International Food Research Journal 20(1): 239-246 (2013)
Journal homepage: *http://www.ifrj.upm.edu.my*

Alleviation of internal browning in pineapple fruit by peduncle infiltration with solutions of calcium chloride or strontium chloride under mild chilling storage

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Article history

Received: 2 May 2012
Received in revised form: 19 June 2012
Accepted:20 June 2012

Keywords

Chilling injury
$CaCl_2$
$SrCl_2$
peduncle infiltration
transpiration

Abstract

The development and control of internal browning (IB, a form of chilling injury) were studied in fruit of two commercial cultivars of pineapples. The symptoms develop in tissues surrounding the core when the fruit are stored at <15 °C for several weeks. It was found that core and the fruitlets of fruit of 'Smooth Cayenne' and 'Trad-Srithong' could be effectively infiltrated with water soluble carmoisine dye or salt solutions by transpiration via the peduncle over three days at storage temperatures of 8, 13, and 20 °C. IB was more severe in 'Trad-Srithong' than in 'Smooth Cayenne' fruit particularly at 8 °C. Infiltration via the peduncle increased calcium or strontium concentrations in the core and adjacent flesh tissue and reduced IB in 'Trad-Srithong' stored at 13 °C. There were no differences in severity of IB between green and quarter ripe fruit. Infiltration with calcium or strontium through the peduncle was more effective when the treatment was applied to freshly harvested fruit under mild chilling conditions.

Introduction

Most cultivars of pineapple (*Ananus comosus* Merr.) develop symptoms of internal browning (IB), a form of chilling injury during storage at <15°C or after removal to room temperature (20-25°C) following several weeks of storage at chilling temperatures (Teisson *et al*., 1979; Smith, 1983; Paull and Rohrbach, 1985; Youryon *et al*., 2011). The symptoms develop mainly in the flesh surrounding the core. Cultivars selected or bred from the Cayenne and Queen groups account for most of the world production of pineapples. 'Smooth Cayenne', the most commonly grown cultivar, has yellow, soft and juicy flesh when mature. It is used both for juicing, canning and fresh consumption. Queen cultivars such as '73-50' and 'Gold' (or 'hybrid 53-116') in Australia, 'Trad-Srithong' in Thailand, and 'Mauritius' in Malaya are popular for consumption as fresh fruit. The flesh of mature fruit is golden-yellow, crisp, sweet, and with lower acidity than 'Smooth Cayenne'. Queen cultivars are said to be tolerant to environmental stresses, pests and diseases but possibly more sensitive to IB compared to 'Smooth Cayenne' (Weerahewa and Adikaram, 2005). The market life of fresh pineapple fruit is short and application of cool storage to extend storage life is limited by the development of IB. Efforts to breed cultivars with lower susceptibility to IB led to the release of '73-50' and 'Gold' in Hawaii, USA. 'Gold' pineapple was reported to be more resistant to IB (Stewart *et al*., 2002), but more, susceptible to fruitlet rot than 'Smooth Cayenne' (Chan *et al*., 2003). In subtropical Southern Queensland, Australia, the industry has been based mainly on the production of 'Smooth Cayenne' for juicing and canning but more recently '73-50' and 'Gold' were introduced for the fresh fruit trade. Fruit of 'Smooth Cayenne' that mature during the winter months are susceptible to black heart (BH), a form of chilling injury (IB) that develops in the field.

Generally immature fruit of susceptible species develop more severe symptoms of CI. Examples

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include immature fruit of mangoes (Mohammed and Brecht, 2002) and peaches (Fernández-Trujillo *et al*., 1998). Fruit of 'Smooth Cayenne' have been classified into 4 maturity stages as judged by the amount of yellowing of the eyes (fruitlets) (Mohammed, 2004): M1 = 5-10 %, M2 = 10-35%, M3 = 35-70%, and M4 = 70-80% yellow eyes. Soares *et al*. (2005) reported that IB was more severe in half ripe (M3) fruit of 'Smooth Cayenne' grown in the Philippines than in fruit harvested at the colour break stage (M1) following cool storage. Green fruit of Cayenne and Queen types grown in Sri Lanka have been reported to be more susceptible to IB than ripe fruit (Weerahewa and Adikaram, 2005). In contrast, Zhou *et al*. (2003) reported immature and over-mature 'Smooth Cayenne' fruits developed less black heart than mature fruit. Time of year can also affect the susceptibility of pineapples to CI. You-Lin *et al*. (1997) reported that following 1 or 2 weeks cool storage, IB was more severe in fruit of 'Smooth Cayenne' and 'Yellow Mauritius' grown in China and harvested during the winter months from November to March. Fruit of '73-50' harvested during winter were reported to exhibit IB in Hawaii, USA, but showed resistance to preharvest induced symptoms of BH when grown in Queensland, Australia (Taniguchi *et al*., 2008).

Deficiencies in calcium have been implicated in the development of physiological disorders in many species of fruit including bitter pit in apples and blossom end rot in tomatoes (Ferguson, 1984; Ho and White, 2005). In addition to its major role in cross linking pectin chains in plant cell walls, calcium also plays an important regulatory role in cell metabolism such as in protein kinase signalling (Gilroy *et al*., 1987). Preharvest or postharvest treatment of several species of fruit with solutions of calcium salts has been shown to reduce storage disorders and to improve shelf-life (Conway and Sams, 1983; Poovaiah, 1986; Picchioni *et al*., 1998; Treeby and Storey, 2002; Lőtze *et al*., 2008; Mahmud *et al*., 2008). Fruit of 'Kew' (Cayenne) and 'Mauritius' (Queen) pineapples were reported to accumulate more calcium in the skin than in the core and adjacent flesh and the incidence of CI was minimal in flesh near the shell (Hewajulige *et al*., 2003). Preharvest calcium treatments of 'Mauritius' pineapple increased fruit calcium content and reduced CI in the stored fruit to commercially acceptable levels (Wijeratnam *et al*., 2006). Preharvest sprays of 'Mauritius' fruit with calcium chloride followed by wax application after harvest prevented IB following storage at 10 °C (Hewajulige *et al*., 2006) whereas postharvest application of potassium sulphate, 2-chloroethyl phosphonic acid, and calcium hydroxide followed by wax sprays and exposure to light also reduced IB in pineapple (Nanayakkara *et al*., 2005). Fruit waxing can induce high internal CO_2 and low O_2 concentrations that delay the development of IB in chilled pineapples (Rohrbach and Paull, 1982; Abdullah *et al*., 1985). Our initial efforts to increase the concentrations of Ca or Sr in whole fruit by vacuum infiltration in aqueous solutions of 0.18 M $CaCl_2$ or 0.18 M $SrCl_2$ failed but subsequently it was found that water soluble dyes and these salt solutions could be drawn into whole fruit via the peduncles by transpiration.

In this paper we compare the effects of postharvest treatments with solutions of $CaCl_2$ or $SrCl_2$ taken up via the peduncle by transpiration on the incidence of IB following cool storage of pineapple fruit. Sr an alkaline earth element with considerably higher mass than Ca was included in this research because Sr has been shown to mimic Ca in plants (Romney *et al*., 1959) and to reduce postharvest disorders in apples (Wills and Scott, 1974; Wills *et al*., 1975). Since Sr is generally present in low concentrations in plants it was expected to be easier to track changes in the distribution of added Sr in pineapple fruit compared to added Ca.

Materials and Methods

Plant materials and experimental design

Four storage experiments on pineapple fruit at mild chilling temperature were conducted. Experiment 1: fruit of cv. 'Trad-Srithong' (Queen group) and 'Smooth Cayenne' were obtained from a central market (Talaad Thai) in Pathum Thani province, Thailand, in June 2008. Fruit of 'Trad-Srithong' for experiments 2 and 3 were also obtained from the central market in July 2008. These fruit that were cultivated in Eastern Thailand (approximately 12°N, 102°E – 13°N, 100°E) were taken to the Postharvest Technology (PHT) Laboratory at King Mongkut's University of Technology Thonburi (KMUTT), Bangkok. Fruit for experiment 3 were selected at two maturity stages; mature green and green with two rows of the fruitlets turning yellow. 'Trad-Srithong' fruit for experiment 4 were harvested at the same two maturity stages as in experiment 3 at a commercial farm in Trad province (12°N, 102°E), Eastern Thailand. These fruit were transported to the PHT laboratory, KMUTT, in a refrigerated truck at 25°C within 4 h after harvest. The crowns were partly removed according to local practice and 2 cm of the peduncles were retained. The fruit were infiltrated by placing the peduncles in water, 0.18 M $CaCl_2$ or 0.18 M $SrCl_2$ for 3 days at the respective storage

temperatures of 8, 13, and 20°C (80-90% RH). The fruit were stored for 14 days at these temperatures, then transferred to 20°C for 3 days and assessed for IB. All experimental treatments comprised 4-10 fruit (replicates).

Assessment of internal browning

Stored fruit were cut longitudinally and the severity of IB was scored subjectively according to the estimated area of flesh with browning: 0 (no browning), 1 (<10 %), 2 (10-25 %), 3 (25-50 %), 4 (50-75%), and 5 (> 75 %) (Teisson *et al*., 1979).

Analyses of calcium and strontium

Ca and Sr concentrations were analysed following wet digestion with 65% high purity nitric acid (Merck, containing 0.00001% Ca). Samples (1.5 g) from each tissue region were placed in Kjeldah flasks (100 mL) and 15 mL of 65% nitric acid were added. The samples were digested on a digestion block Selecta (Selecta S.A., Barcelona, Spain) for 45 min. After cooling to room temperature, 5 mL of 70-72% of perchloric acid (Univar, containing 0.00005% Ca) was added to each flask and the solutions were heated until clear (AOAC, 2000). The reaction mixtures were cooled and filtered with ashless paper (Whatman # 41 filter paper). The concentrations of the minerals were measured with an inductively coupled plasma atomic emission spectrometer (ICP) (Optima 3000, Perkin Elmer). Ca and Sr were detected at the wavelength of 315.9, 460.7 and 766.5 nm, respectively.

Measurement of uptake of calcium and strontium salt solutions

The amount of water and solutions drawn into each fruit by transpiration via the peduncles was estimated by comparison of the changes in weight of undipped control fruit and fruit infiltrated with water or mineral salt solutions after 3 days at each storage temperature.

Measurement of total soluble solids

Fruit were cut longitudinally and juice from 20 g of tissue from the middle of the core and the flesh (pulp) was recovered with stainless steel hand juicer. Samples of juice were filtered through Whatman # 1 filter paper and used to measure total soluble solids (TSS) with a hand refractometre (PAL-1, Japan) standardized at 25°C.

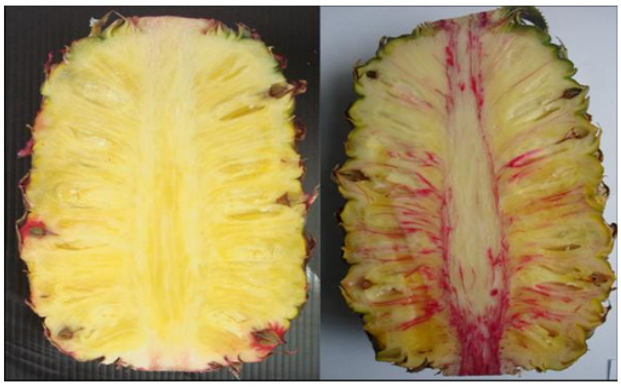
Statistical analysis

A completely randomised design (CRD) was used with at least 4 replications (one fruit per replicate) per treatment. Data were subjected to ANOVA, and the means were compared by least significant differences (LSD) using SPSS software (SPSS version 17.0 for Windows, SPSS Inc., Chicago, IL, USA).

Results

A pilot experiment showed that good uptake and distribution of the water soluble carmoisine dye throughout the core and fruitlets could be achieved by transpiration via the peduncle for 3 days at 25°C (Figure 1) but not by vacuum infiltration at 30 kPa. In the first experiment the fruit were stored at 8, 13 and 20 °C for 14 days. IB was low in 'Smooth Cayenne' fruit (14.2% TSS) and infiltration with water or mineral salts had no effect but IB was severe in fruit of 'Trad-Srithong' (13.9% TSS) and treatment with Ca or Sr gave some reduction in the disorder, especially in fruit stored at 20°C (Table 1). The incidence of IB was high in fruit of 'Trad-Srithong' stored at all temperatures implying that the disorder was initiated preharvest or that IB was also caused by senescence in these particular fruit. Mineral analyses (Table 2) showed no clear increases in Ca concentrations in core and pulp tissue in fruit infiltrated with $CaCl_2$ in either cultivar. Pulp and pulp near skin (s-pulp) tissues contained higher amounts of Ca than the core. However, fruit treated with $CaCl_2$ tended to have higher Ca concentrations in the core. In comparison, Sr concentrations were generally higher in fruit of 'Trad-Srithong' infiltrated with $SrCl_2$, especially in the core tissue (Table 3).

In experiment 2, treatment with both Ca and Sr significantly reduced IB in 'Trad-Srithong' fruit stored at 13°C but no IB was observed in fruit stored at 20°C (Table 1). No mineral analyses were done on these fruit. In experiment 3, fruit of 'Trad-Srithong' were obtained from the central market in July 2008



A B

Figure 1. Distribution of water soluble carmoisine dye 5.66 g/L in 'Trad-Srithong' fruit following vacuum infiltration at 30 kPa for 2 min (A) or infiltration by transpiration via the peduncle for 3 days at 25°C (B). In A, the crown was removed before vacuum infiltration but was removed after infiltration in B

Table 1. Incidence of internal browning in fruit of 'Smooth Cayenne' (SC) and 'Trad-Srithong' (TS) infiltrated with 0.18 M calcium chloride or 0.18 M strontium chloride by transpiration via the peduncle following cool storage for 14 days plus 3 days at 20°C

Treatment	Internal browning (scores) [1]					
	Market	Market	Market		Farm	
	June 2008 Experiment 1	July 2008 Experiment 2	July 2008 Experiment 3		November 2008 Experiment 4	
			green	¼ ripe	green	¼ ripe
SC + 8 °C	e 0.3	-	-	-	-	-
SC + H_2O, 8 °C	e 0.3	-	-	-	-	-
SC + $CaCl_2$, 8 °C	e 0.8	-	-	-	-	-
SC + $SrCl_2$, 8 °C	e 0.8	-	-	-	-	-
SC + 13 °C	e 0.1	-	-	-	-	-
SC + H_2O, 13 °C	e 0.8	-	-	-	-	-
SC + $CaCl_2$, 13 °C	e 0.1	-	-	-	-	-
SC + $SrCl_2$, 13 °C	e 0.1	-	-	-	-	-
SC + 20 °C	e 0.6	-	-	-	-	-
SC + H_2O, 20 °C	e 0.5	-	-	-	-	-
SC + $CaCl_2$, 20 °C	e 0.6	-	-	-	-	-
SC + $SrCl_2$, 20 °C	e 0.3	-	-	-	-	-
TS + 8 °C	a 4.8	-	a 4.9	a 4.8	-	-
TS + H_2O, 8 °C	ab 4.5	-	a 4.9	ab 4.5	-	-
TS + $CaCl_2$, 8 °C	abc 4.3	-	bcd 3.9	a 4.6	-	-
TS + $SrCl_2$, 8 °C	abc 4.3	-	abc 4.4	ab 4.3	-	-
TS + 13 °C	a 4.8	a 4.7	ab 4.6	ab 4.5	b 2.3	a 3.3
TS + H_2O, 13 °C	ab 4.5	a 4.8	ab 4.6	bc 3.4	a 4.7	a 3.2
TS + $CaCl_2$, 13 °C	abc 4.2	b 1.1	e 2.5	d 2.0	c 0.0	c 0.0
TS + $SrCl_2$, 13 °C	bcd 3.7	b 1.7	e 2.4	cd 2.5	c 0.9	b 0.9
TS + 20 °C	a 4.9	-	cd 3.5	bc 3.3	-	-
TS + H_2O, 20 °C	abc 4.3	-	ed 3.1	c 3.1	-	-
TS + $CaCl_2$, 20 °C	d 3.2	-	f 0.7	e 0.7	-	-
TS + $SrCl_2$, 20 °C	cd 3.6	-	f 1.4	de 1.4	-	-
F-test	**	**	**	**	**	**

	Market (June2008)		Market (July 2008)	Farm (November 2008)
Cultivar (V)			-	-
SC	0.3		-	-
TS	4.2		-	-
F-test	**		-	-
Maturity stage (M)	-			
green	-		3.4	1.9
¼ ripe	-		3.2	1.8
F-test	-		ns	ns
Temperature (T)				
8ºC	2.4		a 4.5	-
13ºC	2.8		b 3.3	-
20ºC	2.2		c 2.1	-
F-test	ns		**	-
Chemical (C)				
untreated	a 2.5		a 4.2	b 2.8
H_2O	a 2.4		a 3.9	a 3.9
$CaCl_2$	b 2.1		b 2.4	d 0.0
$SrCl_2$	b 2.1		b 2.7	c 0.9
F-test	*		**	**
V*T	ns	M*T	ns	-
V *C	**	M*C	ns	**
T *C	ns	T*C	**	-
V*T* C	ns	M*T*C	ns	-

Fruit for the first three experiments were sourced from a central market in Bangkok boundary, fruit for the fourth experiment were harvested at a commercial farm in Trad province, Eastern Thailand.[1] Means (n=10) with different lower case letters within the same column are significantly different.
ns: non significant, * Significant difference at P≤0.05, ** Significant difference at P≤0.01

Table 2. Experiment 1, calcium content in 'Smooth Cayenne' and 'Trad-Srithong' fruit infiltrated with 0.18 M $CaCl_2$ or 0.18 M $SrCl_2$ and stored at 8, 13 and 20°C for 14 days plus 3 days at 20°C

Treatment	Calcium content (mg/kg) [1]					
	Smooth			Trad-Srithong		
	Fruit part			Fruit part		
	core	pulp	s-pulp	core	pulp	s-pulp
untreated, 8 °C	179.5	300.0	358.4	b 197.1	201.7	abc 246.0
H_2O, 8 °C	212.4	267.7	282.8	b 189.1	192.6	cd 215.1
$CaCl_2$, 8 °C	224.4	225.5	327.3	b 195.5	197.3	cd 214.3
$SrCl_2$, 8 °C	203.0	317.2	333.2	b 153.0	152.0	d 177.6
untreated, 13 °C	176.0	163.7	263.2	b 147.1	143.7	cd 190.6
H_2O, 13 °C	201.8	194.9	252.9	b 146.6	165.6	abcd 229.5
$CaCl_2$, 13 °C	297.7	168.8	269.2	a 304.8	305.0	a 294.3
$SrCl_2$, 13 °C	159.4	263.5	317.9	b 162.8	182.3	bcd 223.0
untreated, 20 °C	156.7	200.3	259.0	b 170.1	225.1	abcd 231.1
H_2O, 20 °C	161.0	238.1	357.8	b 174.2	199.1	abc 244.1
$CaCl_2$, 20 °C	220.1	210.6	277.2	b 221.3	210.0	ab 281.5
$SrCl_2$, 20 °C	155.9	308.6	325.4	b 181.3	209.6	abc 245.5
F-test	ns	ns	ns	*	ns	*

			Smooth	Trad-Srithong
Cultivar (V)				
Smooth	245.9			
Trad-Srithong	206.2			
F-test	**			
Temperature (T)				
8 °C	232.5		a 269.3	194.0
13 °C	218.4		b 227.3	209.3
20 °C	229.3		ab 241.0	216.1
F-test	ns		*	ns
Chemical (C)				
untreated	b 213.0		228.5	b 196.0
H_2O	b 218.2		241.0	b 192.6
$CaCl_2$	a 247.8		246.6	a 248.9
$SrCl_2$	ab 227.2		268.0	b 187.4
F-test	*		ns	**
Fruit part (F)				
core	c 191.4		c 196.4	b 186.2
pulp	b 219.7		b 238.2	b 199.6
s-pulp	a 267.9		a 302.0	a 232.2
F-test	**	F-test	**	**
V*T	*	-	-	-
V*C	*	-	-	-
V*F	*	-	-	-
T*C	*	T*C	ns	**
T*F	ns	T*F	ns	ns
C*F	ns	C*F	*	ns
V*T*C*F	ns	T*C*F	ns	ns

[1]Means (n=4) with different lower case letters within the same column are significantly different.
ns: non significant, * Significant difference at P≤0.05, ** Significant difference at P≤0.01

at green (13.05% TSS) and quarter ripe maturities (14.04% TSS) stages, infiltrated with water, $CaCl_2$ or $SrCl_2$ and stored at 8, 13 or 20°C. IB developed in fruit stored at all temperatures but was worse in fruit stored at 8 °C. Infiltration with the mineral salts reduced IB in fruit of both maturities No mineral analyses were done on these fruit.

Experiment 4 was conducted on freshly harvested fruit of 'Trad-Srithong' in November 2008. IB developed in fruits stored at 13°C but not at 20°C. IB was severe in control fruit, absent in fruit treated with Ca and low in fruit treated with Sr (Table 3). Tables 4 and 5 show that infiltration by transpiration increased the concentrations of Ca and Sr respectively in all tissue regions with the largest increases in the core tissue. Regression analysis of these data showed that the severity of IB in 'Trad-Srithong' fruit was negatively correlated to the concentrations of Ca (r^2 = 0.7882) or Sr in the flesh (r^2 = 0.9145). Experiments 2 and 4 showed that harvesting at a green and a more mature stage based on skin colour had no significant effect on the incidence of IB (Table 1).

Discussion

Our date confirmed previous research in Thailand (Nukulthornprakit and Siripanich, 2005; Weerahewa and Adikaram, 2005), which showed that fruit of

Table 3. Experiment 1, strontium content in 'Smooth Cayenne' and 'Trad-Srithong' fruit infiltrated with 0.18 M $CaCl_2$ or 0.18 M $SrCl_2$ and stored at 8, 13 and 20°C for 14 days plus 3 days at 20°C

Treatment	Strontium content (mg/kg) [1]					
	Smooth			Trad-Srithong		
	Fruit part			Fruit part		
	core	pulp	s-pulp	core	pulp	s-pulp
untreated, 8 °C	[bcd] 84.5	[cd] 84.7	[de] 87.0	[c] 62.3	[d] 63.8	[abc] 63.6
H_2O, 8 °C	[bc] 93.6	[abc] 95.0	[abcd] 96.5	[cd] 54.1	[de] 54.8	[bcd] 55.8
$CaCl_2$, 8 °C	[b] 102.3	[ab] 102.7	[abc] 104.1	[e] 17.0	[g] 19.5	[f] 19.8
$SrCl_2$, 8 °C	[a] 127.8	[a] 109.5	[ab] 107.5	[a] 211.3	[a] 157.5	[a] 78.6
untreated, 13 °C	[f] 48.4	[f] 50.8	[g] 52.4	[de] 33.5	[edfg] 34.0	[edf] 36.3
H_2O, 13 °C	[de] 66.7	[de] 68.5	[f] 72.2	[cd] 42.6	[edf] 43.3	[ef] 32.8
$CaCl_2$, 13 °C	[cd] 79.2	[cd] 81.1	[ef] 81.1	[b] 49.5	[d] 55.8	[bcd] 54.6
$SrCl_2$, 13 °C	[bc] 89.3	[bc] 86.6	[de] 87.8	[de] 134.1	[b] 116.0	[ab] 71.6
untreated, 20 °C	[bc] 88.4	[abc] 95.9	[a] 109.6	[d] 33.8	[fg] 31.6	[ef] 32.6
H_2O, 20 °C	[bc] 90.0	[bc] 89.5	[cde] 90.8	[cd] 41.3	[edf] 42.6	[de] 41.5
$CaCl_2$, 20 °C	[bc] 91.9	[bc] 92.7	[bcde] 93.9	[b] 50.8	[edf] 49.0	[cde] 46.3
$SrCl_2$, 20 °C	[ef] 57.1	[ef] 60.1	[g] 44.8	122.3	[c] 90.8	[a] 75.6
F-test	**	**	**	**	**	**
Cultivar						
Smooth	85.1					
Trad-Srithong	61.7					
F-test	**					
Temperature					Smooth	Trad- Srithong
8 °C	[a] 85.5				[a] 99.6	[a] 71.5
13 °C	[c] 65.3				[c] 72.0	[b] 58.7
20 °C	[b] 69.3				[b] 83.7	[b] 54.8
F-test	**				**	**
Treatment						
untreated	[c] 60.7				[c] 78.0	[b] 43.5
H_2O	[b] 65.1				[b] 84.8	[b] 45.4
$CaCl_2$	[b] 66.2				[a] 92.1	[b] 40.2
$SrCl_2$	[a] 101.6				[b] 85.6	[a] 117.5
F-test	**				**	**
Fruit part						
core	[a] 78.0				84.9	[a] 71.0
pulp	[b] 74.0				84.8	[b] 63.2
s-pulp	[c] 68.2				85.6	[c] 50.8
F-test	**	F-test			ns	**
V*T	**	-			-	-
V*C	**	-			-	-
V*F	**	-			-	-
T*C	**	T*C			**	**
T*F	ns	T*F			ns	ns
C*F	**	C*F			ns	**
V*T*C*F	**	T*C*F			ns	**

[1] Means (n=4) with different lower case letters within the same column are significantly different.
ns: non significant, * Significant difference at P≤0.05, ** Significant difference at P≤0.01

'Trad-Srithong', a member of the Queen group of pineapples are more susceptible to IB than 'Smooth Cayenne' (Table 1). Endogenous Ca concentrations were generally much higher in 'Trad-Srithong' fruit in experiment 1 than in experiment 4 (Tables 2 and 4), implying that there is there is not a strong association between endogenous Ca concentrations and susceptibility to IB. However, this research showed that concentrations of both Ca (Tables 2 and 4) and Sr (Table 3 and 5) could be increased in the core and adjacent flesh where IB develops by infiltrating the fruit with the salts via the peduncle by transpiration and treatment with both salts reduced IB. The increases in Sr were generally easier to measure because the endogenous levels of this mineral are much lower than for Ca. Seasonal conditions may

Table 4. Experiment 4, calcium content in 'Trad-Srithong' fruit infiltrated with 0.18 M $CaCl_2$ or 0.18 M $SrCl_2$ and stored at 13°C for 14 days plus days at 20°C

Maturity	Treatment	Calcium content (mg/kg) [1,2]			F-test
		Fruit part			
		core	pulp	s-pulp	
green	untreated	[d]	[c] 97.3 B	[bcd]	**
	H_2O	[cd] 80.5 B	[d] 61.4 B	[b]	**
	$CaCl_2$	[a]	[bc] 124.3 B	[a]	**
	$SrCl_2$	[c] 85.9 B	[bc] 113.2 B	[a]	**
¼ ripe	untreated	[b] 135.3	[a] 132.8	[bc] 146.7	ns
	H_2O	[cd] 65.2 B	[d] 65.1 B	[cd]	**
	$CaCl_2$	[a] 222.4	[a] 165.4	[a] 193.3	ns
	$SrCl_2$	[cd] 52.1 B	[d] 65.0 B	[cd] 115.3 A	**
F-test		**	**	**	
Maturity	Treatment	Fruit part			
green	-	-	128.7		
¼ ripe	-	-	122.0		
F-test			ns		
	untreated	-	[b] 115.9		
	H_2O	-	[c] 88.5		
	$CaCl_2$	-	[a] 191.5		
	$SrCl_2$	-	[b] 105.4		
F-test			**		
	-	core	[b] 115.8		
	-	pulp	[c] 103.0		
	-	s-pulp	[a] 157.2		
F-test			**		
Maturity*Treatment			**		
Maturity*Fruit part			**		
Treatment*Fruit part			**		
Maturity*Treatment*Fruit part			ns		

[1] Means (n=5) with different lower case letters within the same column are significantly different.
[2] Means with different capital letters within the same row are significantly different.
ns: non significant, * Significant difference at P≤0.05, ** Significant difference at P≤0.01

cause differences in concentrations of Ca or other elements especially in fruit harvested in November that developed during the rainy season. Variations in the availability of Ca in soils in different growing locations and the timing of the application of mineral fertilizers could also affect Ca concentrations in the fruit.

Treatment of the fruit of 'Trad-Srithong' with Ca or Sr had no effect on IB in fruit harvested in June 2008 (experiment 1), a small effect on fruit harvested in June (experiment 3) but significantly reduced IB in fruit harvested earlier in July (experiment 2) and in November 2008 (experiment 4) (Table 1). The reduction in IB was most marked in fruit treated with Ca or Sr in experiments 2 and 4. We think a key factor influencing the effectiveness of treatments with Ca or Sr is the time from harvest. Fruit for experiment 4 were harvested in November 2008 and treatment of these fruit with salt solutions began on the day of harvest. There were significant increases in Ca (Table 4) and Sr (Table 5) in treated fruit and these increases were negatively correlated with the severity of IB. It is noteworthy that IB was observed in fruit of 'Trad-Srithong' stored at 20°C in experiments 1 and 3 but not in experiments 2 and 4. We think that these symptoms of IB were an expression of senescence not chilling injury. There is no record in Thailand of the occurrence of Black Heart that is a form of field

Table 5. Experiment 4, strontium content in 'Trad-Srithong' pineapple fruit infiltrated with 0.18 M $CaCl_2$ or 0.18 M $SrCl_2$ and stored at 13°C for 14 days plus days at 20°C

Maturity	Treatment	Strontium content (mg/kg) [1,2] Fruit part			F-test
		core	pulp	s-pulp	
green	untreated	[e]	[ed] 45.1 B	[c]	**
	H_2O	[ed] 53.0	[cd] 64.6	[c] 58.9	ns
	$CaCl_2$	[d] 56.9 B	[cd]	[c]	**
	$SrCl_2$	[a]	[a] 195.1 B	[a] 168.7 B	**
¼ ripe	untreated	[c] 73.3	[c] 71.5	[bc] 72.2	ns
	H_2O	[f]	[d] 29.1 B	[c]	**
	$CaCl_2$	[de] 49.6	[c] 57.2	[c] 60.5	ns
	$SrCl_2$	[b]	[b] 100.9 B	[b] 96.4 B	**
F-test		**	**	**	

Maturity	Treatment	Fruit part	
green	-	-	105.5
¼ ripe	-	-	74.7
F-test			**
	untreated	-	[b] 58.6
	H_2O	-	[c] 46.2
	$CaCl_2$	-	[b] 57.6
	$SrCl_2$	-	[a] 194.1
F-test			**
-	-	core	[a] 112.6
-	-	pulp	[b] 78.6
-	-	s-pulp	[b] 77.8
F-test			**
Maturity*Treatment			**
Maturity*Fruit part			**
Treatment*Fruit part			**
Maturity*Treatment*Fruit part			**

[1] Means (n=5) with different lower case letters within the same column are significantly different.
[2] Means with different capital letters within the same row are significantly different.
ns: non significant, * Significant difference at P≤0.05, ** Significant difference at P≤0.01

chilling injury seen in Smooth Cayenne in winter grown fruit in Australia. We had no information on the delay between harvest and purchase of the fruit in the central market.

It is proposed that further experiments are conducted where the fruit are infiltrated with 0.18 M $CaCl_2$ (hypotonic solution) immediately after harvest before placing the fruit at chilling temperatures. The results reported in this paper suggest that IB can be controlled by infiltrating pineapple fruit immediately after harvest with a $CaCl_2$ solution at a rate of 0.01-0.02% of fresh weight (FW). This rate was estimated from measurements of the changes in weight of control and infiltrated fruit during the first three days of storage at each storage temperature. In future work the fruit should be infiltrated with salt solutions at ambient temperatures at 20-25°C and low RH before cool storage to ensure consistent uptake of salt solutions. Uptake of Ca was much greater in experiment 4 than in experiment 1.

An intriguing question is the intracellular location of infiltrated Ca and Sr especially in light of the large variations in endogenous concentrations among different batches of fruit; Ca and Sr concentrations were much higher in fruit used in experiment 1 (Table 2) compared to the fruit used in experiment 4 (Table 4). Staining with carmoisine dye showed that aqueous solutions infiltrated via the peduncle are distributed via the vascular system, first through the core and then to the fruitlets (Figure 1). The network of vasculars serving the fruitlets is located mainly on the periphery of these structures until the vasculars reach the ovarian tissue (the eyes). Clearly, Ca and Sr ions diffuse readily into the parenchyma that comprises the fleshy pulp tissue where the symptoms of IB first appear. It is proposed that the distribution and intracellular location of these ions are examined by ICP spectrometry on a micro scale (Hansen *et al*., 2009) or by laser ablation technology (*http://eetd.lbl.gov/l2m2/laser.html*) to help understand the mechanism of action of Ca and Sr. A clearer picture should be obtained using Sr instead of Ca since this element is naturally lower in pineapple fruit.

Conclusions

Infiltrating freshly harvested pineapple fruit with a $CaCl_2$ or $SrCl_2$ solutions immediately after harvest effectively prevented IB in fruit stored for 14 days at mild temperatures of 13 and 20°C. An increase in Ca was achieved in all tissues by placing the peduncles in 0.18 M solutions $CaCl_2$ or $SrCl_2$ under conditions that promote uptake of solution by transpiration.

Acknowledgements

This research was supported by the award of a pre-doctoral fellowship to Miss Pannipa Youryon by the Commission on Higher Education Programme, Thailand. We thank the Postharvest Technology Innovation Centre, Commission on Higher Education, Bangkok 10400, Thailand for providing laboratory facilities and the Centre for Plants and the Environment, University of Western Sydney, Hawkesbury Campus for training Miss Youryon in analytical methods.

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