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Abstract

This study investigated chilling injury (CI) effects from heat treatment and precooling on the shelf life potential of mango cultivated variety cv. Nam Dok Mai. The first phase involved heat treatment in hot-air ovens of stored mango pulp at both 34° Celsius and 38° Celsius for three separate periods of 24, 48, and 72 hours. The second phase of testing involved the storage of the mango at 5° Celsius for a period of 10, 20, and 30 days to determine the appropriate shelf life. The findings showed that chilling injury (CI) appeared within 30 days of storage at 5° Celsius which included pitting, skin browning, water soaking, and rapid rotting of the fruit that resulted in shorter shelf life. Findings also indicated that chilling injury symptoms from mangos included a lower level of total soluble solid (TSS), higher disease rates, and lower fruit quality when compared with normally ripened mango fruit at 25° Celsius. These results however were improved over non-heat treated fruits when the mangoes were heat treated at 34° Celsius for either 24 or 48 hours or at 38° Celsius for 24 hours prior to cold storage for 10 or 20 days at 5° Celsius. Furthermore, heat treatment did not affect fruit weight loss, firmness, color changes, or water soaking during the storage period at 25° Celsius.

Keywords: chilling injury, heat treatment, shelf life, *Mangifera indica* L., Nam Dok Mai

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Introduction

Globally, there are 160 varieties of mangoes that are cultivated in more than 90 countries and in 2013, if all categories of mangoes are considered, 43.3 million tons were harvested (World Mango Market, 2015). Mango ranks third among the tropical fruits grown in the world with the mango (*Mangifera indica* L.) fruit helping make Thailand the third largest exporter in the world. In 2011 Thailand had an export harvest of 63,827 metric tons representing a value of \$US 57 million dollars (Thailand Foreign Agricultural Trade Statistics, 2011). As this was a significant increase from previous years, one of the main problems became an oversupply during the harvest season which subsequently led to the oversupply being refrigerated. From this came the need for greater awareness of chilling injury (CI) and how long and under what conditions this fruit could be stored.

Pre-cooling effects on CI

According to the Food and Agricultural Organization (FAO) of the United Nations, precooling is 'amongst the most efficient quality enhancements available' and is regarded 'as one of the most value-adding activities in the horticultural chain' (Borompichaichartkul, Kanlayanarat, Rolle and Acedo Jr., 2009). Previous studies have already shown the importance of temperature on shelf life storage. Wang et al. (2008) indicated that mango fruit shelf life CI can be significantly alleviated along with disease incidence after 7 days of storage at 4 °C, followed by an additional 14 days at 20 °C. This is consistent with Gonzakez-Aguilar et al. (2008) which showed that mango fruit kept at 5°C showed considerably less symptoms of water soaking compared to storage at a lesser temperature of 2°C.

Industry research has already determined that the use of low temperatures prior to storage reduces the effects of CI on mango fruit with most harvesters, warehouses, and retail merchants making efforts to preserve their commodities and prolong shelf life by use of lower temperatures (Gonzakez-Aguilaret et al., 2008; Ghasemnezhad et al., 2008; Dea et al., 2010)

Heat treatment effects on CI

Dea, Brecht, Nunes, and Baldwin (2010) stated that it is known that heat treatment has a positive effect on fruit quality such as extending storability and marketing by inhibiting ripening processes or inducing resistance to chilling injury (CI). Ghasemnezhad, Marsh, Shilton, Babbalar, and Woolf (2008) also confirmed this indicating that CI was reduced and durability was increased by using hot air ovens prior to storage with temperatures ranging from 30 - 40 °C before storage.

CI can also be reduced by storing mango fruit at 38 $^{\circ}$ C with a 90 percent relative humidity for 24 and 48 hours. This is followed by a storage period of 11 days at 5 $^{\circ}$ C which is followed by storage at 21 $^{\circ}$ C (Tasneem, 2004).

According to Field (1984), ethylene is involved in many aspects of fruit ripening and its production is inhibited by high temperatures. Tasneem (2004) confirmed this when mango fruits cv. Nam Dokmai were kept at 38 °C for 3 days and then transferred to 20 °C. According to Wang (2010) heat treatment induces heat shock proteins (HSP), suppresses oxidative activity, and maintains membrane stability. HSP are a family of proteins that are produced by cells in response to exposure to stressful conditions. This is consistent with Luengwilai and Beckle (2010) which discovered that tomatoes which underwent heat treatment at 38°C for 3 days before storage showed a reduction of CI symptoms. Also, tomatoes treated with heat treatment had increasing HSP and became endurable to CI.

The objective of this study therefore was to investigate the effects of heat treatment and pre-cooling on shelf life CI of mango cv. (cultivated variety) Nam Dok Mai under various conditions.

Material and methods

Mango fruit test sample preparation

For the study the researchers used mango fruit that weighed 300-350 grams and were 115 days old from their initial flowering. Samples were collected from the district of Bangkla located in Chachoengsao Province just east of Bangkok in Thailand. All of the selected samples were of the same maturity and were initially disease-free and exhibited no skin wounds selected by using a floating technique in 3% salt solution but downward sunk in 1% salt solutions. The mango fruits were subsequently cleaned with water and air dried in ambient temperature.

Experiments were designed so that there were 0, 10, 20, and 30 days storage times at 5°C. Effects of chilling injury (CI) from shelf storage were determined on days 0, 3, 6, and 9 after the start of the storage experiment.

Heat effects experimentation at both 34°C and 38°C at 5°C was also carried out to determine mango fruit quality by varying the time increments to 24, 48, and 72 hours.

The third experiment used pre-cooling at 5°C for 10, 20, and 30 days to determine the quality of mango fruit against various conditions of shelf life.

The lay out was planned by Completely Randomized Design (CRD) (Jayaraman, 1999). There were three components to the experiment which have been labeled 'Factors'. They consisted of the following:

Factor 1 contained two level of heat treatment at 34°C and 38°C (Table 3).

Factor 2 also used three levels of heat treatment but was measured in time units of 24, 48, and 72 hours (Table 3).

Factor 3 used three levels of storage time at 5°C which consisted of 10, 20 and 30 days (Table 3).

Each testing period had 12 mango fruits packed in 20x30 inch plastic bags with some small pores for aeration.

Data collection

All data were records according to the protocols that listed in the previously mentioned experiments, and used for the calculation, where % (percentage) of weight loss was equivalent to 100 times of the difference between the weight detected before and after storage and then divided by the weight detected before storage. Mango fruit firmness was determined by fruit firmness testing equipment with 0.65 centimeter in diameter and 0.7 centimeter on slant high.

The value of Total Solid Solution (TSS) was carried out using the liquid solution that was derived from squeezing the mango pulp into distillated water. A refractometer measures TSS as °Brix in 0.1% graduations (OECD, 2009). The degree of disease symptom of mango fruits was rated depending on the severity of the appearance, i.e., 1 indicated no disease symptoms, 2 represented 40 millimeters or less of discoloration, 3 indicated 40-60 millimeters of discoloration, and 4 was greater than 60 millimeters.

Results and discussion

Research results from the heating and refrigeration of mango fruit on shelf life and the related chilling injury (CI) of Nam Dok Mai mango cv. are summarized in Table 1. Results also indicated that the CI effect on shelf life was not significant when refrigerated at 5°C for 0, 10 and 20 days but when stored for a longer period of 30 days; there was a significant difference as shown in Figure 1. Results from chilling at 5°C on mango shelf life showed that the shelf life could last for 20 days. This agrees with the results of other researchers including Kasim and Kasim (2011), Dea et al. (2010) and Wang (2010).

Storage time at 5°C	Mango shelf life chilling injury (CI) score			I) score
(days)	0 day	3 days	6 days	9 days
0 day	1.00 ^b	1.00 ^b	1.19°	1.28°
10 days	1.00 ^b	1.28 ^b	1.95 ^b	2.20 ^b
20 days	1.00 ^b	1.45 ^b	3.53ª	3.95ª
30 days	1.70ª	3.36ª	-	-
LSD0.05	0.41*	0.50*	0.40*	1.01^{*}
%CV	15.21	15.35	12.12	14.65

Table 1 Results on shelf life after storing the test sample at 0, 3, 6, and 9 days at 5°C.

Note. * represents a statistical significance level of 0.05. Numbers followed by a letter in the column represent the statistical significance of the mean comparison according to Least Square Difference (LSD) ($p \le 0.05$). Chilling injury scores: 1=no chilling injury, 2= chilling injury symptom 1-40 centimeter², 3= chilling injury symptom 40-60 centimeter², 4= chilling injury symptom 60 centimeter².



Figure 1 Effects of refrigeration on mango fruit after cooling at 5°C for 30 days.

Table 2 shows the effects on weight loss and fruit firmness from pre-heating and precooling treatment of mango fruit. Results indicated that heat treatment at both 34°C and 38°C gave better results. This is consistent with Nair and Singh (2003), where it was concluded that the application of heat treatment to plant material was a means of controlling pests which provided a non-chemical method of control.

The effect of heat treatment can reduce the rate of ripening as well, while any damage by heat may be immediately or develop after a period of storage. From the research, mangoes stored at 38°C showed a significant difference to mangoes stored at 34°C in both weight lost and fruit firmness.

In weight loss, the lowest score was 14.68 % when the test sample was kept at 5°C for 20 hours. The highest score for fruit firmness was 1.15 kg/cm² after the test sample was kept at 5°C for 20 hours. This agrees with other research from Tasneem (2004) and Dea et al. (2010).

Temp. (°C)	Heat treatment time (hours.)	Pre-cooling time 5 °C time (days)	Weight loss (%)	Fruit firmness (kg/cm ²)
(0)	time (nours.)			
	24	10	18.02°	0.47°
	24	20	18.97°	0.49°
		30	26.75ª	0.36°
24	40	10	16.34 ^d	0.66 ^b
34	48	20	18.44°	0.56
		30	24.55ª	0.41°
		10	15.77 ^d	0.52 ^b
	72	20	14.68 ^d	1.15ª
		30	25.58ª	0.39°
		10	20.19°	0.44°
	24	20	17.21 ^d	0.45°
		30	27.34ª	0.41°
		10	18.54°	0.65 ^b
38	48	20	16.21 ^d	0.56 ^b
		30	26.15ª	0.36°
		10	14.95 ^d	0.74 ^b
	72	20	14.85 ^d	0.36°
		30	22.87 ^b	0.54 ^b
		10	15.85 ^d	0.41°
,	control	20	17.77°	0.60 ^b
(no	pre-heating)	30	19.31°	0.32°
	control (25°C or normal T.	= check)	9.66ª	1.02ª
	LSD ₀ .		3.05*	0.23*
	%CV		10.25	15.94

Table 2 Results of weight lost and fruit firmness at both 34° C and 38° C as well as refrigeration storage effects for 10, 20, and 30 days at 5°C after which, the samples were stored at 25 ° C for 6 days.

Note. * represents a statistical significance level of 0.05. Numbers followed by a letter in the column represent the statistical significance of the mean comparison according to Least Square Difference (LSD) ($p \le 0.05$).

Table 3 reveals the results of mango heating at 34°C and 38°C and mango cooling at 5°C for 10, 20 and 30 days. Table 3 also indicated temperature as Factor 1, heating time in hours as Factor 2, and cooling time at 5°C time in days as Factor 3. Factor 1's contribution to mango fruit weight loss and fruit firmness was insignificant. Factor 2's contribution to weight loss and fruit firmness however was significant. For Factor 3, storage also played a significant role on weight loss and fruit firmness.

Temperature in °Celsius	Weight loss	Fruit firmness
(factor 1)	(%)	(kg/cm ²)
34	19.90	0.56
38	19.81	0.50
LSD _{0.05}	ns	ns
Heat treatment time in hours	Weight loss	Fruit firmness
(factor 2)	(%)	(kg/cm ²)
24	21.41ª	0.44°
48	20.04 ^b	0.53 ^b
72	18.12°	0.61ª
LSD0.05	1.05*	0.98*
Pre-cooling at 5°C in days	Weight loss	Fruit firmness
(factor 3)	(%)	(kg/cm ²)
10	17.30 ^b	0.58ª
20	16.73 ^b	0.59ª
30	25.54ª	0.41 ^b
LSD 0.05	1.12*	0.08*
factor 1	ns	ns
factor 2	*	*
factor 3	*	*
1x2	ns	ns
1x3	ns	*
2x3	ns	ns
1x2x3	ns	*
%CV	9.83	17.21

Table 3 The results of mango heating at 34°C and 38°C and mango cooling at 5°C for 10, 20 and 30 days.

Table 4 and Table 5 show the effects of heat treatment and pre-cooling on mango TSS and disease symptoms of the test sample of Nam Dok Mai mango fruit. It was shown that the time that was used for heat treatment at 34°C and 38°C was not significantly different in the values and scores for TSS samples and disease symptoms. However, as time increased, the values changed significantly (Luengwilai and Beckle, 2010). Finally, the pre-cooling treatment at 5°C for 10, 20 and 30 days showed a significant difference among them.

Note. * represents a statistical significance level of 0.05. ng = no statistically significant level 0.05. Numbers followed by a letter in the column represent the statistical significance of the mean comparison according to Least Square Difference (LSD) ($p \le 0.05$).

Temperature in °Celsius	Heat treatment time (hours.)	Pre-cooling time 5°C time (days)	TSS (brix)	Disease symptom (scores)
		10	18.39ª	2.24 ^b
	24	20	19.76ª	2.36 ^b
		30	17.61 ^b	2.86 ^b
		10	17.01°	2.24 ^b
34	40	20	17.76 ^b	2.74 ^b
	48	30	16.71°	3.24ª
	70	10	15.36 ^d	1.86°
		20	14.31°	2.61 ^b
	72	30	15.01°	3.89ª
		10	18.56ª	1.99°
	24	20	18.51ª	2.24 ^b
	24	30	17.96 ^b	2.74 ^b
38 4		10	17.51 ^b	1.99°
	48	20	18.91ª	2.86 ^b
		30	16.21°	2.99 ^b
		10	15.81 ^d	1.99°
72	72	20	16.01 ^d	2.86 ^b
		30	14.56°	3.49ª
contro	l (25 °C or normal	T.=check)	17.71b	1.00°
	LSD 0.05	,	1.64*	1.22*
	%CV		6.42	18.20

Table 4 Results of the determination of TSS and disease symptom testing after heating at both 34° C and 38° C, and pre-cooling at 5° C for 10, 20, and 30 days after which, the samples were stored at 25° C for 6 days.

Note. * represents a statistical significance level of 0.05. Numbers followed by a letter in the column represent the statistical significance of the mean comparison according to Least Square Difference (LSD) ($p \le 0.05$). TSS (brix) is total soluble solid.

Table 5 Conclusion of TSS and disease symptom testing after pre-heating at both 34°C and 38°C, and cooling at 5°C for 10, 20, and 30 days after which, the samples were stored at 25 ° C for 6 days.

Temperature in °Celsius	TSS	Disease symptom
(factor 1)	(brix)	(scores)
34	16.88	2.62
38	17.12	2.43
LSD 0.05	ns	ns
Heat treatment time in hours	TSS	Disease symptom
(factor 2)	(brix)	(scores)
24	18.46ª	2.40
48	17.35 ^b	2.46
72	15.18°	2.71
LSD 0.05	0.57*	ns
Cooling at 5°C in days	TSS	Disease symptom
(factor 3)	(brix)	(scores)
10	17.11ª	2.05 ^b
20	17.54ª	2.40 ^b
30	16.34 ^b	3.13ª
LSD 0.05	0.57*	0.46*
factor 1	ns	ns
factor 2	*	ns
factor 3	*	*
1x2	ns	ns
1x3	ns	ns
2x3	ns	ns
1x2x3	ns	ns
%CV	3.01	17.05

Note. * represents a statistical significance level of 0.05. ns = no statistically significant level .05. Numbers followed by a letter in the column represent the statistical significance of the mean comparison according to Least Square Difference (LSD) ($p \le 0.05$). TSS (brix) is total soluble solid.

Conclusion

Results demonstrated that the storage time at 5°C and shelf life of mango fruits affected the test sample, which could cause damages after chilling treatment that relevant to the storage time, where longer storage time could reduce the shelf life or increase the chilling injury symptoms.

The pre heating treatment on the test sample at 34°C for either 24 or 48 days or at 38°C for 24 hours could reduce the damage on the test sample after chilling treatment in mango after the test sample had been kept after pre cooling treatment at 5°C for 10 and 20 days.

The test sample that had been kept for 30 days after pre-cooling treatment *could not* maintain weight lost and fruit firmness, whereas the value and score of total solid solution and disease symptom were increased when the comparisons were made to those of 10 and 20 days, and the control group.

From the 10, 20, and 30 day test groups, it was determined that exceeding 20 days showed a rapid deterioration of the sample group including weight loss and disease symptoms even though it had been exposed to pre-cooling. Storage of fruits at 5 $^{\circ}$ C for 30 days also caused CI which included darker fruit, tissue collapse and rotten fruit.

The mango fruits that treated by heat treatment for 72 hours gave an abnormal ripening. The cooling treatment at 5°C that kept for 30 days after treatment showed some changes in color of the skin of mango fruits, *become a lofted fruit*, and reducing in fruit quality in a short time. The mango fruits that had been kept after the chilling treatment would have all qualities lower than the control group.

Mango fruit exposed to heat treatment for 72 hours had an abnormal ripening. Additionally, pre-cooling treatment at 5°C for 30 days contributed to skin color changes, darker fruit, tissue collapse, and rotten fruit and reduced the fruit quality quickly. All fruit kept after chilling treatments had qualities lower than the control group.

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