CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Nutritional value and composition of fresh noni fruits

The chemical composition and physical properties of three harvesting stages of fresh noni fruits, including unripen fruits (green noni fruit), half-ripen (light yellow noni fruit) and fully ripen (light brown fruit) are shown in Table 4.1. Significant ($p<0.05$) difference were observed among fully ripen, half-ripen and unripen fruits in their content of moisture, ash, fiber, carbohydrate, reducing sugar, and total sugar. Not significant ($p>0.05$) difference were observed in content of fat and protein.

Fully ripen had higher amounts of moisture content (88.30 $\pm$ 0.32%), fiber (1.60 $\pm$ 0.02%), reducing sugar (4.45 $\pm$ 0.01%) and total sugar (5.33 $\pm$ 0.01%) than half-ripen and unripen fruits. It was found that moisture, fiber, reducing sugar and total sugar was slightly increased during ripening. However, fully ripen fruits had lower amounts of ash (0.39 $\pm$ 0.01%) and carbohydrate (14.05 $\pm$ 0.40%) than half-ripen and unripen fruits. Ash and carbohydrate content was slightly decreased during ripening. The most significant difference between unripen and fully ripen was noted in the pH and titratable acidity. Titratable acidity was higher and pH was lower in the unripen than in the fully ripen fruit. With regard to organic acid (titratable acidity), citric acid content declined slightly during ripening from 0.98 $\pm$ 0.01 to 0.37 $\pm$ 0.02 g/100g, whereas pH slightly increase from 4.11 $\pm$ 0.00 to 4.47 $\pm$ 0.01. The trend of citric acid content during ripening was similar to that reported by Raffo *et al.* (2002). Total acidity, citric acid content declined during ripening due to organic acid were used as substrate in the respiration processed for build energy that can change starch to sugar, thus, total sugar increasing but carbohydrate content decreasing during ripening were observed (Sirisakulwat, 2001). Fully ripen and half-ripen have high levels of reducing sugar and total sugar. Due to this finding, the application of half-ripen and fully ripen fruits as noni fruit salad, fermented noni juice and noni wine was done because higher amount of the sugar that give a better taste (Sabiletou, 2002).

Table 4.1 Chemical and physical properties of fresh noni fruits at different harvesting stage

Parameters	Fruit Full ripen	Fruit Half-ripen	Fruit Unripen
Moisture content (%) (w/w)	88.30 ± 0.32^{a}	78.37 ± 0.66^{b}	77.03 ± 0.62^{c}
Ash (%) (w/w)	0.39 ± 0.01^{c}	0.68 ± 0.01^{b}	1.16 ± 0.06^{a}
Fat (%)(w/w)	0.17 ± 0.00^{ns}	0.18 ± 0.01^{ns}	0.16 ± 0.00^{ns}
Protein [x6.25] (%)(w/w)	0.48 ± 0.07^{ns}	0.52 ± 0.05^{ns}	0.54 ± 0.03^{ns}
Fiber (%)(w/w)	1.60 ± 0.02^{a}	1.50 ± 0.02^{b}	1.36 ± 0.02^{c}
Carbohydrate (%)(w/w)	14.05 ± 0.40^{b}	18.75 ± 0.64^{b}	19.74 ± 0.65^{a}
Reducing sugar (%)(w/w)	4.45 ± 0.01^{a}	3.17 ± 0.01^{b}	1.41 ± 0.01^{c}
Total sugar (w/w)	5.33 ± 0.01^{a}	4.01 ± 0.01^{b}	1.93 ± 0.01^{c}
Total acidity : citric acid (g/100g)	0.37 ± 0.02^{c}	0.53 ± 0.01^{b}	0.98 ± 0.01^{a}
pH	4.47 ± 0.01^{a}	4.46 ± 0.01^{a}	4.11 ± 0.00^{b}
Colour L^* (Light–dark)	54.93 ± 2.66^{b}	73.92 ± 2.86^{a}	45.49 ± 1.84^{c}
a^* (Red–green)	-0.38 ± 1.68^{a}	-3.05 ± 1.65^{b}	-15.11 ± 0.86^{c}
b^* (Yellow -blue)	22.17 ± 2.31^{c}	30.84 ± 3.83^{a}	25.65 ± 2.10^{b}
Weight of individual fruit (g)	78.94 ± 3.02^{a}	75.02 ± 2.96^{b}	48.97 ± 2.64^{c}

Data were expressed as mean of 3 replications $\pm$ SD. Similar letters in each row indicated treatments were not significantly different ($p > 0.05$).

Color lightness (L^*) value of unripen fruits was lowest (45.49 $\pm$ 1.48) and increase to highest value at half-ripen fruits (73.92 $\pm$ 2.86) but then decrease at fully ripen (54.93 $\pm$ 2.66). Color a^* (red-green) value of unripen fruits was lowest (-15.11 $\pm$ 0.86) that mean it was more green than other ripening stage. Since the unripen fruit have the higher amount of chlorophyll pigments that exhibited the lowest a^* color (red-green) value. The greenness of unripen fruit was due to the color of chlorophyll pigments which declined during ripening. The brown pigment from polymerization of polyphenols which can accumulate during ripening may the case of highest value of color L^* (Light-dark) in fully ripen fruit (Sirisakulwat, 2001).

Unripen fruits had the lowest weight (48.97 ± 2.64 g) and increase during ripening to 78.94 ± 3.02 g of fully ripen. During fruits ripening, it was observed that increased of the individual weight of fruit because the maturation of fruit could accumulate the elements for development the cells structure and produced the compounds for used in maturation processes (Siripanish, 2006).

Direct observation by hands showed that the firmness, another physical parameter related to ripening stage was declined with the ripening stage.

Table 4.2 Ascorbic acid, carotenoid, flavonoid and total phenolic compounds of noni fruit at different harvesting stages

Fruit	Ascorbic acid (mg/100g)	Carotenoid (µg/g)	Flavonoid (mg/100g)	Total phenolic (mg/100g)
Unripen	97.4 ± 1.47^{b}	18.30 ± 0.56^{a}	221.8 ± 3.1^{a}	369.1 ± 2.2^{a}
Half-ripen	110.0 ± 3.05^{a}	15.58 ± 0.30^{b}	154.4 ± 4.3^{b}	290.0 ± 3.3^{b}
Fully ripen	69.8 ± 2.18^{c}	3.96 ± 0.58^{c}	58.1 ± 2.5^{c}	69.5 ± 2.9^{c}

Data were expressed as mean of 3 replications ± SD. Similar letters in each row indicated treatments were not significantly different ($p > 0.05$).

Table 4.2 showed ascorbic acid content, carotenoid content, flavonoid content and total phenolic compounds of noni fruit harvested at 3 different ripening stages. Significant ($p<0.05$) changes in the content during ripening were recorded. Although antioxidative activity of noni fruit have been studies (Zin *et al.*, 2002), no studies have demonstrated their antioxidant content and stability during ripening, processing and storage.

Changes in ascorbic acid content during ripening

Noni fruits are a rich source of ascorbic acid. An increase in the ascorbic acid content was observed from unripen to half-ripen (97.4 ± 1.47 to 110.0 ± 3.05 mg/100g). Also, the content of ascorbic acid was the highest in the half-ripen stage,

whereas then decreased to a lowest of fully ripen stage (69.8 ± 2.18 mg/100g). Ascorbic acid decreased in fully ripen may due to the oxidation reaction which can deform ascorbic acid to dehydroascorbic acid (Sirisakulwat, 2001). These results are in consistent with other studies. In other fruits such as tomato, a rather large vitamin C increase was observed from green stage up to the red stage with a subsequent decrease as the fruit over matured (Yahai *et al.*, 2001). The increase in ascorbic acid content with increasing ripening stage is in agreement with other studies. Olsson *et al.* (2004) reported the increase in ascorbic acid content with increasing ripening stage of strawberries.

The amount and intensity of light during the growing season have also been described to have a definite influence increasing the ascorbic acid formed (Lee and Kader, 2000). The light exposure could explain the increase in ascorbic acid content of red fruits as compared to the green ones. However, some contradictory results were observed in bell pepper fruits where an increase during development was described with a maximum of 136.1 mg/100g at 51 days from fruit set and then decreased suddenly to a maximum of 65.5 mg/100g at 64 days (Yahai *et al.*, 2001).

Ascorbic acid was the main form of vitamin C, and its content increased as the sweet peppers reached maturity. Vitamin C levels depend on several factors including cultivar, production practice, maturity at harvest, and storage conditions (Marin *et al.*, 2004).

Changes in carotenoid content during ripening

During noni ripening, the carotenoid content were declined. Unripen noni fruit had the highest content of carotenoid (18.30 ± 0.56 µg/100g) then decreased into half-ripen (15.58 ± 0.30 µg/100g) and lowest in fully ripen (3.96 ± 0.58 µg/100g). The carotenoid content of noni fruit decreased during ripening. However, the mechanism of the carotenoid degradation is unclear. It may occur due to an isomerization which change *trans*-isomers to *cis*-isomers. Carotenoid in fruits may also be destroyed by heat, light and oxygen from the environments (Marin *et al.*,2004).

These results for carotenoid quantified were no consistent with other fruits such as tomatoes. Raffo *et al.* (2002) reported that cherry tomatoes have the great increase of carotenoid content during ripening stage, which at full ripeness accounted for 90% increasing. Hart and Scott (1995) reported that the content of carotenoids

may be affected by variety, maturity, growing conditions, growing season and the part of the root sample. Although climate had a major influenced the variability of carotenoids in carrots. Marin *et al.* (2004) reported that sweet pepper carotenoid were quantified during maturation. Lutein was the predominant carotenoid for immature green pepper while β-carotene was the predominant pigment for green peppers. The total carotenoid pigments increased four times for red ripe fruits. Lutein and *cis*-lutein contents decreased during maturation to non detectable values in immature red and red ripen stages.

Changes in flavonoid and total phenolic content during ripening

A decrease in the flavonoid content and total phenolic content during ripening was observed (Table 4.2). The total flavonoid content of noni fruits was considerably lower than total phenolic content, which was expected because flavonoid are the major subgroup of total phenolic (Siripanish, 2006).

The flavonoid content decreased during ripening from 221.8 ± 3.1 to 58.1 ± 2.5 mg/100g, the total phenolic content decreased from 369.1 ± 2.2 to 69.5 ± 2.9 mg/100g. This results was not due to the fruit size decrease with advancing maturity since similar sizes were used for all maturity stages. In general, immature fruits contained the highest concentration of flavonoid and phenolic while ripe fruits contained the lowest (Marin *et al.*, 2004).

Gonçalves *et al.* (2004) reported cultivar, ripeness stage, harvest year, and storage condition have a major influence on the quantitative individual phenolic composition of cherries. With regard to flavonoid, in Naomi tomatoes, the highest flavonoid content was observed at the green-orange stage, 5.96 mg/100g, whereas at full ripeness it decreased to 3.56 mg/100g.

The declining content of the main flavonoid could be associated with their involvement in fruit defense mechanisms against reactive oxygen species, which are produced in high amounts during the climacteric peak as a consequence of increasing rate of respiration (Raffo *et al.*, 2002). The changes of phenolic compound content, as for other fruits and vegetables, may be influenced by external factors, such as light and average temperature, although genetic control is the primary factor (Macheix *et al.*, 1990).

The optimum ripening stage for noni fruits harvest based on carotenoid content, flavonoid content and total phenolic content was the unripe stage. However, it could be considered the total phenolic compounds of the unripen fruit, their content so high amounts (369.1 ± 2.2 mg/100g) and may including tannin, a subgroup in total phenolic, they may cause an astringency and bitterness of unripen fruits (Siripanish, 2006). If noni fruits are harvested in a half-ripen stage, they have considerably higher content of moisture, reducing sugar, total sugar and ascorbic acid and also less a astringency and bitterness, more good taste, aroma and textural than unripen and fully ripen fruits (Boonyakiat, 2005). Therefore, the half-ripen fruits were used to produce the noni juices.

Table 4.3 Antioxidant activity of noni fruit

Fruit	Antioxidant Activity (IC_{50}) (mg/g DPPH)
Unripen	12.30 ± 2.44^{b}
Half-ripen	16.67 ± 2.74^{c}
Fully ripen	37.92 ± 2.78^{d}
Gallic acid	1.78 ± 0.00^{a}

Data were expressed as mean of 3 replications ± SD. Similar letters in each row indicated treatments were not significantly different ($p > 0.05$).

Table 4.3 shows the antioxidant activity of noni fruit express as IC_{50} value. In this study, the DPPH radical scavenging activity of noni fruits were measured. A stronger radical quenching agent generally resulted in a lower IC_{50} value. IC_{50} is the amount of the antioxidant necessary to decrease the initial concentration of DPPH radical which reacted with the antioxidant at the steady state (Argolo *et al.*, 2004). Thus, the lowest IC_{50} value are the highest antioxidant activity.

Unripen noni fruit had the lowest IC_{50} value (12.3 ± 2.44 mg/g DPPH), half-ripen had more than the unripen. Fully ripen had the highest IC_{50} value (37.9 ± 3.90 mg/g DPPH). The results exhibited unripen fruit had the highest antioxidant activity

whereas fully ripen had the lowest. In this result, consistent with the antioxidant content of fruit. Since unripen fruit had the highest content of carotenoid, flavonoid and total phenolic. In this case, the DPPH radical acavenging activities were correlated with the carotenoid content, flavonoid content and total phenolic content at all ripening stage. In the literature, the correlation between radical scavenging activity and total phenolic is reported (Benvenuti *et al.*, 2004).

The result indicated that unripen noni fruit have a very potent antioxidant activity, compared with the pure gallic acid used as positive control.

4.2 Nutritional value and composition of fresh noni juices

Analysis of noni juice

Noni fruit juice was produce using half-ripen of noni fruits. The chemical composition of fresh raw juice are shown in Table 4.4. Compared to the result in Table 4.1, only moisture content (half-ripen fruit 78.37 $\pm$ 0.66%) was increasing (raw juice 90.22%), whereas others component (ash, fat, protein, fiber, carbohydrate, reducing sugar and total sugar) was decreasing. Raw noni juice contains moisture 90.22 $\pm$ 0.32%, ash 0.13 $\pm$ 0.02%, fat 0.11 $\pm$ 0.00%, protein 0.29 $\pm$ 0.07%, fiber 1.10 $\pm$ 0.02%, and carbohydrate 8.15 $\pm$ 0.66%. Due to low sugar content, the most commercial noni juice always add sweeteners such as sucrose or honey.

Raw noni juice had pH 4.48 which the optimize conditions to pasteurization for fruit juice at pH < 4.5 would be 63°C for 30 minutes, 71°C for 1 minute and 88°C for 15 seconds (Rattanapanon, 2002).

The main antioxidant content of fresh noni juice comprise ascorbic acid (87.8 $\pm$ 1.64 mg/100g), carotenoid (13.32 $\pm$ 0.20 µg/g), flavonoid (175.1 $\pm$ 16.2 mg/100g) and total phenolic (336.8 $\pm$ 19.2 mg/100g). Noni juice had high antioxidant activity which represent in IC_{50} as 13.60 mg/g DPPH. When compared to the half-ripen in Table 4.2, noni juice had lower antioxidant content than half-ripen fruit. This could be due to the juice extraction in a juicer could destroy the cell wall and some organelle of fruit components (Sirisakulwat, 2001). Oxidation by oxygen could also be happened, especially for ascorbic acid and carotenoid. In addition, some antioxidant components might loss during the extraction process (Yadav and Sehgal, 1997).

Table 4.4 Chemical composition and antioxidant content of fresh noni juices

component	Raw juice
Moisture content (%) (w/v)	90.22 ± 0.32
Ash (%) (w/v)	0.13 ± 0.02
Fat (%)(w/v)	0.11 ± 0.00
Protein (%)(w/v)	0.29 ± 0.07
Fiber (%)(w/v)	1.10 ± 0.02
Carbohydrate (%)(w/v)	8.15 ± 0.66
Reducing sugar (%)(w/v)	1.99 ± 0.01
Total sugar (%)(w/v)	2.13 ± 0.02
Total acidity (g/100g)	0.42 ± 0.02
pH	4.48 ± 0.01
Ascorbic acid content (mg/100g)	87.8 ± 1.64
Carotenoid content (μg/g)	13.32 ± 0.20
Flavonoid content (mg/100g)	175.1 ± 16.2
Total phenolic content (mg/100g)	336.8 ± 19.2
Antioxidant activity (IC_{50}, mg/g DPPH)	13.60 ± 0.50

Data were expressed as mean of 3 replications ± SD.

4.3 The effect of different pasteurization conditions and boiling on the antioxidant content and antioxidant activity of noni juices

4.3.1 The effect of different pasteurization conditions and boiling on the ascorbic acid content

The unprocessed raw juice had the highest ascorbic acid content (87.8 ± 1.64 mg/100ml). After pasteurization at 88°C for 16 seconds, 71°C for 1 minute, 63°C for 30 minutes and boiled at 100°C for 10 minutes, the ascorbic acid content dropped to 72.6 ± 5.23, 62.5 ± 1.77, 54.0 ± 2.43, and 18.9 ± 1.62 mg/100ml, respectively, with significant decreases by 17.3 %, 28.8%, 38.5%, and 78.5%, respectively, compared with unprocessed raw juice (Figure 4.1). Whereas the 2 brands commercial pasteurized noni juice had lower ascorbic acid contents (38.4 ± 1.03 and 23.0 ± 1.37 mg/100ml) than results in this study. The different of the ascorbic acid content depended on several factors, including production practice, maturity at harvest and storage condition (Gil *et al.*, 2002).

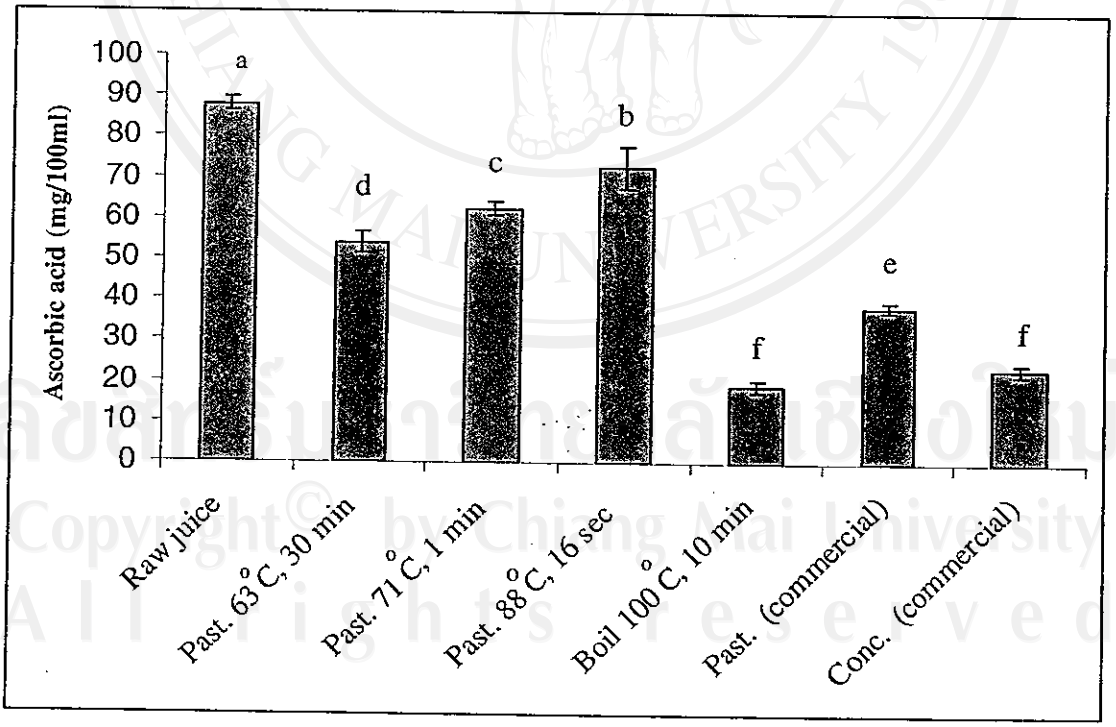

Figure 4.1 Effect of different pasteurization conditions and boiling on ascorbic acid content of noni juices (mean ± SD, n = 3). Similar letters in each bar indicated treatments were not significantly different (p>0.05).

It was found that the loss of ascorbic acid content by pasteurization is the range from 17.3% to 38.5%. With increasing time, 16 seconds to 30 minutes, the ascorbic acid content decreased. Ascorbic acid is a heat instable vitamin; thus, high temperatures led to a loss of ascorbic acid. Differences in temperature had a minor influence on the losses of ascorbic acid. Many studies showed the decline in ascorbic acid during the production and cooking (Dewanto *et al.*, 2002a; Giovanelli *et al.*, 2001). Gahler *et al.* (2003) reported with increasing heating time and processing steps of different tomato products (tomato juice, tomato sauce and tomato soup), a continuous loss of the water soluble vitamin C was observed. The highest decreased of ascorbic acid content after boiled at 100°C for 10 minutes might be based on the long time processed and the interaction of the juice with oxygen during the processing.

Assuncao and Mercadante (2003) reported that cashew apple products (concentrated juice, frozen pulp, nectar, ready-to-drink, and sweetened concentrated juice) showing ascorbic acid contents from 13.7 to 121.7 mg/100g, suggested that cashew apple products were proved to be excellent sources of vitamin C. Talcott *et al.* (2003) reported that pasteurization (85°C for 30 minutes) fruit juice resulted in a 25% loss in L-ascorbic acid, which was completely destroyed after 14 days of storage; losses coincided with increased juice browning and formation of 5-hydroxymethylfurfural. Jiratanan and Liu (2004) reported that thermal processing of beets showed a slight linear degradation of free vitamin C content. Total vitamin C content of processed beets did decrease with thermal processing, as was seen with free vitamin C content. Thermal processing of green beans under constant temperature of 115°C at various time intervals of 10, 20 and 40 minutes showed a reduction of vitamin C.

Processed fruits have long been perceived to have lower nutritional value than the fresh commodities because of the decline in vitamin C (Dewento *et al.*, 2002b). Loss of vitamin C occurs primarily by chemical degradation involving oxidation of ascorbic acid to dehydroascorbic acid (DHAA) and 2,3-diketogulonic acid and further polymerization to other nutritionally inactive products. Because heat is known to speed the oxidation process of ascorbic acid, thermal processing results in a loss of vitamin C content in fruits (Gregory, 1996).

4.3.2 The effect of different pasteurization conditions and boiling on the on carotenoid content

The unprocessed raw juice had the highest carotenoid content (13.32 ± 0.20 μg/ml). After pasteurization at 88°C for 16 second, 71°C for 1 minute, 63°C for 30 minute and boiled at 100°C for 10 minute, the carotenoid content dropped to 12.61 ± 0.17, 12.00 ± 0.61, 10.43 ± 0.29, and 4.27 ± 0.46 μg/ml, respectively, with decrease by 5.3%, 9.9%, 21.7%, and 67.9%, respectively, compared with unprocessed raw juice. Whereas, the 2 brands of commercial noni juices resulted in 6.66 ± 0.42 and 10.41 ± 0.54 μg/ml, respectively (Figure 4.2). The commercial pasteurization had the lower carotenoid content than this study. Their content were significantly different, which could due to differences in the fruit source, production practice and also processing methods (Giovanelli *et al.*, 2001).

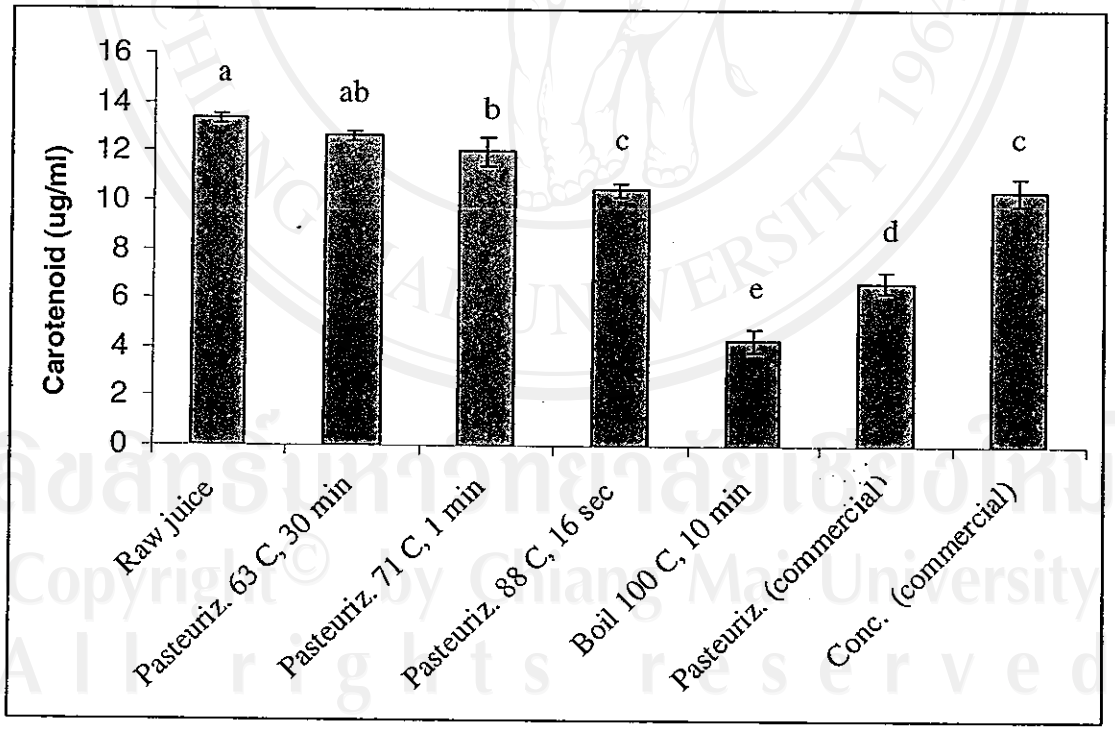

Figure 4.2 Effect of different pasteurization conditions and boiling on carotenoid content of noni juices (mean ± SD, n = 3). Similar letters in each bar indicated treatments were not significantly different (p>0.05).

In this study, the effect of heat treatments on the stability of the total carotenoid content was investigated. Total carotenoid content was measured during heat treatment at different conditions. Pasteurization and boiling did affect the stability of carotenoid. The slightly decrease of carotenoid content by pasteurization is the range from 5.3% to 21.7%, these content decreased with increased heating conditions. Carotenoid content decreased with increasing heating conditions could be due to heat can induce the isomerization of carotenoid structure, from *trans*-isomer to *cis*-isomer, which occurred after heat treatment and also carotenoid can be degraded by oxidation (Siripanish, 2006). This results showed a consistent trend for the effects of thermal processing on carotenoid content as compared to other studies.

Zhang and Hamauzu (2004) reported that both microwave cooking and conventional boiling caused loss of total carotenoid in broccoli florets and stems. The florets cooked conventionally boiling for 30, 60, 90, 120 and 300 seconds lost 2.7%, 12.0%, 14.4%, 17.1% and 22.9% of total carotenoid, respectively, while the stems cooked for 60, 120 and 300 seconds lost 10%, 20% and 20%, respectively. In the microwave cooking, total carotenoid in the florets and stems of broccoli also declined continuously. β-carotene in the florets was quite heat labile and a substantial level of this compound was lost in the first 60 seconds of the cooking. Yadav and Sehgal (1997) reported losses of β-carotene from vegetables, including spinach, amaranth and fenugreek, during cooking procedures, such as boiling, stewing, frying, blanching and pressure cooking. Takeoka *et al.* (2001) reported that lycopene losses during thermal processing of tomatoes products. Comparison of carotenoid levels throughout processing (raw tomatoes, tomato juice and final paste) losses range from 9% to 28%. Longer processing times, required to achive the desired final solid levels, may be associated with increased losses. Abushita *et al.* (2000) observed that ascorbic acid and β-carotene levels decreased as function of thermal processing. However, no consistent changes in lycopene levels were observed. The *trans*-lycopene of raw tomato had increased from 2.01 to 3.11, 5.45 and 5.32 mg of *trans*-lycopene/g of tomato after 2, 15 and 30 minute of heating at 88°C, respectively. Thermal processing enhanced the nutritional value of tomatoes by increasing the bioaccessible lycopene content and total antioxidant activity and are against the notion that processed fruits have lower nutritional value than fresh produce (Dewanto *et al.*, 2002a).

4.3.3 The effect of different pasteurization conditions and boiling on the flavonoid content and total phenolic content of noni juices

Pasteurization treatments did not show significant changes in the flavonoid content and total phenolic content. The total flavonoid content of the unprocessed raw juice was 175.1 ± 16.2 mg/100ml. After pasteurization at 63°C for 30 minutes, 71°C for 1 minutes, 88°C for 16 seconds and boiled at 100°C for 10 minutes, the flavonoid content were 174.8 ± 18.8, 186.8 ± 20.1, 181.7 ± 16.6 and 168.4 ± 14.9 mg/100ml, respectively (Figure 4.3 (A)). The total phenolic content of the unprocessed raw juice was 336.8 ± 19.2 mg/100ml. After pasteurization at 63°C for 30 minutes, 71°C for 1 minutes, 88°C for 16 seconds and boiled at 100°C for 10 minutes, the total phenolic content were 341.5 ± 19.6, 352.9 ± 25.6, 331.2 ± 17.9 and 268.2 ± 15.1 mg/100ml, respectively (Figure 4.3 (B)). The commercial pasteurized noni products had lower flavonoid content and total phenolic content than results in this study. This could be due to the differences in fruit sources, production practice and also processing methods (Giovanelli *et al.*, 2001).

There were no change in the total flavonoid and total phenolic contents in noni juices with pasteurization. Although the heat-treated had slightly higher contents of flavonoid and total phenolic, there were no significant differences among all treatments. Therefore, there was no loss or gain in content of both total flavonoid and phenolic in noni juice with pasteurization.

In this study, the stability of flavonoid and phenolic were consistent with many other studies. Dewanto *et al.* (2002a) reported no loss or gain in content of both total phenolics and flavonoids in tomatoes with thermal processing at 88°C for 2, 15 and 30 minutes. There were no significant changes in either total phenolics or total flavonoids. Turker *et al.* (2004) reported that there were no significant positive or negative effects of pasteurization on total anthocyanin content nor on the anthocyanin profile with respect to control at any storage temperature.

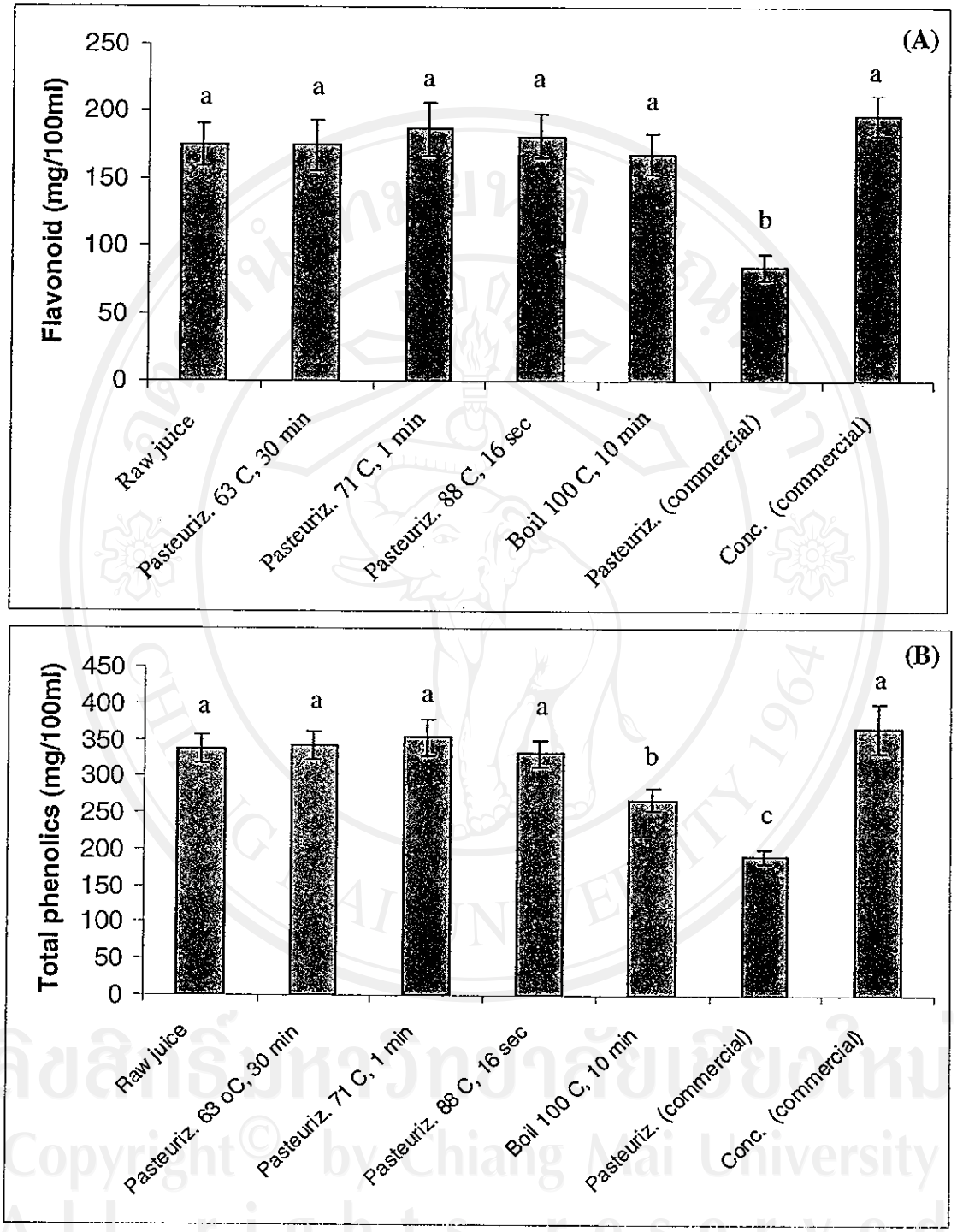

Figure 4.3 Effect of different pasteurization conditions and boiling on total flavonoid content (A) and total phenolic content (B) of noni juices (mean $\pm$ SD, n = 3). Similar letters in each bar indicated treatments were not significantly different (p>0.05).

Murakami *et al.* (2004) reported that polyphenolic compound (retin, luteolin, luteolin-7-glucoside and chlorogenic acid) are relatively stable during heating at 100°C. Even though these compounds decomposed at 180°C, some decomposition products still had radical-scavenging activity. Ewald *et al.* (1999) reported that, for blanched onions, green beans and peas, various cooking procedures including boiling, microwaving, frying or further warm holding, did not significantly affect the levels of quercertin and kaempferol, major compounds of flavonoid.

The ability of flavonols to resist degradation during heat processing is believed to be highly associated with their structure but is also substantially dependent on other factors, such as the presence of oxygen or oxidizing agents (Makris and Rossiter, 2000). There were no change in total flavonoid and total phenolic contents in noni juices with pasteurization. Phenolic acids occurs in plants as metabolic intermediates, and they also accumulate in the vacuoles. Thermal processing may release more bound phenolic acids from the breakdown of cellular constituents. Although disruption of cell walls also releases the oxidative and hydrolytic enzymes that can destroy the antioxidants in fruits (Chism and Haard, 1996), pasteurization treatment deactivates these enzymes to avoid the loss of phenolic acids.

However, many studies reported no consistent results. Dewanto *et al.* (2002b) reported that thermal processing at 115°C for 25 minutes significantly elevated the total antioxidant activity of sweet corn by 44% and increased total phenolics by 54%. Dewanto *et al.* (2002a) reported the *trans*-lycopene content of tomato had increased after heating at 88°C for 2, 15 and 30 minutes. Jiratanan and Liu (2004) reported that processed green beans at 100, 115 and 120°C for 20 min showed a decrease of 65%, 58% and 55% of total flavonoid. In this study, the results did not show a consistent trend of effects of thermal processing on phenolic, flavonoid and carotenoid in noni juice when compared to the thermally processed sweet corn, tomatoe and green beans. This suggest that the effect of thermall processing on phenolic, flavonoid and carotenoid will be different in different produce and is worth further investigation. In addition, difference processing methods used may have impacted result. It can only conclude that, depending upon the particular produce, processing parameters and method thermal processing may enhance, reduce or in some cases no change in total antioxidant components from that of fresh produce.

4.3.4 The effect of different pasteurization conditions and boiling on the antioxidant activity of noni juice

Table 4.5 shows the antioxidant activity (express as IC_{50} value, mg/g DPPH) of noni raw juice, pasteurized juice, boiled juice and 2 brands commercial juice. There were significant difference among pasteurization treatments and boiling had slightly lower antioxidant activity than pasteurization. Therefore, heat-treatments did affect antioxidant activity of noni juice.

Table 4.5 Antioxidant activity of noni raw juice, pasteurized juice, boiled juice and 2 brands commercial juice

Treatment	Antioxidant Activity IC_{50} (mg/g DPPH)
Raw juice	13.60 ± 0.28^{d}
Pasteurization at 63°C for 30 minutes	11.98 ± 0.12^{b}
Pasteurization at 71°C for 1 minutes	9.23 ± 0.14^{a}
Pasteurization at 88°C for 16 seconds	12.75 ± 0.32^{c}
Boiling at 100°C for 10 minutes	15.56 ± 0.36^{e}
Pasteurization (Commercial)	27.34 ± 0.27^{f}
Conc. (Commercial)	13.36 ± 0.11^{d}

Data were expressed as mean of 3 replications $\pm$ SD

Similar letters in each row indicated treatments were not significantly different (p>0.05).

In this result, a positive effect of thermal processing on antioxidant activity of noni juice was displayed. After pasteurization at 71°C for 1 minute the antioxidant activity of the juice was higher than the pasteurization conditions at 63°C for 30 minutes, at 88°C for 16 seconds and boiling at 100°C for 10 minutes. These results consistent with flavonoid and total phenolic content of the noni juices pasteurized at 71°C for 1 minute, which was higher than other conditions (Figure 4.3). Whereas the

commercial pasteurized noni juice had the lowest antioxidant activity which consistent with its lower flavonoid and total phenolic contents. The concentrated commercial noni juice was not significantly different in the term of flavonoid content, total phenolic content and antioxidant activity compared with the raw noni juice.

Lee *et al.* (2003) reported that the total antioxidant capacities of cocoa from DPPH assays were highly correlated with phenolic content and flavonoid content. Therefore, various factors such as experimental conditions, sample preparation method and physiological relevance of the assays should be considered in the evaluation of antioxidant activity.

Dewanto *et al.* (2002b) reported that thermal processing at 115°C for 25 minutes significantly elevated the total antioxidant activity of sweet corn by 44%. Dewanto *et al.* (2002a) also demonstrated that thermal processing of tomatoes singnificantly increased the lycopene content and the total antioxidant activity, whereas no significant change on the total phenolics content was observed.

It is conventional wisdom that thermal processed fruits have a lower nutritional values than their respective fresh commodities due to the loss of vitamin C content in the processing (Dewanto *et al.*, 2002a). In this study, both of ascorbic acid and carotenoid content decreased after thermal processing but the total antioxidant activity did not decrease, indicating most of the activity comes from the natural combination of phytochemicals especially flavonoid and total phenolic. This suggests that pasteurized noni juices may retain their antioxidant activity despite the loss of ascorbic acid and carotenoid.

4.4 The effect of fermentation periods on the antioxidant content and antioxidant activity of noni juice

4.4.1 The effect of fermentation periods on the antioxidant content

During a fermentation period of 120 days at room temperature, samples of noni juice were taken on 1, 30, 60, 90 and 120 days to be analyzed for ascorbic acid and carotenoid contents (Figure 4.4), flavonoid and total phenolic contents (Figure 4.5). At the same time, a commercial brand of fermented noni juice was also analyzed. The content of ascorbic acid after fermentation 1, 30, 60, 90 and 120 days was 61.1 $\pm$ 3.54, 57.9 $\pm$ 2.56, 57.5 $\pm$ 1.86, 56.1 $\pm$ 2.12 and 51.4 $\pm$ 1.76 mg/100ml, respectively. A slightly decrease of ascorbic acid content after 30 days fermented period and then stable constant until 90 days. Slightly decrease again at 120 days fermented period (Figure 4.4 (A)). A slightly decrease of ascorbic acid content after fermentation could due to the changing form of ascorbic acid to dehydroascorbic acid (Gregory, 1996). The ascorbic acid content of a commercial brand of fermented noni juice was 37.1 $\pm$ 2.09 mg/100ml which lower than this study, which could be due to differences in fruit sources, production practice, maturity at harvest and storage condition (Gil *et al.*, 2002). In this study, it was accounted that 16% lossed of ascorbic acid content by 120 days fermentation. This results was consistent with Zhou *et al.* (2000) study. In the study, there was about 80 – 100 ppm ascorbic acid in the fresh cucumbers, which, after dilution with cover liquid, was reduced to about 40 – 50 ppm after fermentation.

The content of carotenoid after fermentation 1, 30, 60, 90 and 120 days was 12.67 $\pm$ 0.68, 10.15 $\pm$ 0.31, 9.83 $\pm$ 0.14, 9.59 $\pm$ 0.16 and 8.76 $\pm$ 0.40 µg/ml, respectively. Similar to ascorbic acid content, a slightly decrease of carotenoid content after 30 days fermented period and follow by stable constant until 90 days, finally decreased again at 120 days fermented period (Figure 4.4 (B)). However, fermented noni contained carotenoids within a narrow range of 8.76 $\pm$ 0.40 to 12.67 $\pm$ 0.68 µg/ml. This results indicated both of ascorbic acid and carotenoid were slowly decreased during fermentation. Since the carotenoid is a water insoluble compound, fermentation may not affect this compound. There was not any clear explanation for this reduction finding.

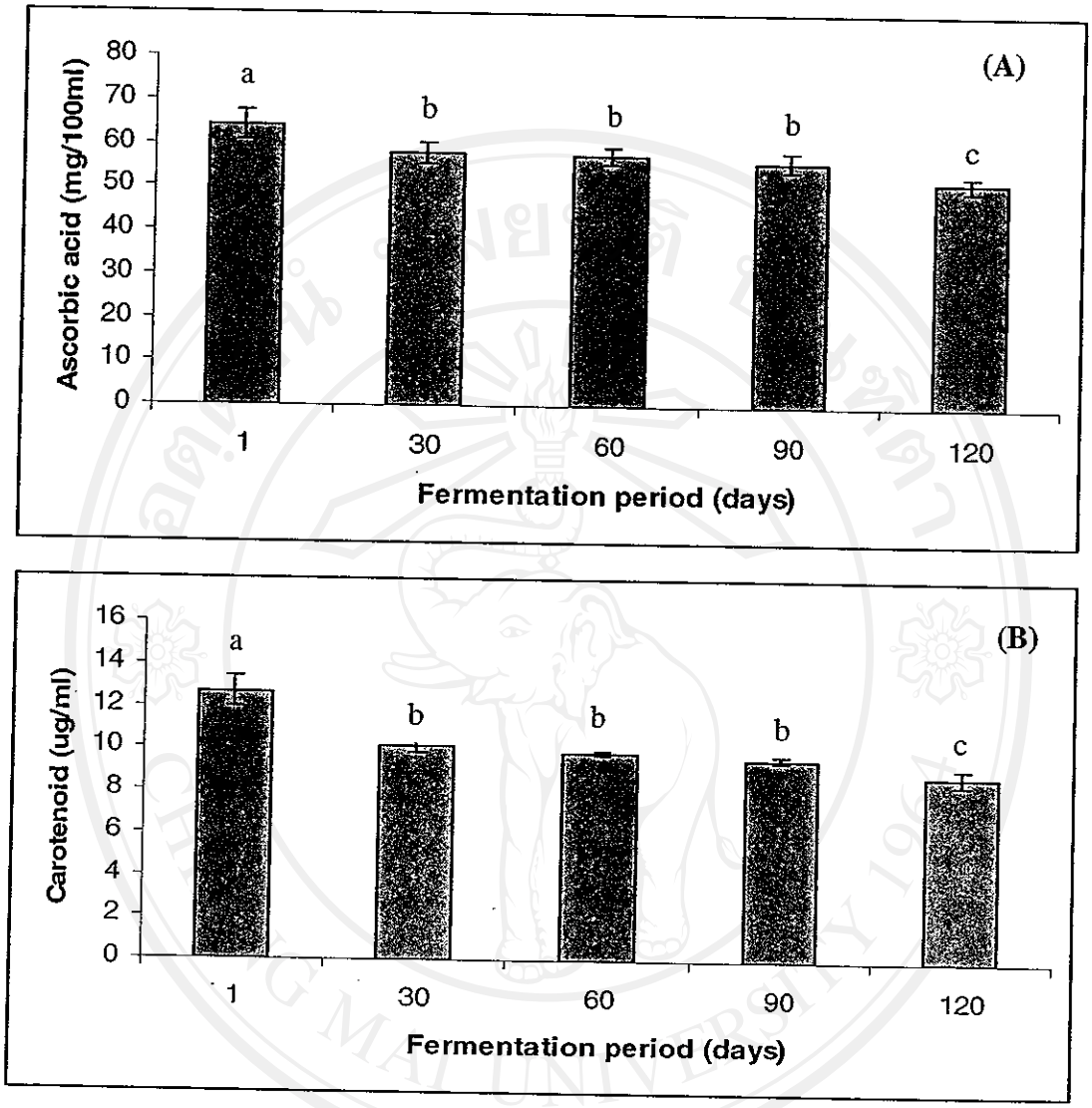

Figure 4.4 Effect of fermentation period on ascorbic acid content (A) and total carotenoid content (B) of noni juices (mean ± SD, n = 3). Similar letters in each bar indicated treatments were not significantly different (p>0.05).

In this study, carotenoid content decreased during fermentation. So if longer fermentation less carotenoid was observed. Carotenoid of the commercial fermentation (16.73 ± 0.29 µg/ml) is higher than this study although the commercial product may have longer fermentation period. This result could due to the commercial fermentation may have a higher initial carotenoid content by using unripen noni fruit which contributed the higher carotenoid content than half-ripen in the fermentation production (Sabiletou, 2002). Although, their content may slightly decreased during fermentation, but it was still higher than this study.

During a fermentation period of 120 days at room temperature, samples of noni juice were taken on 1, 30, 60, 90 and 120 days to be analyzed flavonoid and total phenolic content (Figure 4.5). At the same time, a commercial brand of fermented noni juice was also analyzed.

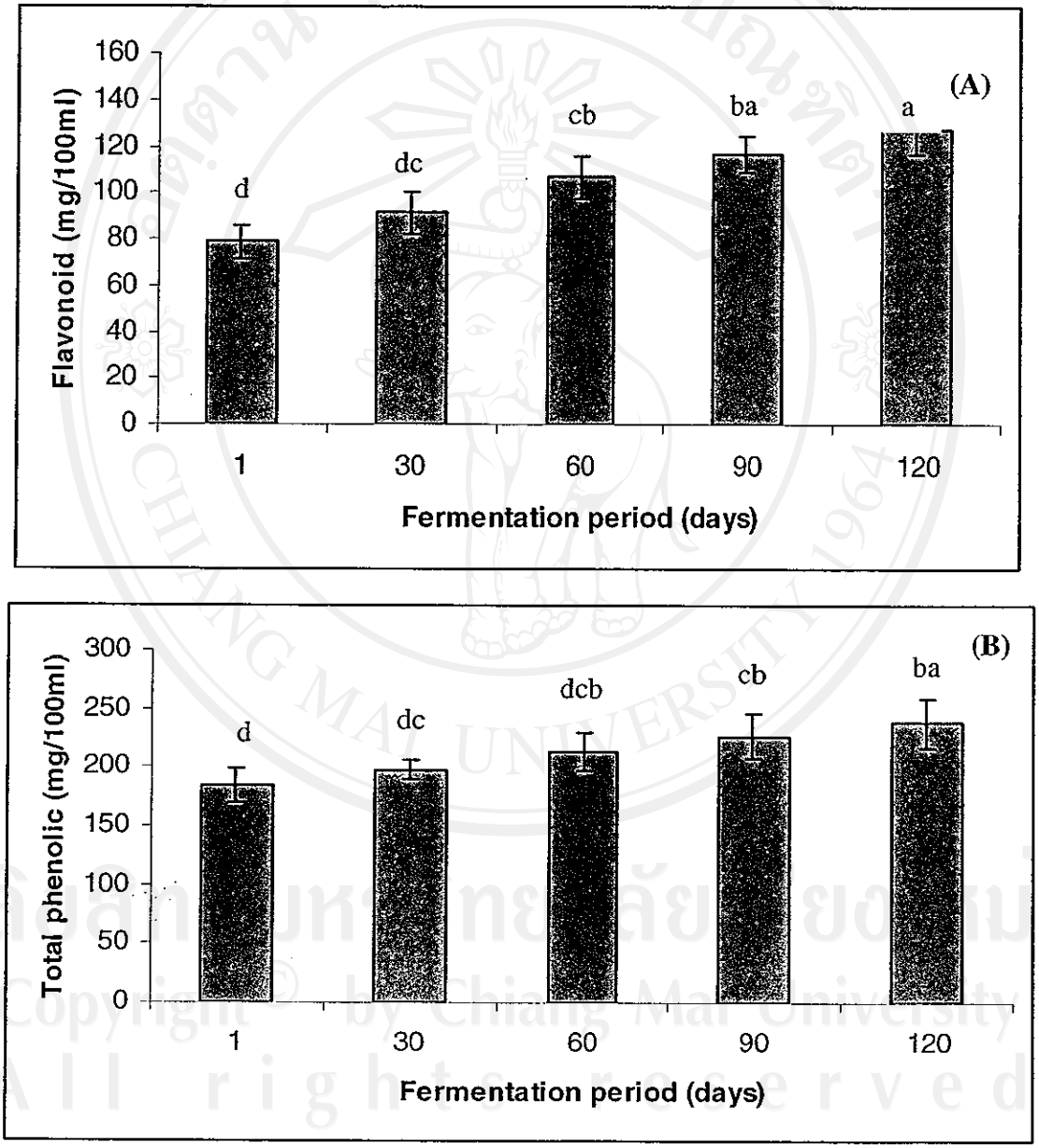

Figure 4.5 Effect of fermentation period on flavonoid content (A) and total phenolic content (B) of noni juices (mean $\pm$ SD, n = 3). Similar letters in each bar indicated treatments were not significantly different (p>0.05).

The content of flavonoid after fermentation at 1, 30, 60, 90 and 120 days was 78.7 ± 6.9, 91.1 ± 9.4, 106.6 ± 9.3, 116.9 ± 7.7 and 126.8 ± 10.5 mg/100ml, respectively (Figure 4.5 (A)). A significant increase of flavonoid content during fermentation was observed. For the total phenolic content, the result showed 184.3 ± 14.2, 197.3 ± 8.5, 213.4 ± 16.3, 226.5 ± 19.2 and 237.9 ± 20.4 mg/100 ml, respectively (Figure 4.5 (B)).

The flavonoid content and total phenolic content increased with increasing time as a function of fermentation. After 120 days fermentation period, flavonoid content increased by 38% and total phenolic increased by 23%. Statistical evaluations showed that there were significant positive effect of fermentation period on flavonoid and total phenolic content. Factors that affect the stability of anthocyanins include structure, pH, temperature, light, copigments, self-association, metallic ions, oxygen, ascorbic aicd, sugar and their degradation products (Rodriguez-Saona *et al.*, 1999). In this study, light, metallic ions could not affect the flavonoid and phenolic content but structure, water- soluble compounds and copigments might have a positive effect and also self-association. The presence of ascorbic acid and sugar might also be another reason for increasing of the flavonoid and total phenolic contents (Turker *et al.*, 2004).

The commercial fermented noni juice had the highest content of total phenolic compound (271.2 ± 36.7 mg/100g), flavonoid (148.4 ± 17.3 mg/100 ml) and carotenoid (16.73 ± 0.29 µg/ ml) when compared to the results in this experiments. In general, commercial fermentation noni juice have a longer fermentation period, usually more than 365 days (Sablietou, 2002). With increasing time of fermentation, increasing total phenolic and flavonoid contents were observed. This could explain the finding in this research.

4.4.2 The effect of fermentation periods on the antioxidant activity of noni juice

Table 4.6 showed the antioxidant activity of noni juice during fermentation. After fermented of 1, 30, 60, 90 and 120 days the antioxidant activity was significantly increased. With increasing time of fermentation, the increase in antioxidant activity was occurred. Therefore, it was expected that antioxidant activity may increase more after 120 days. In general, the commercial fermented noni juice usually ferments for more than a year (Sablietou, 2002). It was not surprising that the commercial fermented noni juice had the highest antioxidant activity. Although the ascorbic acid and caroteonoid content was decreased during fermentation, the antioxidant activity was increased. Also antioxidant activity was correlated with flavonoid and total phenolic contents, their contents were increased during fermentation. This result suggest that the fermentation did the positive effect on the antioxidant activity.

Table 4.6 Antioxidant activity of fermented noni juice and commercial juice

Treatment	Antioxidant Activity IC_{50} (mg/g DPPH)
Fermented 1 days	31.48 ± 0.95^{de}
Fermented 30 days	29.23 ± 0.87^{d}
Fermented 60 days	25.67 ± 0.67^{c}
Fermented 90 days	23.78 ± 0.44^{bc}
Fermented 120 days	19.33 ± 0.52^{b}
Commercial ferment.	15.89 ± 0.34^{a}

Data were expressed as mean of 3 replications $\pm$ SD

Similar letters in each row indicate indicated treatments were not significantly different ($p > 0.05$).

4.5 The effect of storage temperature on the antioxidant contents of noni juices

Since the pasteurization at 71°C for 1 minute retained the highest content of flavonoid and total phenolic compounds and also a high antioxidant activity of noni juice, this pasteurization condition was selected to be further studied during a storage period.

The result are shown in Figure 4.6 (A). The ascorbic acid contents of the juice which pasteurized and stored at 4°C for 1, 7, 14 and 21 days were 58.9 ± 1.39, 49.2 ± 2.13, 46.2 ± 0.45 and 41.1 ± 0.92 mg/100ml, respectively. Ascorbic acid content was declined during storage at 4°C. Stored the juices at room temperature for 1, 7, 14 and 21 days has ascorbic acid content of 56.7 ± 1.18, 39.4 ± 1.47, 27.1 ± 2.68 and 24.2 ± 1.89 mg/100ml, respectively. Both of 4°C and room temperature storage showed decreasing of ascorbic acid contents by 30% and 57%, respectively. The reasons for ascorbic acid losses may be due to the ascorbic acid enhanced the stability of flavonoid and other phenolic compounds, which oxidized the asocorbic acid to dehydroascorbic acid and also reaction with others copigments to produced their degradation products (Gregory, 1996). Therefore, the degradation products of ascorbic acid were accumulated, they may enhance the stability of other phenolic compounds (Rodriguez-Saona *et al.*, 1999).

Figure 4.6 (B) shows that the decreased of carotenoid content after storage at 4°C for 1, 7, 14 and 21 days which were 11.78 ± 0.26, 11.00 ± 0.10, 10.56 ± 0.23 and 10.37 ± 0.29 ug/ml, respectively. For storage at room temperature for 1, 7, 14, 21 days, the reductions were 10.17 ± 0.31, ± 7.81 ± 0.10, 7.20 ± 0.23 and 6.03 ± 0.36 ug/ml, respectively. Both of 4°C and room temperature storage showed decreasing of carotenoid contents by 12% and 41%, respectively. This might be due to oxidation reaction after heat treatments. Conjugated double bond system of carotenoid could be destroyed after heating (Gregory, 1996). On the other hands, carotenoid may quench singlet oxygen and peroxyl radicals from the oxidation of ascorbic acid. Oxidative degradation of carotenoid by ascorbic acid has been previously reported (Bohm *et al.*, 1997).

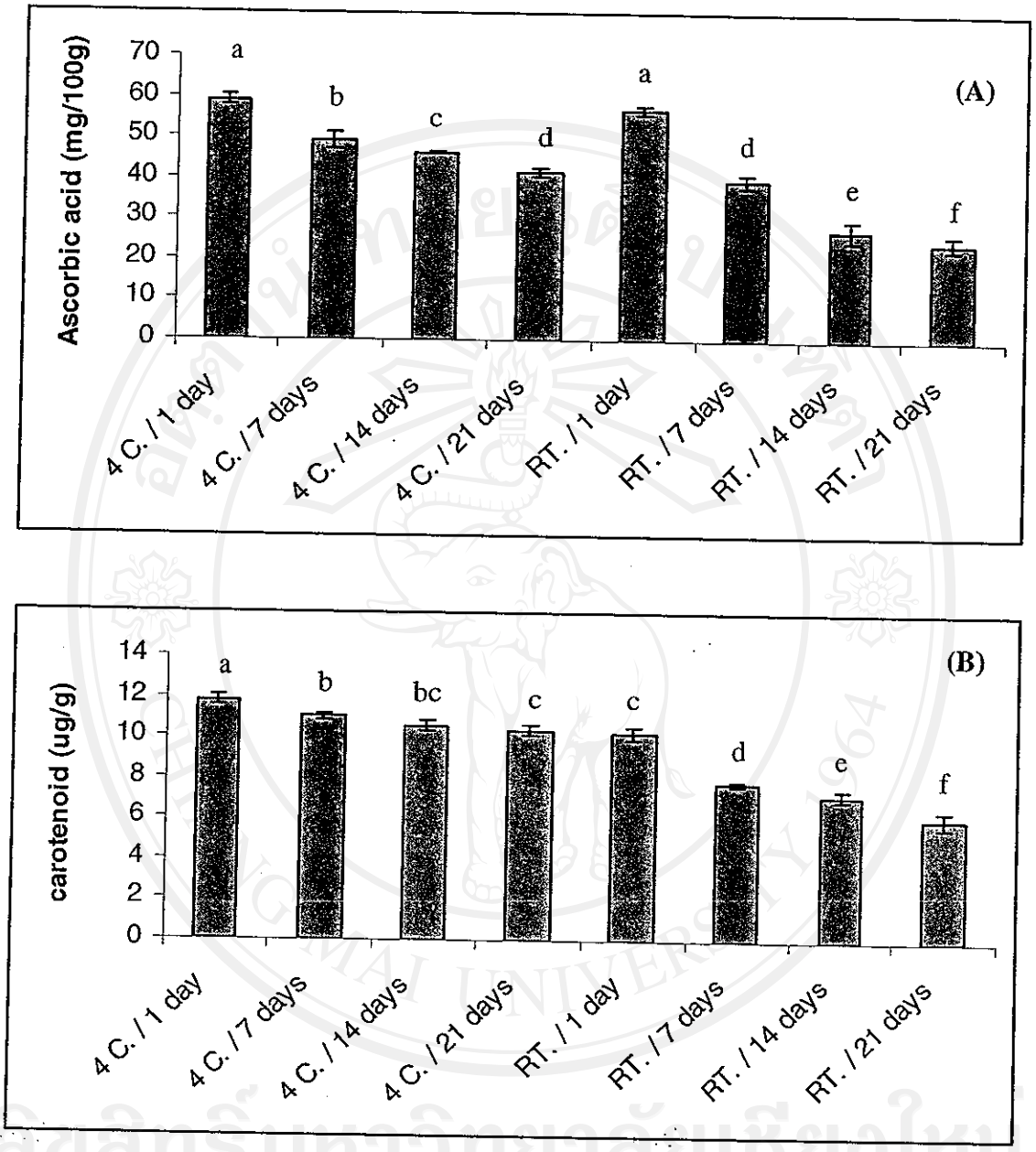

Figure 4.6 Effects of storage temperature on the ascorbic acid content (A) and carotenoid content (B) of noni juices (mean ± SD, n = 3). Similar letters in each bar indicated treatments were not significantly different (p>0.05).

Figure 4.7 (A) showed both at 4°C and room temperature storage, the flavonoid contents of noni juices did not change significantly, within a narrow range of 176.9 ± 10.8 to 180.8 ± 7.8 mg/100ml. These results suggested that storage temperature did not affect the flavonoid content. Similar to Figure 4.7 (A), both at 4°C and room temperature storage produced not significant change of total phenolic contents, which were within a narrow range of 342.9 ± 18.5 to 349.6 ± 27.7 mg/100ml. These results suggested that storage temperature did not affect the total phenolic content.

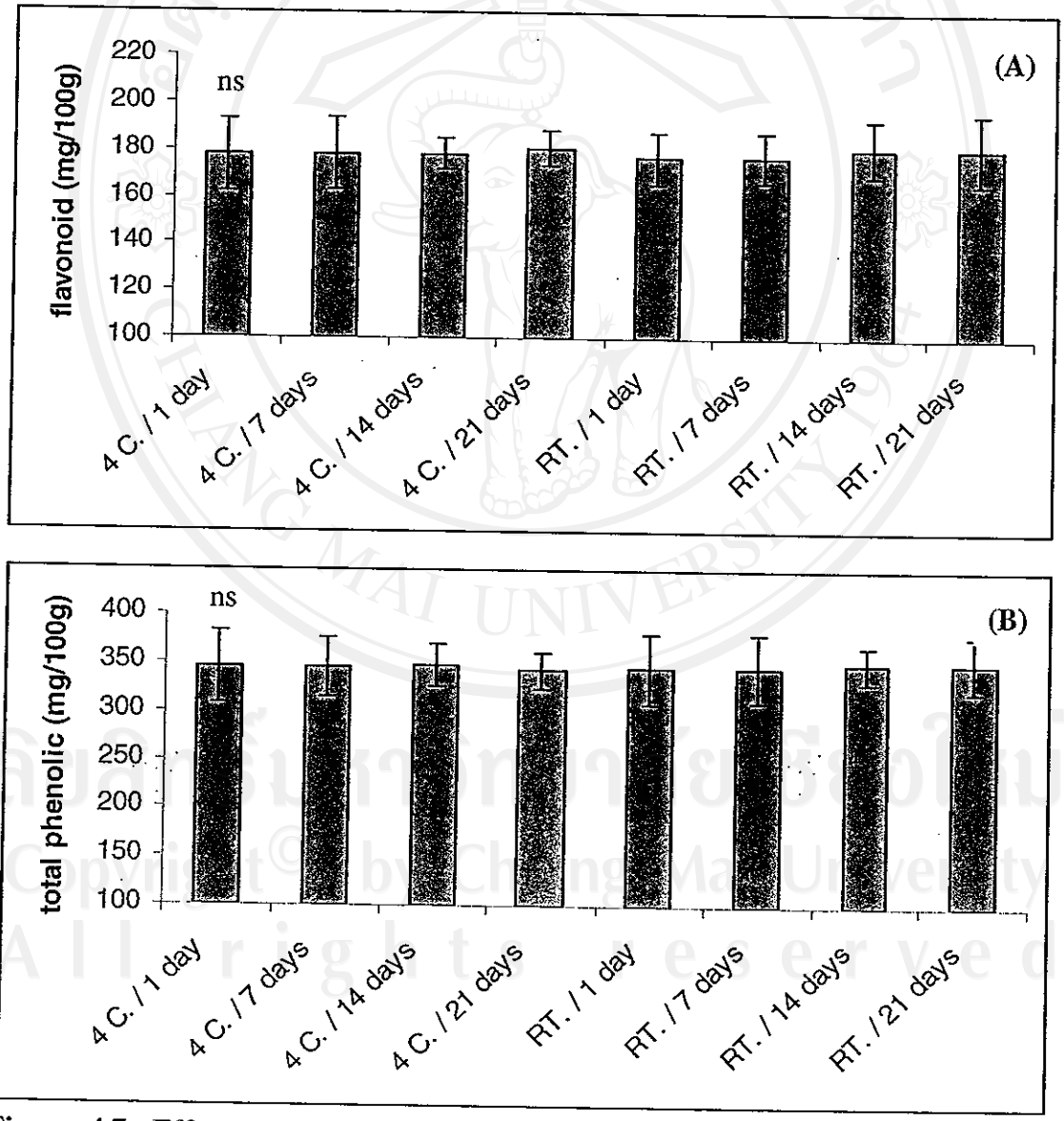

Figure 4.7 Effects of storage temperature on the flavonoid content (A) and total phenolic content (B) of noni juices (mean ± SD, n = 3). Similar letters in each bar indicated treatments were not significantly different (p>0.05).

Antioxidant activity of pasteurized noni juice after storage at 4°C and room temperature is shown in Table 4.7. No significant difference among all treatments. Antioxidant activity correlated with the content of flavonoid and total phenolic. The antioxidant activity did not change during storage despite some losses of ascorbic acid and carotenoid contents.

Table 4.7 Antioxidant activity of pasteurized noni juice during storage

Treatment	Antioxidant Activity IC_{50} (mg/g DPPH)
Storage at 4°C for 1 day	10.50 ± 0.95^a
7 days	11.02 ± 0.87^a
14 days	10.99 ± 0.67^a
21 days	10.39 ± 0.44^a
Storage at roomtemperature for 1 day	9.98 ± 0.16^a
7 days	10.13 ± 0.25^a
14 days	11.00 ± 0.01^a
21 days	11.77 ± 0.33^a

Data were expressed as mean of 3 replications $\pm$ SD
Similar letters in each row indicated treatments were not significantly different ($p > 0.05$).