure A2 - Chapter 5
Figure A3 - Chapter 6
Figure A4 - Chapter 7



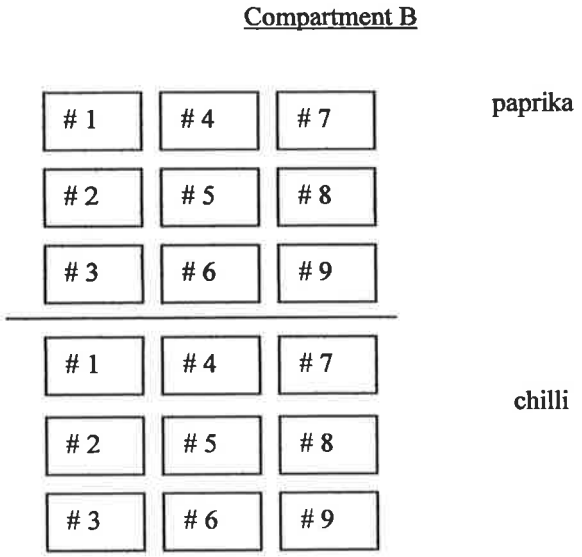


Figure A1. Plot layout for air temperature study (Chapter 4) of potted paprika and chilli plants which were grown in two separate compartments; (A) 22/17°C and (B) 30/25°C (day/night) at the Waite Campus, Adelaide University. Each cultivar was randomly located in each compartment in separate blocks. Nine plants were used and coded by plant number (#).

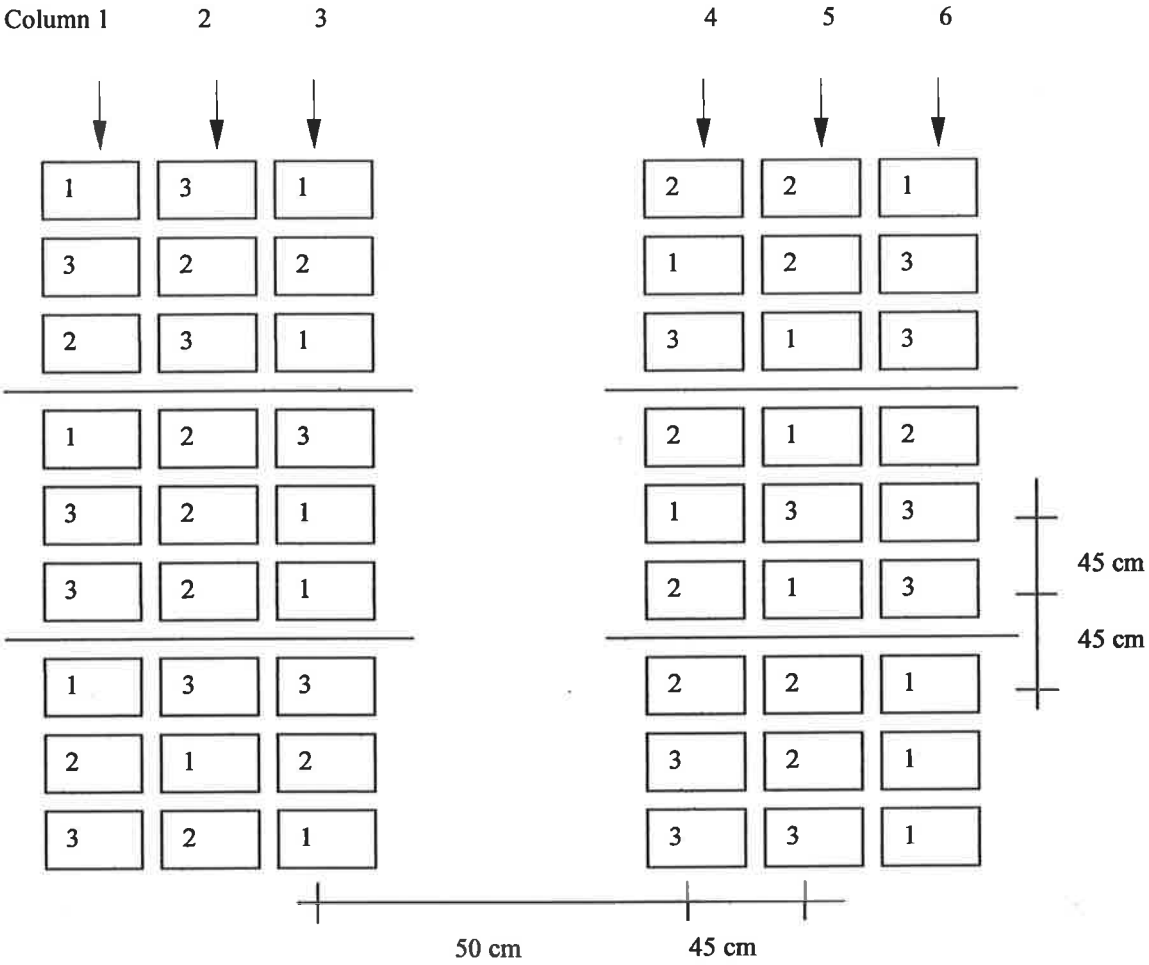


Figure A2. Plot layout for water stress study (Chapter 5) located in block replicates of potted paprika (three columns on the left) and chilli (three columns on the right) plants without border plants in the glasshouse at the Waite Campus, Adelaide University. The horizontal lines indicate boundaries of block replicates.

Treatment no.	Treatment
1	2D irrigation frequency
2	4D "
3	6D "

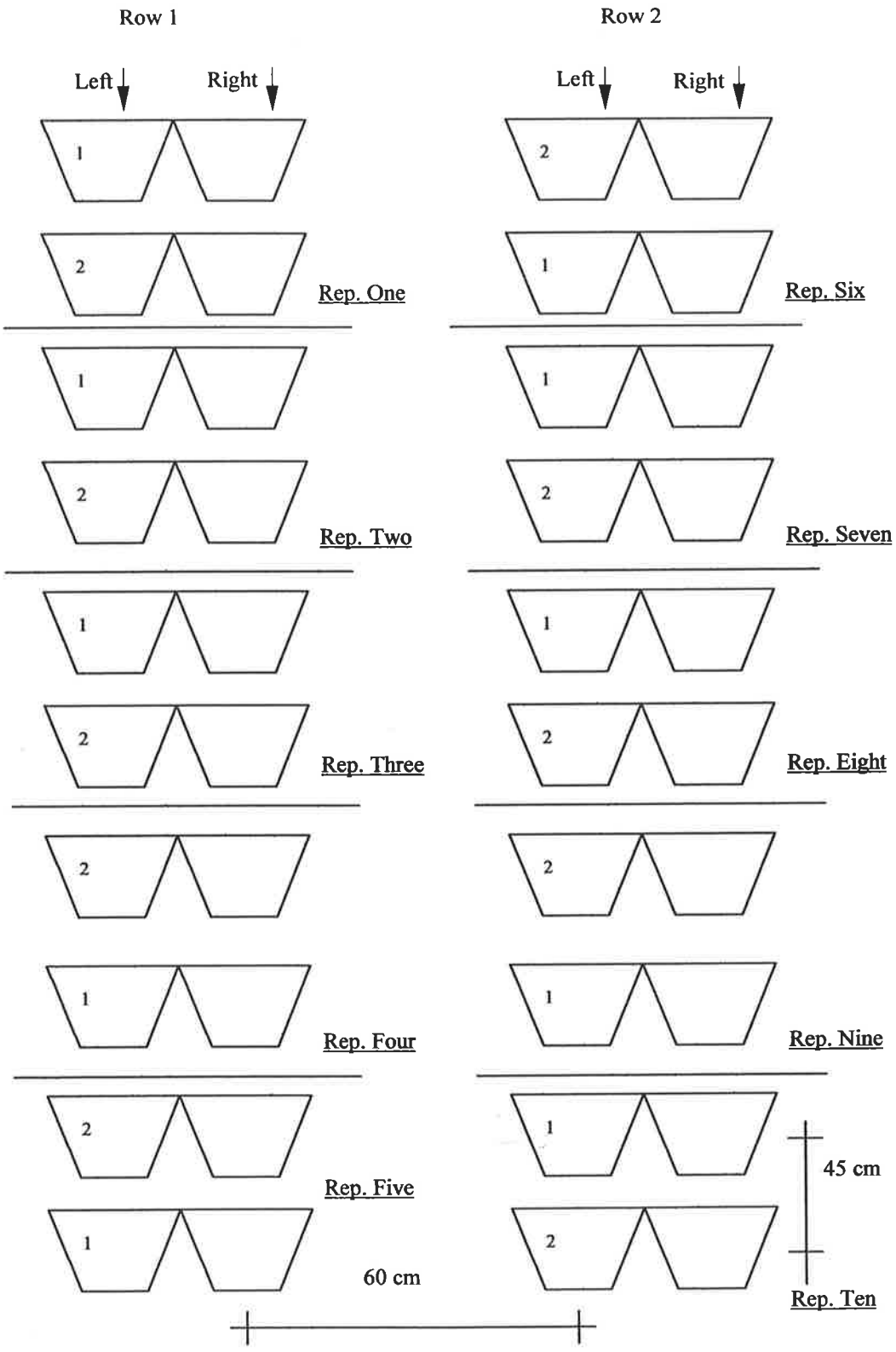


Figure A3. Plot layout for PRD study (Chapter 6) of pot-grown chilli plants located in a glasshouse at the Waite Campus, Adelaide University. The twin pot-shape represent containers where each side was termed 'left' and 'right' for convenient designation of half irrigation treatments. Details of treatments are described in Chapter 6. The horizontal lines separate block replicates.

Treatment no.	Treatment
1	Control
2	PRD

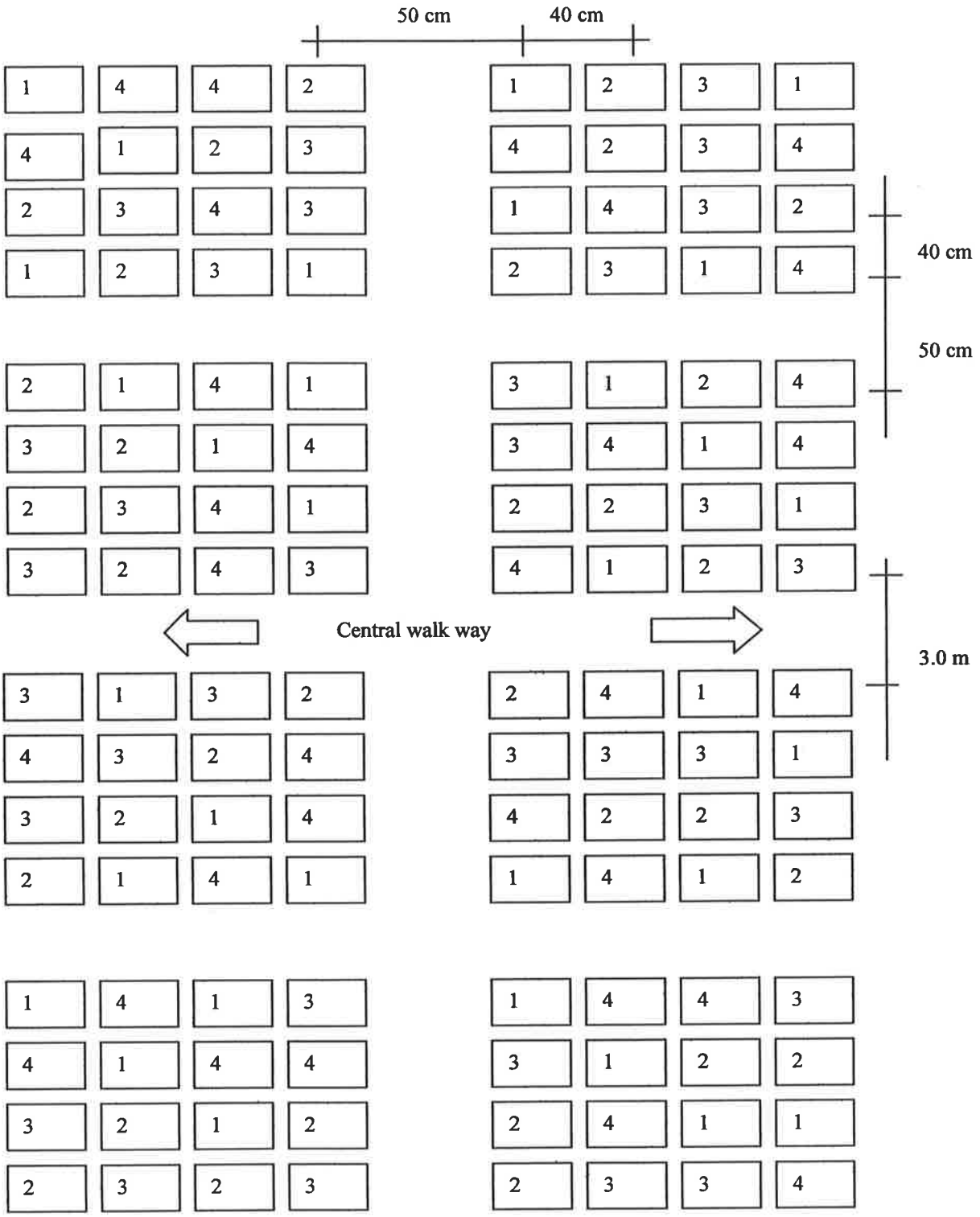


Figure A4. Plot layout of ethephon experiment (Chapter 7) of potted paprika (four top replicates) and chilli (four bottom replicates) plants which were grown in the shadehouse at the Waite Campus, Adelaide University. Each cultivar was analysed individually, and separated by a central walk way. Treatments were randomly arranged into block replicates of 16 plants.

Treatment no.	Treatment
1	Control (plain water)
2	1000 $\mu L.L^{-1}$ ethephon concentration
3	3000 $\mu L.L^{-1}$ "
4	5000 $\mu L.L^{-1}$ "

III. Climatic data

Summary of climatic data recorded in the shadehouse at the Waite Campus, the University of Adelaide, for the 1996/97 growing season.

Table A3: Summary of climatic data.

Parameter [a]	Sep	Oct	Nov	Dec	Jan	Feb
Max Temp	28.1	32.6	31.2	35.9	37.9	28.0
Min Temp	12.4	14.4	16.8	17.9	20.8	17.2
Rainfall	86.2	23.4	5.0	13.8	9.8	30.0

[a] Max Temp = maximum daily temperature (°C); Min Temp = minimum daily temperature (°C); Rainfall = total rainfall for month (mm).

IV. Characteristics of potting mix (UC, Waite Version)

The University of California potting mix or the UC mix was firstly introduced to Waite by a Professor from California in the 1950s. Recently, the UC mix (Waite Version) has been developed. In general, its soil mix contained two third of a cubic meter of washed coarse sand is sterilised at 100°C for half an hour in a sterilising mixer. One third of a cubic meter of peatmoss is added and mixed for ten seconds (E. Nagy, 1996, pers. comm.). After approximately 10-15 min, the following fertilisers are added; 700 g calcium hydroxide, 480 g calcium carbonate and 600 g Nitrophoska 15-4-12, and mixed for 20 seconds. This mix has been previously classified as a loamy sand soil type having 47.3% available water holding capacity and 2.9% air-filled porosity (Malinda, 1996). Characteristics of potting mix are shown below:

Table A4: Characteristics of potting mix.

Sample	**pH**	**E.C.**	**pH**	**Total**	**HCO_3^-**	**HCO_3^-**	**DTPA extraction[a]**			
		Soil:water (1:5)	(0.01 M $CaCl_2$)	N	Extracted P	Extracted K	Cu	Fe	Mn	Zn
(#)		($dS.m^{-1}$)		(%)	($mg.kg^{-1}$)	($mg.kg^{-1}$)	($mg.kg^{-1}$)			
1	6.0	0.40	5.5	0.10	37	2880	0.03	9.9	0.49	0.47
2	6.0	0.36	5.5	0.11	26	675	0.03	9.1	0.48	0.44
3	6.1	0.40	5.5	0.11	30	363	0.03	9.7	0.52	0.49

[a] DTPA extraction = a method for determining nutrient levels in potting mixes by using diethylenetriamine-N, N, N′, N″, N″- pentaacetic acid (DTPA) as an extracting solution.

Source of analysis: Department of Soil and Water, Adelaide University, Waite Campus, January 1998.

V. Soil water retention curve

Soil water retention curve for UC mix (Waite Version) used in this study was fitted to the data by exponential regression to allow estimation of soil water content at different soil water potentials: $SWC = 8.17 + 13.57\ (1.17)^{\Psi_{soil}}$ ($r^2 = 0.67$, $P<0.001$). Data are summarised in Table A5.

Table A5. Relationship between soil water potential (Ψ_{soil}, kPa) and volumetric soil water content (SWC, %) of UC mix at the Waite Campus. Plant responses were investigated for 'Caysan SPS705' chilli plants that were grown in the UC mix located in a greenhouse.

Ψ_{soil}	SWC	Plant response	Note
Near 0	21.7	No visible wilting	Close to field capacity
-10	11.0	"	Close to field capacity
-20	8.8	Visible leaf wilt	Stress start
-30	8.3	Visible wilt (more leaf wilt)	Mild stress
-40	8.2	Severe wilt (whole plant parts)	Severe stress
-50	8.2	"	"
-60	8.2	"	"
-70	8.2	"	"
-80	8.2	"	"

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Colour at harvest and post-harvest behaviour influence paprika and chilli spice quality.

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Abstract

Capsicum annuum L. paprika and cayenne chilli pepper fruit were grown for red spice production and harvested at various colour stages on the same day. Fruit of each stage were allowed to change colour at room temperature with or without the addition of 100 μl l^{-1} ethylene. Fruit appearance and colour development, and respiration and ethylene production were measured during the colouring period. Ethylene treatment had no effect on colour development or pungency for both cultivars, even though it easily crossed the cuticle, epidermis and flesh tissues into the fruit cavity. Green or deep green harvested fruit failed to fully colour red, while fruit that were harvested at or after the colour break stage visually completed their red colour development within 7–9 days. However, the colour intensity of spice powder was low for all fruit that had not developed a deep red colour prior to harvest. For paprika no difference between deep red fruit that were succulent or that had partially dried on the plant was found, but chilli fruit that had partially dried before harvest produced the most intense colour. American Spice Trade Association (ASTA) extractable red colour was the best measure of spice colour quality, compared to reflected lightness (L*), chroma (C*) and hue angle (h°) colour measurements. Pungency did not change between ripeness stages for chilli and was absent in paprika. Paprika and chilli fruit showed climacteric behaviour as long as they were attached to the plant, but when detached were non-climacteric.

Keywords: *Capsicum annuum* L.; Ethylene; Respiration; Colour; Pungency; Spice

1. Introduction

Worldwide interest in capsicum spices is increasing (Bosland, 1993). These are either pungent or non-pungent spices, such as pimiento, paprika, and chilli that are produced from dried fruit and are ground into powders. To achieve a good spice colour quality, completely red fruit are processed.

Since fruit in different positions on the chilli or paprika plant mature at different times in the growing season, cost-effective once-over mechanical harvesting yields a mixture of fruit of different ripeness stages. Pre- or post-harvest treatments may increase the number of red ripe fruit by

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inducing fruit ripening (Saltveit, 1977; Gomez, et al., 1998). However, results from these treatments are often unsatisfactory (Knavel and Kemp, 1973; Krajayklang et al., 1999). While preharvest studies indicate ethylene involvement in colour development (Osterli et al., 1975), overall information on the ripening behaviour of harvested *Capsicum* species is limited.

Fruit in the genus of *Capsicum* have been classified as non-climacteric (Lurie et al., 1986; Biles et al., 1993), but the hot chilli cv. 'Chooraehong' (*Capsicum frutescens*) was reported as climacteric (Gross et al., 1986). By definition, in non-climacteric fruit an increase in respiration and ethylene production during ripening is absent (Biale, 1964). Treatment with exogenous ethylene in climacteric fruit such as banana leads to an autocatalytic biosynthesis of ethylene and this accelerates fruit ripening, but ethylene synthesis does not occur spontaneously in non-climacteric fruit after treatment with exogenous ethylene (Biale, 1948).

The aim of this study was to investigate the effects of colour stage at harvest and ethylene on paprika and chilli fruit colour changes to improve colour quality, pungency, and yield of red fruit after once-over harvesting.

2. Materials and methods

2.1. Plant material

Healthy fruit of PS72285 paprika (*Capsicum annuum* L.) and Caysan SPS705 chilli (*Capsicum annuum* L.) were hand-harvested on the same day at different colour stages. Seven colour stages were harvested; light green, deep green, breaker (slight colouration), breaker red (some red colour), bright red (100% red), deep red and succulent, and deep red and partially dried. Ten fruit of each colour category were randomly selected and separated into two groups, one as a control and one for ethylene treatment. The experiment was replicated three or four times for paprika or chilli, respectively.

2.2. Ethylene treatment and storage conditions

Within one hour after harvest, five fruit were weighed in bulk and enclosed in 2.2 l plastic containers with 5 g of calcium hydroxide ($Ca(OH)_2$) to absorb evolved CO_2. Ethylene was injected through a septum port into the sealed ethylene C_2H_4 treatment containers, to obtain 100 $\mu l\ l^{-1}$. Control containers were vented every 12 h for 5 min, followed by ethylene containers to prevent cross-contamination. Ethylene was then re-injected until 48 h. Thereafter, containers were opened and fruit stored in the same container in a well-ventilated room at 22°C under normal fluorescent room light for 7–10 days depending on their external appearance. Visual colour, weight loss, CO_2 and C_2H_4 evolution were determined during storage.

2.3. External and internal quality assessments

Skin colour was assessed daily on a subjective scale from 0 (light green) to 11 (deep red and partially dried), using a modified scale from Lownds et al. (1994). External quality was judged at the end of storage based on fruit skin shrivelling (water loss) and calyx yellowing. Decay incidence was also evaluated at the end of storage as percent of fruit manifesting stem-end rots or other rots, and on the inside of cut fruit.

2.4. Colour and pungency determination of spice

After quality evaluation and gas measurements, fruit were dried to constant weight in a hot air oven at 45°C and ground with a Culatti electric mill (1.5×10^{-8} m mesh size). Ground samples were kept in airtight plastic bags in the dark at room temperature and used for colour and pungency measurements.

2.4.1. Reflected colour

Surface colour of sample powder was measured as reflected colour in the CIELAB (L*a*b*) colour space using a Minolta model CR-300 Colorimeter (Minolta, Osaka). One reading was performed for each sample. Lightness L*, chroma C* and hue angle h° were determined, with L* rang-

ing from 0 = black to 100 = white. C*, $((a^*)^2 + (b^*)^2)^{1/2}$) and represents colour saturation which varies from dull (low value) to vivid colour (high value), and $h^\circ = \tan^{-1}(b^*/a^*)$ and is defined as a colour wheel, with red-purple at an angle of 0°, yellow at 90°, bluish-green at 180°, and blue at 270° (McGuire, 1992).

2.4.2. Extractable colour

Extractable red colour was measured in the units of the American Spice Trade Association (ASTA) (Woodbury, 1997). A representative ground sample (~70–100 mg) was extracted in 100 ml of acetone for 16 h at room temperature in the dark. Absorption of this solution was measured at 460 nm in comparison to a standard glass reference. The final ASTA value for each measurement was calculated on a dry weight basis as previously described by Krajayklang et al. (1999).

2.4.3. Pungency

The pungency of each sample was estimated by determining the gross capsaicin and dihydrocapsaicin content (Todd et al., 1977), using a high performance liquid chromatographic (HPLC) procedure measuring absorbance at 280 nm (Krajayklang et al., 1999). Scoville values were calculated by multiplying the amount of capsaicin and dihydrocapsaicin per gram dry weight by 16 million Scoville heat units for pure capsaicin and dihydrocapsaicin (Todd et al., 1977).

2.5. Gas measurements

Rates of C_2H_4 and CO_2 production were measured daily by gas chromatograph. A static, closed system was employed, whereby the storage container for the above fruit was sealed for a set period at 22°C before taking gas samples for assessment.

2.5.1. C_2H_4 measurement

To measure C_2H_4, a 1-ml gas sample was collected from the container after 4 h of sealing the containers. C_2H_4 was quantified using a Varian chromatograph model 3400 equipped with a flame ionisation detector (Varian Australia, Mulgrave, Vic.) and a Porapak Q stainless steel column (60 cm × 3.1 mm i.d.) of 80/100 mesh. Temperature conditions were 50°C for the column, 135°C for the injector and 150°C for the detector. Flow rates of the carrier gas nitrogen, air and hydrogen were 50, 300 and 40ml/min, respectively. A gas standard containing 95 nmoles l^{-1} C_2H_4 standard (BOC Gases, Torrensville, SA) was used for calibration. Results were expressed as nmoles of ethylene produced per kg fresh weight and h (nmoles.kg^{-1} h^{-1}).

2.5.2. CO_2 measurement

CO_2 was measured at hourly intervals for 4 h by injecting another 1-ml gas into a Varian 3300 chromatograph equipped with a thermal conductivity detector (Varian Australia, Mulgrave, Vic.) and a silica column (35 cm × 3.1 nm i.d.) of 80/100 mesh. Temperature conditions were 28°C for the column and 90°C for the injector and detector, and the flow rate of the carrier gas helium was 5ml/min. A calibration was performed using a 0.5% CO_2 standard (BOC Gases). Results were expressed as mmoles of CO_2 produced per kg of fresh weight and h (mmoles.kg^{-1} h^{-1}).

2.5.3. Ethylene uptake by fruit

The internal C_2H_4 concentration in the fruit cavity was measured in contrast to the external one surrounding the fruit, in order to determine fruit ethylene uptake. Two groups of five fruit were enclosed in separate containers. One was used for a control (no C_2H_4 injection) and the other for a 100 μl l^{-1} C_2H_4 treatment. C_2H_4 was injected into the sealed container, and it was left at room temperature for 12 h. Two 1-ml gas samples were taken from a container, and used to determine the external C_2H_4 concentration. Internal fruit atmosphere samples were taken by inserting the needle of a 1 ml syringe through the ovary wall into the fruit cavity. C_2H_4 concentrations were determined as above.

2.6. Statistical analyses

The experiment was conducted as a completely randomised factorial design, with seven colours at harvest × two ethylene applications. Experiments were replicated using 3–4 different harvests over a

month period. Each cultivar was evaluated as a separate experiment. Statistical analyses were performed using Genstat 5 for Windows Release 4.1 (3rd edition, Rothamsted Experimental Station, England). Data were subjected to two ways analysis of variance, and treatment means were compared using least significant differences ($P < 0.05$).

3. Results

3.1. Fruit appearance

External quality of the green and the deep green harvested fruit after storage was very poor for both primarily from significant shrivelling, but that of the breaker to the red harvested fruit was acceptable after 10 days of storage. Ethylene had no effect on quality or fungal spoilage in paprika or chilli (data not shown).

Harvested chilli fruit of all colour stages developed a slight discolouration on the seed after storage, indicating possible internal fungal contamination, while harvested paprika fruit showed no sign of any fungal development throughout the experiment. There was no visible disease for any fruit after storage.

3.2. Fruit colour development

Ethylene application did not influence the final colour of fresh paprika and chilli fruit (Table 1). However, maximum colour for paprika was slightly delayed by ethylene application.

Green and deep green harvested fruit of both cultivars achieved less than 50% red colouration during storage (Table 1), even after ethylene treatment. Up to 8 days additional exposure to ethylene did not further promote colour development of these fruit (data not shown). Fruit harvested at

Table 1
Colour development of *Capsicum* fruit harvested at different colour stages during storage at room temperature with or without ethylene application

Treatment factor		Final colour[z]	Time to final colour (days)
Paprika cv. PS72285			
Colour at harvest	Green	4.4b[y]	8.2abc
	Deep green	4.7b	9.3a
	Breaker	10.2a	8.7ab
	Breaker red	10.2a	7.7bc
	Bright red	10.4a	7.0c
	Deep red	11.0a	3.5d
	Deep red + dried	11.0a	0.0e
Ethylene	Without (−)	8.9a	5.9b
	With (+)	8.8a	6.7a
Chilli cv. Caysan SPS705			
Colour at harvest	Green	2.1e	9.5a
	Deep green	4.3d	9.0a
	Breaker	9.4c	8.9a
	Breaker red	9.8bc	8.8a
	Bright red	10.8ab	8.5a
	Deep red	11.0a	4.9b
	Deep red + dried	11.0a	0.0c
Ethylene	Without (−)	8.3a	6.8a
	With (+)	8.4a	7.3a

[z] Skin colour was scored on a scale from 0 to 11: 0, green; 1, deep green; 3, 25% red; 5, 50% red; 7, 75% red; 9, bright (full) red; 10, deep red and succulent; 11, deep red and partially dried.

[y] Different letters within columns for each treatment factor and cultivar show significant differences ($P < 0.05$) using LSD.

Table 2
Reflected and extracted colour, and pungency of *Capsicum* powder made from fruit that were harvested at different colour stages and ripened with or without ethylene

Treatment factor		Colour characteristics[z]				Pungency ($\times 10^3$ SHU)[y]
		L*	C*	h°	ASTA	
Paprika cv. PS72285						
Colour at harvest	Green	52a[x]	52b	69a	50d	0
	Deep green	52a	57b	65b	63d	0
	Breaker	45b	66a	58c	104c	0
	Breaker red	43b	70a	54d	139b	0
	Bright red	42bc	71a	54cd	139b	0
	Deep red	41bc	68a	53d	169a	0
	Deep red+dried	38c	67a	51d	194a	0
Ethylene	Without (−)	45a	64a	58a	123a	0
	With (+)	45a	65a	57a	123a	0
Chilli cv. Caysan SPS705						
Colour at harvest	Green	52a	44c	79a	25f	16a
	Deep green	52a	53b	70b	38e	16a
	Breaker	47b	71a	56c	74d	17a
	Breaker red	46b	71a	56c	89c	17a
	Bright red	45b	72a	55c	90c	18a
	Deep red	42c	71a	54c	106b	17a
	Deep red+dried	42c	70a	53c	120a	22a
Ethylene	Without (−)	47a	64a	60a	76b	18a
	With (+)	46a	65a	60a	79a	18a

[z] Lightness (L*) ranged from 0 = black to 100 = white, chroma ($C^* = (a^2+b^2)^{1/2}$ with 0 = least intense, hue angle ($h^\circ = \tan^{-1}(b/a)$ with 0° = red-purple and 90° = yellow in the CIELAB colour space. American Spice Trade Association (ASTA) colour units measured extractable red colour/g dry weight.

[y] Scoville heat units (SHU) were calculated by multiplying the amount of capsaicin and dihydrocapsaicin per gram dry weight by 16 million Scoville units for pure compounds.

[x] Different letters within columns for each treatment factor and cultivar show significant differences ($P<0.05$) using LSD.

breaker stage or later developed a dark red colouration (stage 10), except for breaker chilli fruit that only turned bright red (stage 9). Fruit harvested at breaker to bright red stage reached their final colour within seven to nine days (Table 1).

3.3. Colour of spice powder

Reflected colour of the powder was not affected by ethylene treatment for either cultivar (Table 2).

As colour stage at harvest increased, paprika powder of redder fruit obtained a darker colour as indicated by a reduction of L* value, from 52 to 38, for the green and deep red and partially dry harvested fruit, respectively (Table 2). Colour saturation (C*) increased for green to breaker-harvested fruit, and then remained constant (Table 2). The higher C* value for red harvested fruit represents a more vivid colour (Table 2). The hue angle of green harvested fruit after storage describes a more yellow colour with the highest angle of 69, while an increase in colour stage at harvest resulted in an increase in red colour with the lowest angle of 51 in red harvested fruit (Table 2).

Powder colour of chilli fruit showed the same characteristics. With increasing colour stage at harvest, powder of stored fruit was more dark, vivid and deep red in colour. The L* values ranged from 52 to 42, C* ranged from 44 to 70, and hue angle was about 70–53 for green harvested fruit to deep red harvested fruit, respectively (Table 2).

Deep-red coloured fruit at harvest (stage 10 or 11) achieved the maximum extractable red colour, 194 and 120 ASTA units for partially dry paprika and chilli fruit, respectively (Table 2). There was no difference in extractable colour intensity between powders made from succulent and partially dried paprika fruit, but for chilli, letting fruit partially dry on the bush increased extractable colour intensity of its powder. Green and deep green fruit at harvest obtained a very low colour intensity of 50–60 ASTA units for paprika and about 25 to 40 ASTA units for chilli (Table 2). Breaker to bright red fruit at harvest, while appearing visually as red or deep red after storage, had reduced extractable red colour of the spice powder.

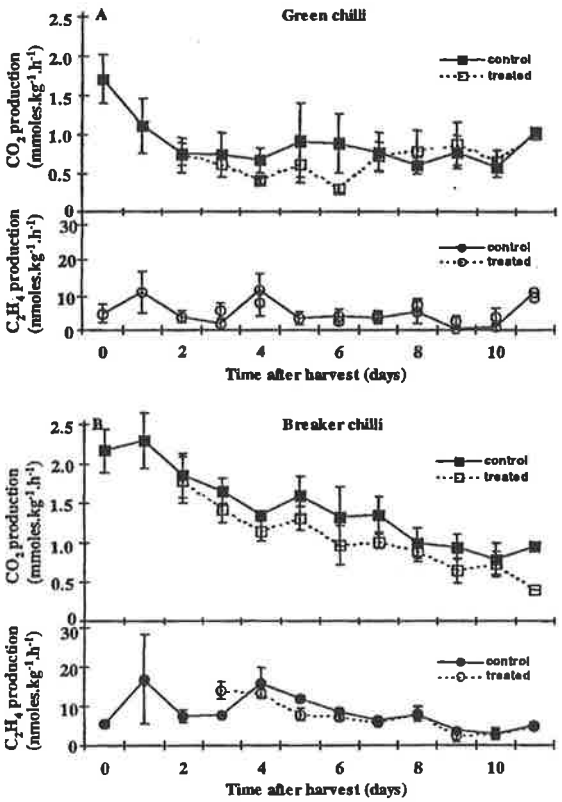

Fig. 1. Ethylene and carbon dioxide production at 22°C of green mature (A) and breaker (B) harvested chilli fruit exposed for 48 h to air (control) or 100 μl l^{-1} ethylene (treated). Four replications of five fruit were used for each measurement; error bars represent the SE of the mean.

3.4. Pungency

Pungency or hotness was absent in paprika fruit. Pungency of chilli powder did not increase significantly with colour stage at harvest (Table 2). Ethylene had no effect on pungency (Table 2).

3.5. CO_2 and C_2H_4 production

3.5.1. During post-harvest storage

Typical data for respiration and ethylene production of chilli fruit are shown in Fig. 1, as the behaviour of chilli and paprika fruit was very similar. In general, neither respiration nor ethylene production of green or breaker harvested fruit was affected by exogenous ethylene treatment, but these data were not available during ethylene treatments (Fig. 1). Respiration declined directly after harvest in all fruit without a significant respiratory peak, and ethylene production did not change markedly throughout the experiment (Fig. 1). Green fruit did not reach a fully red colour before measurements concluded, whereas breaker fruit did.

3.5.2. At harvest

Typical data for respiration and ethylene production rates of different colour stages of chilli fruit immediately after harvest are shown in Fig. 2, as paprika and chilli showed very similar behaviour. A distinct respiratory climacteric pattern was apparent, with respiration peaking at dark green to breaker red colour stages (Fig. 2). Ethylene production peaked later, increasing from breaker red and peaking at the bright red colour stage (Fig. 2).

3.5.3. Ethylene uptake by fruit

At harvest, both control and treated fruit had a similar internal ethylene atmosphere concentration at 0.01–0.02 μl l^{-1}. After ethylene was injected into the container, internal and external ethylene atmosphere was markedly different between treated and untreated fruit. Ethylene levels of 0.02–0.06 and 18.2–22.8 μl l^{-1} were detected in the internal atmosphere inside the cavity of control and treated fruit, respectively. The external

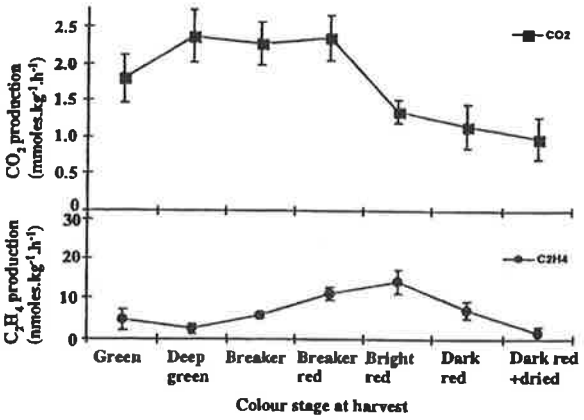

Fig. 2. Ethylene and carbon dioxide production at 22°C of differently coloured cv. Caysan SPS705 chilli fruit directly after harvest. Four replications of five fruit were used for each measurement; error bars represent the SE of the mean.

atmosphere surrounding control fruit contained 0.04–0.05 μl l^{-1} ethylene, while that of treated fruit was about double that in the fruit cavity at about 40.2–43.9 μl l^{-1}. The difference may be caused by air leakage into the syringe during sampling and after retracting the needle due to reduced pressure within the syringe and the small internal fruit volume during sampling.

4. Discussion

4.1. Fruit appearance

Green harvested fruit appeared to be more susceptible to water loss compared to red harvested fruit after 10 days of storage. It has been noted that during the growing season green chilli fruit are highly sensitive to water loss and disorders influenced by environmental conditions such as heat damage (Wall and Biles, 1994). Therefore, if fruit are to be stored for the fresh market water loss control, especially for green fruit, is essential.

4.2. Fruit colour development

Exogenous ethylene treatment was not effective in inducing red colour development of paprika and chilli fruit under the conditions of this study. Green mature harvested fruit failed to fully colour even when treated with ethylene. Similar results were found in detached green pimiento (Knavel and Kemp, 1973) or bell pepper fruit (Lockwood and Vines, 1972) after they were treated with either ethylene (500 μl l^{-1}) or ethephon (1000 μl l^{-1}). Lockwood and Vines (1972) suggested that the thick cuticle of capsicum fruit is a barrier to ethylene thus preventing its action. However, we observed that a high level of internal ethylene uptake occurred under ethylene treatment, indicating that ethylene was able to cross the cuticle. Therefore ethylene is not able to promote postharvest colour development. In our study ethylene even slightly delayed paprika fruit colour development, similarly to a previous report (Lockwood and Vines, 1972) on fruit colour development of pimiento fruit. The reason for this is unknown.

Nevertheless, once fruit were harvested at or near the breaker colour stage, they coloured normally in both cultivars. While on the plant, fruit were able to change colour satisfactory and also exhibited ethylene and respiratory peaks. Colour change involves both the degradation of chlorophyll and the *de novo* production of ketocarotenoids capsanthin and capsorubin; also xanthophylls and carotenoids are present and these often become esterified (Minguez-Mosquera and Hornero-Mendez, 1994). Therefore, red colouring on the plant appears to involve some additional factor, such as other hormones or presence of sufficient pigment precursors, that interacts with ethylene to induce full colour changes from green to deep red. In addition the chlorophyll degradation process is impaired in harvested fruit.

4.3. Colour of spice powder

The concentration of extractable colour pigment in dried paprika and chilli powder was not affected by ethylene application, mirroring the

lack of effect on visual fruit colour. Extractable colour on a dry weight basis was highest in fruit allowed to dry on the bush (pre-harvest dehydration). Similar findings were reported in red harvested paprika fruit (Kanner et al., 1977) and field-dried cayenne chilli (Lease and Lease, 1956). In addition, we found similar high levels for paprika fruit that were succulent but deep red. These colour stages have been previously mentioned as optimum for harvesting spice paprika in Hungary (Markus et al., 1999) and for spice paprika and Cayenne chilli in the USA (Lease and Lease, 1956). In addition to better initial colour intensity, letting fruit partially dry improves colour retention in spice (Lease and Lease, 1956), as β-carotene is converted to more stable red coloured xanthophylls (Markus *et al.*, 1999).

L*, C* and h° values indicated general changes in colour for different colour stages at harvest. However, C* and h° in general only showed differences between green or partially to fully red fruit. While L* was better able to differentiate between colour stages at harvest, these measurements did not always mirror changes in ASTA colour values. Therefore, L* values cannot be used to replace the industry standard of measuring extractable red colour using the ASTA method.

4.4. Pungency

Pungency of chilli powder in this study did not vary with different colour stages. Some reports agree with this, for example Somos (1984), but Balbaa et al. (1968) and Mathew et al. (cited in Cotter, 1980) found increases in pungency as the fruit reaches the red colour stage. As only red fruit are processed into spice, always the maximum possible pungency will be achieved for the spice as influenced by colour stage.

4.5. Respiration and ethylene production

We found no response of chilli or paprika fruit to applied external ethylene. It has been reported that an application of ethylene or propylene to climacteric fruit can stimulate both respiration and autocatalytic ethylene production, while in non-climacteric fruit exogenous ethylene stimulates respiration only (McGlasson, 1978). While our paprika and chilli fruit behaved in a non-climacteric fashion after harvest, we were not able to determine respiratory stimulation by ethylene as a CO_2 scrubber had to be included during ethylene exposure to prevent CO_2 interfering with ethylene effects. However, just after treatment application these fruit had similar rates to control fruit. Contrary to us and other reports (Lurie et al., 1986; Biles et al., 1993), Gross et al. (1986) found 'Chooraehong' chilli (*C. frutescens*) to be climacteric as they found a respiratory climacteric; however, they found no C_2H_4 peak.

When comparing the respiration and ethylene production rates of different fruit colour stages on the plant, a climacteric pattern was apparent for both chilli and paprika cultivars. Respiration peaks occurred during the initial colour change, while C_2H_4 peaks occurred later as fruit completed their colour change to red. This concurs with peaks found by Wall and Biles (1994) for just harvested fruit. It therefore appears that the fruit from our study behaved differently on and off the plant, and at this stage the reason is unclear as discussed above.

The highest C_2H_4 production level for paprika and chilli fruit was about 17 nmoles.kg^{-1} h^{-1} at 22°C in this study. This was similar to other studies (Wall and Biles, 1994), but the climacteric 'Chooraehong' chillies peaked at about 30 nmoles.kg^{-1} h^{-1} (Gross et al., 1986).

Fruit harvested at an active growth stage such as green fruit tend to have high respiration rates and it is also temporarily elevated at harvest due to the harvest wound (Kays, 1991). This was also observed in this study. Maximum CO_2 production in paprika fruit at 22°C was 3.5 mmoles.kg^{-1} h^{-1} and in chilli fruit 2.3 mmoles.kg^{-1} h^{-1}; this is comparable to other studies such as of Biles et al. (1993) and Gross et al. (1986).

5. Conclusion

Different colour stages at harvest significantly affected chilli and paprika fruit colour development and spice colour quality, but not pungency

levels of chilli spice. Green or deep green harvested fruit failed to fully colour red after harvest, while fruit that were harvested at or after the breaker stage completed their colour change to fully red. Exogenous ethylene application did not affect red colour development or pungency of any colour stage at harvest. Allowing fruit of both cultivars to fully ripen and/or partially dry on the bush resulted in the maximum colour intensity after processing. Extractable red colour measured using the ASTA technique was the most suitable method of spice colour assessment, compared to reflected L*, C* and h° colour measurements. A distinct climacteric pattern was found during colour change as long as fruit were attached to the mother plant. However, once fruit were harvested, green and breaker harvested fruit behaved in a non-climacteric manner.

Therefore completely red fruit at harvest, either succulent or preferably partially dry, are needed to produce the best quality spice. After once-over machine harvesting, fruit that have not achieved this colour stage should be culled before processing.

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