Chapter 4

Results and Discussion

4.1 Proximate analysis and chemical characteristics of fresh lime juice

Proximate analysis and chemical characteristics of the fresh lime juice samples were shown in Table 4.1.

Table 4.1 Proximate analysis, physical and chemical characteristics of fresh lime juice

Parameters	Values*
Protein (%)	0.42 ± 0.06
Fat (%)	0.03 ± 0.05
Ash (%)	0.32 ± 0.03
Fiber (%)	0.11 ± 0.03
Carbohydrate (%)	9.44 ± 1.52
Moisture (%)	89.68 ± 1.48
pH	2.19 ± 0.21
Total Soluble Solid (^{o}Brix)	7.27 ± 0.5
Total solid (%)	10.32 ± 1.48
Total acidity (% citric acid)	7.09 ± 0.19
Ascorbic acid (mg / 100 ml)	37.18 ± 2.78
Yield (%)	34.42 ± 2.46
Reducing sugar (%)	0.35 ± 0.23
Total sugar (%)	0.58 ± 0.13

*) mean ± SD from triplicate of fresh lime juice samples

Table 4.1 (Cont.)

Parameters	Values*
Color (L*)	54.57 ± 2.63
(a*)	-2.58 ± 0.13
(b*)	8.45 ± 0.27

*) mean ± SD from triplicate of fresh lime juice samples

From Table 4.1, it could be seen that the fresh lime juice contained a lot of water and total solid, which was dominated by carbohydrate. The juice itself was an acidic solution with a pH value of 2.19 ± 0.21 and contained 7.09 ± 0.19% citric acid. High ascorbic acid content and a low total sugar content might contribute to sour taste of the juice when it was tested by sensory panelists (section 4.7, Tables 4.28 4.31). The juices had total soluble solid of 7.27 ± 0.50^{o}Brix, which consisted of all soluble materials in citrus juices, including reducing sugar and organic acids (Kimball, 1999).

The lime juice had a yellowish green color, which was represented by negative a* and positive b*-values. The color of citrus juice was mainly affected by the presence of two carotenoids, carotene and xanthophylls in juice (Ting and Rouseff, 1980). β-carotenene imparts a light yellow to orange color to citrus juice drink (Kimball, 1999).

Low acidity of the lime juice might affect the spoilage microorganisms that could grow in the product, such as yeast and mold and also some lactic/acetic bacteria that could tolerate the low pH of the juice (Hocking and Jensen, 2001). At the same time, the presence of protein and reducing sugar in juice might facilitate a reaction of non-enzymatic browning reaction during a prolong storage of lime juice, although this reaction could be delayed by the presence of ascorbic acid in juice (Burdurlu *et al*., 2006).

4.2 Physical, chemical, nutritional and microbial qualities of fresh lime juice (non-processed lime juice) during storage at 4-6°C and at ambient temperature for 1 month period

4.2.1 Chemical characteristics of fresh lime juice

Chemical characteristics of the fresh lime juice, including pH, % total acidity and total soluble solid, during storage at 4-6°C and at ambient temperature could be seen in Tables 4.2 and 4.3, respectively. The pH values of the fresh lime juices were significantly affected by the storage temperatures and storage time. However, the pH values of the juices kept at ambient temperature was increased at a higher rate than those stored at 4-6°C. Storage of the fresh lime juices at different storage temperatures for 1 month also affected the total soluble solid and total acidity of the juices. The total soluble solid of the lime juices did not significantly change when the juice was stored at chilled temperature. However, the total soluble solid was significantly reduced when the juices were kept at ambient temperature. This result could be affected by the growth of microorganisms in the juices when the juice was kept in the higher storage temperature (section 4.2.4).

For the total acidity, the juice acidities were significantly decreased during storage with a higher decreasing rate in the samples stored at ambient temperature compared to those that kept at 4-6°C. This result was consistent with the increase in the juice pH values and could be due to chemical reactions, including Maillard reactions (Koca *et al*., 2003), that occurred slowly during the storage period. Clegg

(1966) also reported that at pH about 2.5, citric acid was able to change in brown substances and accelerated oxidation of ascorbic acid.

Table 4.2 Chemical characteristics of fresh lime juice during one month storage at 4-6°C

Chemical Parameters		Storage period (weeks)			
	0	1	2	3	4
Total acidity (% citric acid)	7.03 ± 0.12^{a}	6.87 ± 0.11^{ab}	6.82 ± 0.08^{b}	6.76 ± 0.06^{b}	6.72 ± 0.05^{b}
pH value	2.34 ± 0.08^{a}	2.34 ± 0.05^{ab}	2.37 ± 0.03^{b}	2.37 ± 0.05^{b}	2.37 ± 0.03^{b}
Total soluble solid (°Brix)^{NS}	7.83 ± 0.06^{a}	7.83 ± 0.06^{a}	7.83 ± 0.11^{a}	7.80 ± 0.06^{a}	7.80 ± 0.06^{a}

Values within a row followed by different letters were significantly different ($p<0.05$)
Mean ± SD
NS = not significant different

Table 4.3 Chemical characteristics of fresh lime juice during one month storage at ambient temperature

Chemical Parameters		Storage period (weeks)			
	0	1	2	3	4
Total acidity (% citric acid)	7.03 ± 0.12^{a}	6.86 ± 0.06^{b}	6.77 ± 0.02^{bc}	6.66 ± 0.06^{cd}	6.58 ± 0.07^{d}
pH value	2.34 ± 0.08^{a}	2.39 ± 0.04^{b}	2.41 ± 0.03^{b}	2.43 ± 0.04^{b}	2.43 ± 0.05^{b}
Total soluble solid (°Brix)	7.83 ± 0.06^{a}	7.80 ± 0.03^{a}	7.73 ± 0.05^{b}	7.67 ± 0.06^{bc}	7.58 ± 0.06^{c}

Values within a row followed by different letters were significantly different ($p<0.05$)
Mean ± SD

4.2.2 Nutritional value of fresh lime juice

Nutritional value of the fresh lime juice during one month storage represented by the ascorbic acid content was displayed in Figure 4.1. The results showed clearly that the ascorbic acid content of the lime juices was significantly decreased during the storage period. Keeping the juice at chilled temperature only produced a slightly better retention of ascorbic acid within one week storage followed by a further reduction in ascorbic acid content causing different storage temperatures to be insignificantly in affected the ascorbic acid content in the lime juice.

A higher decreasing rate of ascorbic acid at the beginning of the storage period could be attributed to the immediate reaction of the compound with dissolved oxygen that was incorporated into juices during processing (Polydera *et al*., 2003). This was in an agreement with characteristic of ascorbic acid that was reported to be an unstable compound and highly sensitive to changes by environments, such as pH, oxygen, light, temperature, the presence of trace metal catalysts and enzyme. The structure of ascorbic acid has a two-electron oxidation and hydrogen dissociation convert L-ascorbic acid to L-dehydroascorbic acid (DHAA). The main chemical degradation of ascorbic acid involves oxidation of the compound to DHAA, followed by hydrolysis to 2,3-diketoglulonic acid and further oxidation, dehydration and polymerization (Fennema, 1996). Storage time and storage temperature could also affect the stability of the ascorbic acid (Gordon and Samaniego-Esguerra, 1990). An additional explanation can be seen in the next section.

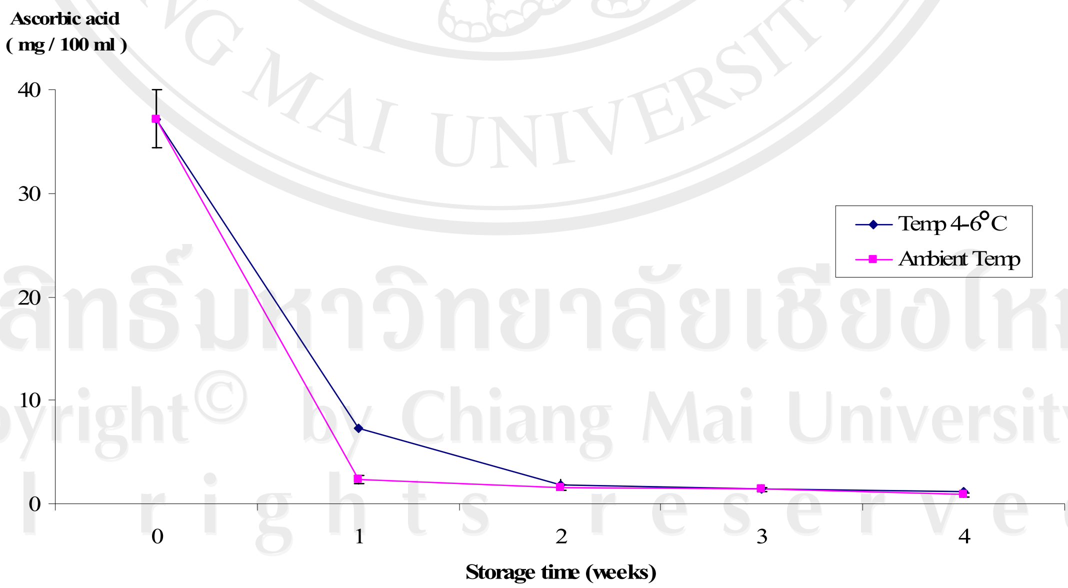

Figure 4.1 The content of ascorbic acid in fresh lime juices during one month storage at different storage temperatures

4.2.3 Physical characteristics of lime juice

The physical characteristics of lime juice measured from the juice color was reported based on L*, a* and b*-values. The L*-value represents the lightness of the juice, the a*-value measures the green or red direction of the juice and the b*-value shows the blue or yellow direction of the sample.

4.2.3.1 L*-value (Lightness)

The monitoring results for the L*-value of the lime juice during storage that were shown in Figure 4.2 demonstrated that storage temperature did not significantly affect the L*-value of the juice. At any storage temperature, the L*-value of the juices was slightly changed within the first week of storage followed by a significant reduction into a brown, darker and deeper color, especially for the juice that stored at ambient temperature, in the middle of the storage period before it had a significant increase again at the end of the storage. The lime juice samples at the end of the storage period was bright and clear and experienced loss of its cloudiness, which could be due to precipitation of the colloidal substances of lime juices. This result was similar to the observation of Wattanaphahu (2002).

Changing in the L*-value of the lime juice could be affected by the precipitation and extraction process of the juice. During these processes, oxidation of the juice compounds might be occurred causing the juice to have dark and brown color (Nagy *et al*., 1977). At the same time, during the extraction, a pectin methyl esterase (PME) (Nagy *et al*., 1977) enzyme was released into the juice. The presence of this enzyme in the juice caused a hydrolysis in the pectin chain, which was a colloid substance of the juice, into low methoxy pectin. The low methoxy pectin was then formed complexes with calcium producing insoluble calcium flocculation that resulted in the loss of the cloudiness and made the juice became a bright and clear juice (Joslyn and Pilnik, 1961).

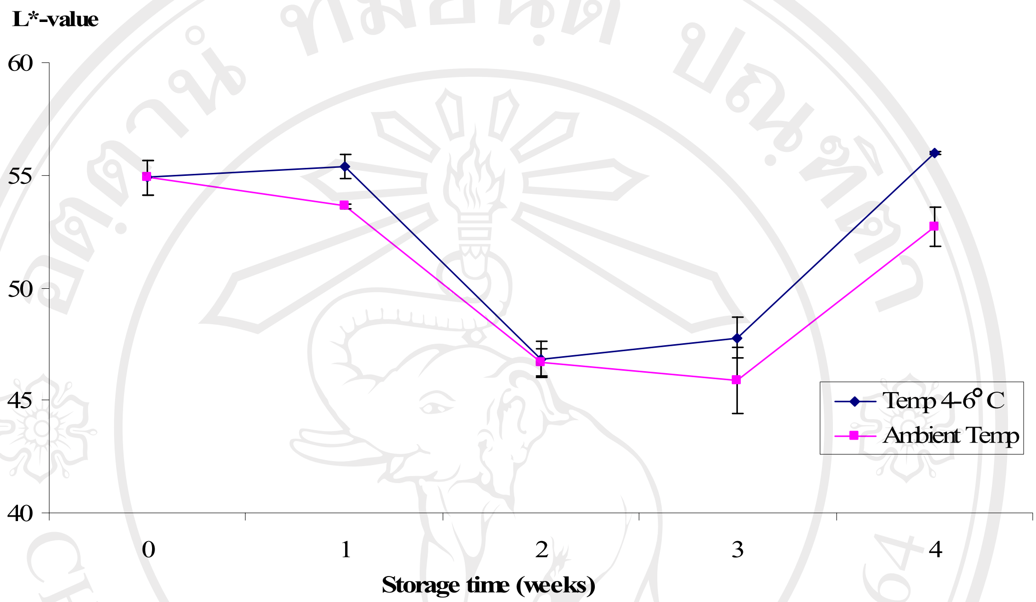

Figure 4.2 The L*-value of fresh lime juices during 1 month at different storage temperatures

4.2.3.2 a*-value (green to red scale)

Measurement of the a*-value of the lime juice samples during storage for 1 month illustrated in Figure 4.3 clearly displayed that the juice samples significantly had an increase in its a*-value during storage and was significantly affected by the storage temperature. Keeping the juice samples at ambient temperature significantly changed the color direction of the a*-value from a green color (negative a*-value) at the beginning of the storage period into a red color (positive a*-value) at the end of the storage time. At the same time, for the lime juice samples kept at chilled temperature, a significant reduction in the green color was recorded.

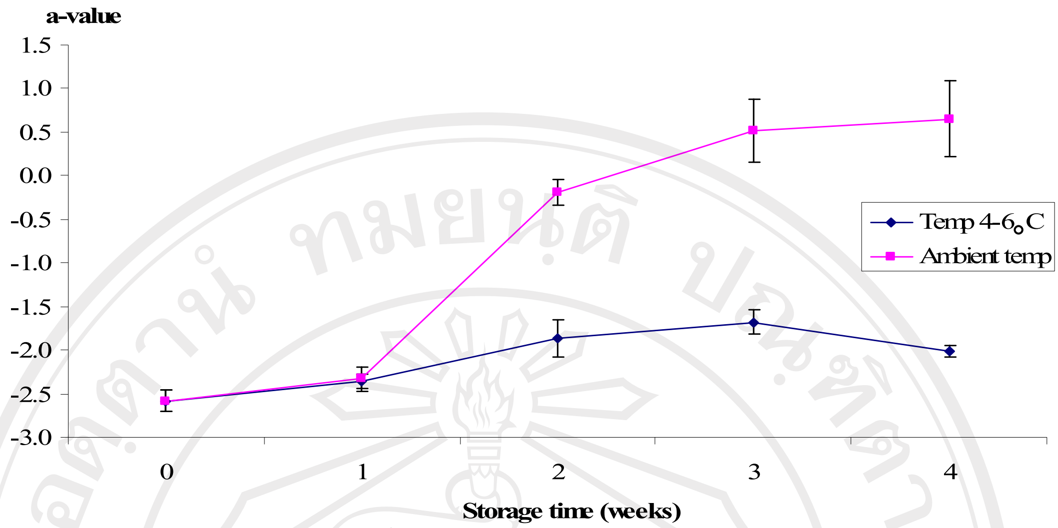

Figure 4.3 a*-value of fresh lime juices during 1 month at different storage temperatures

Reduction in the green color or even a development of a red color in the lime juice samples during storage could be due to non-enzymatic browning reactions, which gave undesirable off-taste and off-color in citrus juice (Handwerk and Coleman, 1988).

The non-enzymatic browning in acidic media such as juice products, is primarily due to the ascorbic acid degradation rather than the reaction between the reducing sugars and amino acids (Kaanane *et al*., 1988).

Browning in the citrus juices as a result of ascorbic acid degradation and is known as a cause of non-enzymatic browning in food (Marcy *et al*., 1984) involves a formation of furfural. The polymerization of the last compound is responsible for the development of browning in citrus juices and other food products. It has been known that uronic acid decomposes in acid solutions, first into pentose and carbon dioxide and subsequently into furfural (Braverman, 1949).

The production of furfural from ascorbic acid in the presence of acid is accompanied with a development of CO_2. The polymerization of furfural causes a production of brown substances. This fact has been observed from concentrated orange juice that was stored at ambient temperature and produced CO_2 without any signs of fermentation (Braverman, 1949).

In addition, hydroxymethylfurfural (HMF) is another product from ascorbic acid (Burdurlu *et al.,* 2006) and is suggested as a precursor of brown pigments (Solomon *et al*., 1995). The accumulation of HMF can also come from a degradation of reducing sugars and Maillard reactions (Yaylayan, 1990). Darkening of citrus juices during storage occurs after ascorbic acid has been transformed into its dehydroascorbic acid form and when no readily oxidizable substances are left in the juice (Braverman, 1949).

4.2.3.3 b*-value (blue to yellow scale)

The b*-value indicated a blue color direction if negative value and a yellow color direction if positive values. For the b*-values of the lime juice samples during storage for 1 month shown in Figure 4.4 clearly demonstrated a significant increase in the b*-value of the juice samples throughout the storage period. Different storage temperatures significantly affected the development of the yellow color in the juice samples with higher increasing rate of the b*-value when the juice samples were kept at higher storage temperature.

Changes in the color values of the lime juice samples during storage were correspondent with the concentration of ascorbic acid in the juice (section 4.2.2). A rapid degradation of the ascorbic acid within the first week of storage (Figure 4.1) due to chemical reactions that caused ascorbic acid degradation was found to significantly affect the color values (L*, a* and b*-values) of the lime juice. The color of the juice samples became dark and developed a brown color. Changes in this section were in an agreement with reports of Roig *et al*. (1999) and Burdurlu *et al*. (2006), who reported that an extensive change, especially in color and flavor, occurred in fruit juices during storage and run parallel with the progressive decrease in the amount of ascorbic acid.

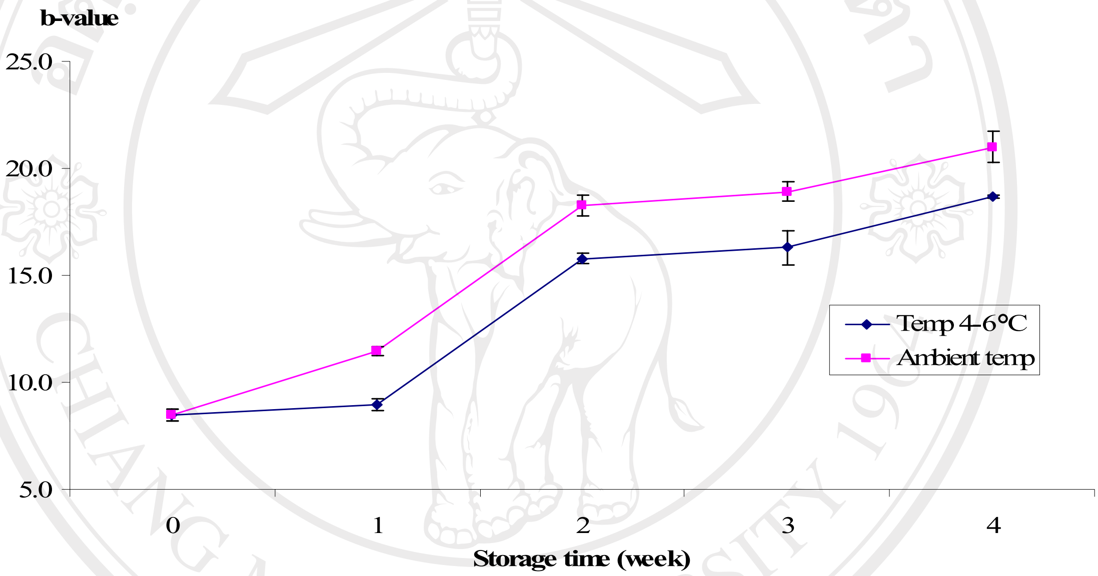

Figure 4.4 b*-value of fresh lime juices during 1 month at different storage temperatures

4.2.4 Microbial stability during storages

The microbial quality of fresh lime juice, including total aerobic plate count and the count of yeast and mold, during one month storage could be seen in Figure 4.5. The weekly monitoring displayed that the fresh lime juice contained low initial numbers of microbial counts, in which the number of total plate count together with yeast & mold were less than 25 and 15 CFU/ml, respectively. No coliform bacteria and *Escherichia coli* was detected in the fresh lime juice samples.

During a month storage, the growth of microorganisms in the fresh lime juices was significantly be affected by storage temperature. Keeping the juice samples at refrigerated temperature maintained the microorganisms number in the samples as low as the initial microbial count. Whereas, a significant microorganisms growth was detected when the juice samples were kept at ambient temperature.

An increase for up to $6.60x10^2$ and $1.30x10^2$ CFU/ml of total plate count together with yeast and mold, respectively, was recorded in the juice samples kept at high storage temperature and being accompanied with undesirable changes in the juice characteristics, including gas formation; alcoholic production, which could be due to yeast fermentation (Braverman, 1949) and development of off-flavor, which might be due to lactic acid fermentation that produced abnormal flavor and odor (Nagy *et al*., 1977).

The significant increase in the microbial number of the fresh lime juices stored at high storage temperature indicated that mesophilic spoilages microorganisms, such as yeast, mold and some lactic acid bacteria could be responsible for the spoilage developed in the juice samples. Yeast and mold have been reported to be the major microorganisms that spoil citrus juice products due to their survival and growth at low pH environments and their capability to use of sugar and vitamin in the juice (Ogawa *et al*., 1990). Hocking and Jensen (2001) reported to identify several yeast and mold genera, including *Saccharomyces cerevisiae*, *Aspergillus* sp., *Penicillium glaucum*, etc. from citrus juice. They also found more than 100 bacteria cultures in the juice samples.

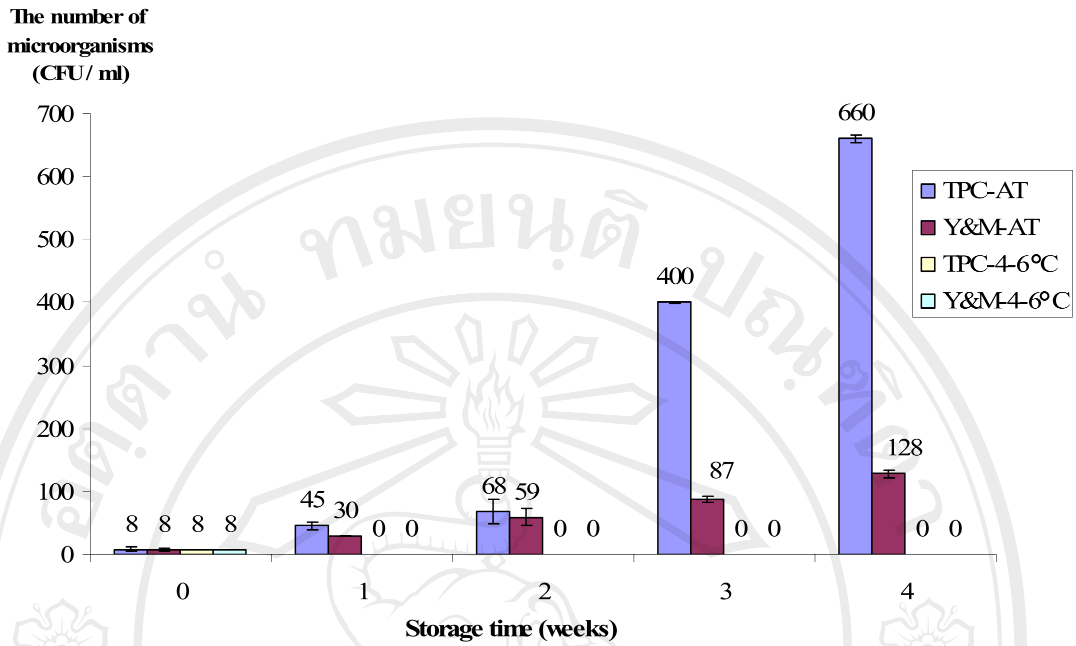

Figure 4.5 Total plate count (TPC) and the count of yeast and mold (Y&M) of fresh lime juice during one month storage at different storage temperatures

Citrus juice is sterile when it is inside the endocarp. However, after an extraction, the juice invariably shows quite a rich microflora, consisting of yeast, bacteria and spores (Parish, 1998b). Usually the fruit surface carries an assortment of dead and living fungi and bacteria, particularly when the first is grown in a humid and climate temperature (Nagy *et al*., 1977). The fungal mycelium can be intimately associated with the surface wax, epidermal cells and stomatal chambers (Hocking and Jensen, 2001).

All of these microorganisms can find their way into the expressed juice by various means, including a contact with the peel or with various parts of machinery and probably also from environment (Braverman, 1949). This is a major source of potential contamination in extracted juices for processed citrus products.

4.3 Distribution of d-limonin in lime fruit

The distribution of d-limonin in each lime fruit parts showed that the seed had the highest d-limonin content of 194.1 ± 5.33 ppm, followed by the segment membrane, albedo, flavedo and juice sacs with 19.28 ± 0.34, 16.48 ± 1.34, 8.58 ± 0.26 and 7.14 ± 0.46, respectively (Figures 4.6 and 4.7).

Similar results had been reported by Hasegawa *et al*. (1980), Matthews *et al.* (1990) and McIntosh and Mansell (1997) who found that the highest amount of d-limonin was in seeds of citrus fruits and smaller amounts in the central core and segment membrane. Kasemsuksakul and Noomhorm (1992) analysed the d-limonin contents in different parts of Thai tangerine using a thin-layer chromatography and reported that the highest limonin contents was in seed followed by the segment membrane, albedo, flavedo and juice vesicles.

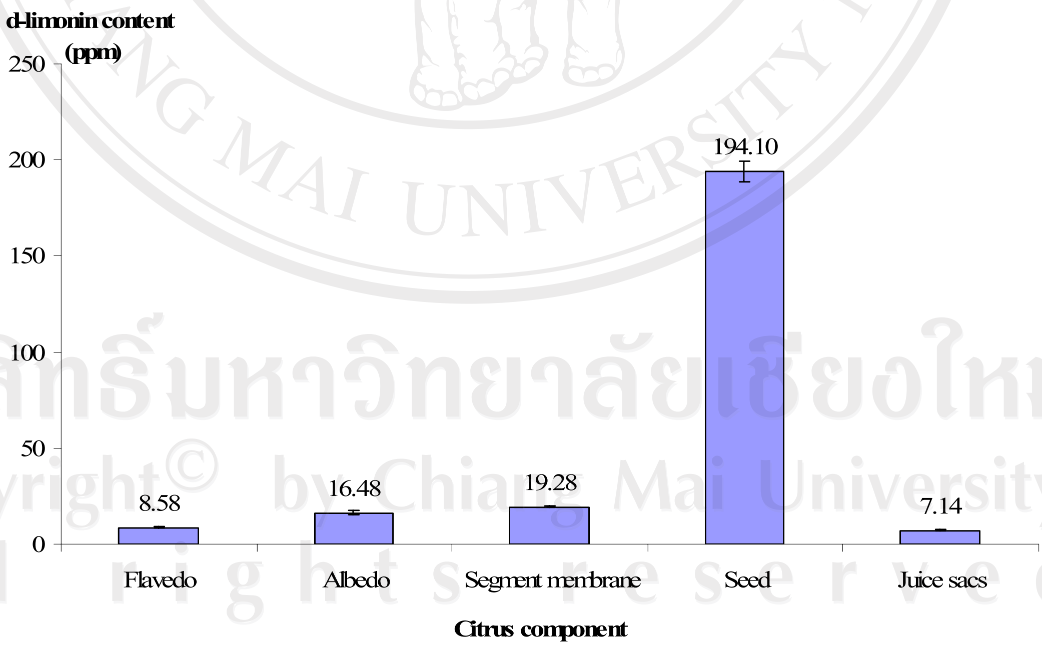

Figure 4.6 The distribution of d-limonin in lime fruits

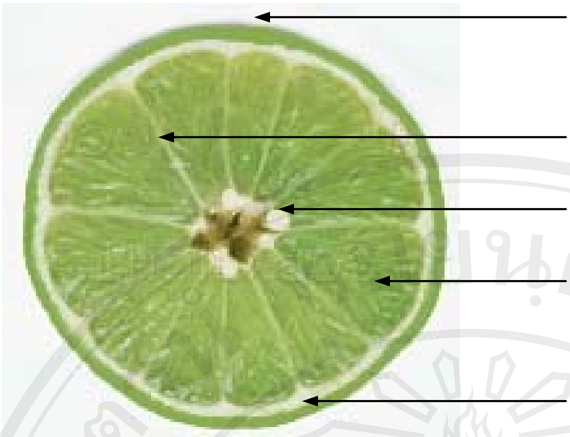

Figure 4.7 Cross-section of lime fruit, showing the d-limonin distribution in each lime fruit part

4.4 The level of d-limonin in fresh lime juice during one month storage at different storage temperatures

The freshly squeezed lime juices had an initial d-limonin level of 6.85 ± 0.06 ppm, which was around the human detection for the compound. Guadagni *et al.* (1974) reported that the least sensitive individual had a d-limonin threshold detection limit of 5-6 ppm. Due to these reasons, some of the sensory panelists could not detect the bitterness in the fresh lime juice (section 4.7.1). However, within a few hours after juicing at ambient temperature or overnight if stored in a refrigerator, the extracted juice became bitter (Hasegawa and Maier, 1990). Therefore, after one week storage, the d-limonin content of fresh lime juices increased significantly to 34.89 ± 0.16 and 23.34 ± 0.53 ppm when the juice samples kept at chilled and ambient temperature, respectively (Figure 4.8).

Result in this section was similar to Hasegawa *et al*. (1992) and Pao *et al*. (1997) who found the bitterness in citrus juice develops gradually after extraction and referred as delayed bitterness.

The limonoate-A-ring lactone (non-bitter precursor-tasteless) was formed into d-limonin within 2-3 hours and increased to 9 ppm after 1 week storage stored at 4-6°C in fresh-cut of Valencia orange. The reaction proceeds under acidic conditions below pH 6.5 and is accelerated by the enzyme limonoid D-ring lactone hydrolase. This enzyme has been isolated from citrus and shown to be an extremely heat stable enzyme (Hasegawa, 1995). Limonoid bitterness develops slowly during storage of citrus juices and made them unacceptable to the consumers (Maier *et al*., 1977).

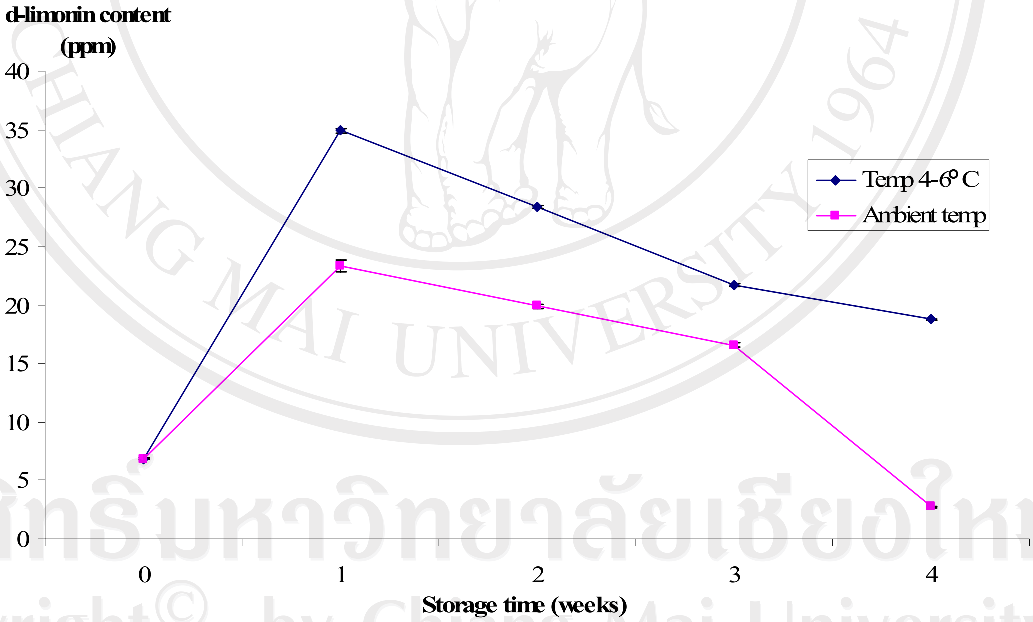

Figure 4.8 The d-limonin content of fresh lime juice during one month storage at different storage temperatures

After one week storage, the levels of d-limonin in fresh lime juice were significantly decreased, especially for the juices that kept at ambient temperature. The changing could be due to the degradable properties of d-limonin (Maier *et al*., 1980). Rouseff (1980) reported that naringin and d-limonin were precipitated out of the tangerine juice samples during storage due to precipitation of solid particle in juice.

In addition, the growth of spoilage microorganisms in the juice that capable to use d-limonin as a sole carbon source (Vaks and Liftshitz, 1981) might contribute to a higher decreasing rate of d-limonin in the lime juices stored at ambient temperature compared to those that stored at refrigerator temperature.

4.5 The effect of hydrocolloids on physical, chemical, nutritional characteristic and d-limonin content of lime juice during one month storage

4.5.1 Effect of different types and concentrations of hydrocolloids on the d-limonin content of lime juice during one month storage at ambient temperature

Four hydrocolloid types, including gum acacia, pectin, CMC and κ-carrageenan were added at levels of 0, 0.5, 1.0 and 1.5 g/l (w/v) into the fresh lime juices to understand whether the hydrocolloids could reduce the bitterness problem of the lime juice. After mixed samples thoroughly, the hydrocolloid added-lime juice were stored at ambient temperature for a month.

4.5.1.1 Gum acasia

Different levels of gum acacia in the lime juices were found to significantly reduce the d-limonin content of the juice at the beginning of storage period (Appendix

E, Table 1E). Higher reduction of the d-limonin content was recorded at higher levels of gum acasia. A reduction for slightly more than half of the d-limonin content in the control treatment could be achieved when 1.5 g/l (w/v) gum acasia was added into the juice at 0 week. There was a significant ($p<0.05$) reduction on the d-limonin content when different levels of gum acasia was added in the lime juice during the storage period.

The presence of gum acasia in the lime juices could significantly produce lower level of d-limonin compared to the control treatment throughout the storage period. The highest gum acasia level of 1.5 g/l (w/v) was found to be the best addition level in maintaining the d-limonin content to be below 7.52 ± 0.02 ppm.

4.5.1.2 Pectin

The addition of pectin was not as effective as gum acasia in reducing the levels of d-limonin at the beginning of the storage period (Appendix E, Table 2E). No significant difference was found for the d-limonin content when different levels of pectin were added into the lime juices before the storage period. However, the effectiveness of pectin to decrease the d-limonin content was significantly shown during the storage period. Higher addition levels of pectin was more effective in decreasing the d-limonin content. At the highest addition levels of pectin of 1.5 g/l (w/v), the amount of d-limonin content could be maintained to be lower than the initial level of 6.01 ± 0.32 ppm throughout the storage period.

4.5.1.3 Carboxymethylcellulose (CMC)

Interestingly, the addition of CMC into the lime juice was shown to be optimum in reducing the d-limonin content at a level of 1.0 g/l (w/v) at the storage period (Appendix E, Table 3E). During the storage period, the CMC could significantly decrease the d-limonin content compared to the control treatment, but different CMC levels did not significantly produce different reduction levels, except after one week storage period.

The conversion of limonoate-A-ring lactone (a non-bitter precursor) into d-limonin might have been slowed down due to an increase in pH with the addition of CMC (section 4.5.2.1). It was observed that the storage lime juices with higher pH values could significantly had lower d-limonin content than the control treatment, which could be due to unfavorable conditions for conversion of limonoate A-ring lactone to d-limonin (Premi *et al*., 1995).

4.5.1.4 κ-carrageenan

The presence of κ-carrageenan in the lime juice produced higher d-limonin content at the beginning of the storage period (Appendix E, Table 4E). This result could be affected by the gel formation formed by κ-carrageenan in the juice causing the juice to be more concentrated. However, during the storage period the κ-carrageenan was still effective in reducing the level of d-limonin in the lime juices. The optimum level of κ-carrageenan to reduce the d-limonin content was found at 0.5 g/l (w/v) at the end of the storage period.

Similar results to the finding in this research were had been reported by Aggarwal and Sandhu (2004) who found that hydrocolloids could reduce d-limonin content in kinnow juice. The hydrocolloids of CMC and sodium alginate were found to be more effective than the control treatment in masking the bitterness from limonin content after six months storage.

4.5.1.5 The overall effect of different types and levels of hydrocolloids on the d-limonin content of lime juice during storage

To understand the effect of different types and levels of hydrocolloids on the d-limonin content of lime juice, collected data was displayed in Figures 4.9-4.11 and statistically analyzed (Appendix E, Tables 1E-4E). The statistical analysis clearly displayed that CMC (in the form of sodium salt) significantly produced the lowest level of d-limonin in the lime juice compared to those of the other hydrocolloids throughout the storage period.

The CMC effect could be due to an increase in the lime juice pH producing unfavorable conditions for conversion of limonoate A-ring lactone to d-limonin (Aggarwal and Sandhu, 2004 and Premi *et al*., 1995). At the same time, the d-limonin could be encapsulated by solid particles of the juices. This possibility was based on a report that demonstrated that sodium alginate and CMC could act as protective colloid by covering the solid particles and making a bridge between the continuous phase and the particles they enveloped (Aggarwal and Sandhu, 2004 and Glicksman, 1982). It could not be ruled out that because of the covering of the suspended particles, the diffusion of limonoate-A-ring lactone to d-limonin from the suspended solids or pulp to juice could also be reduced.

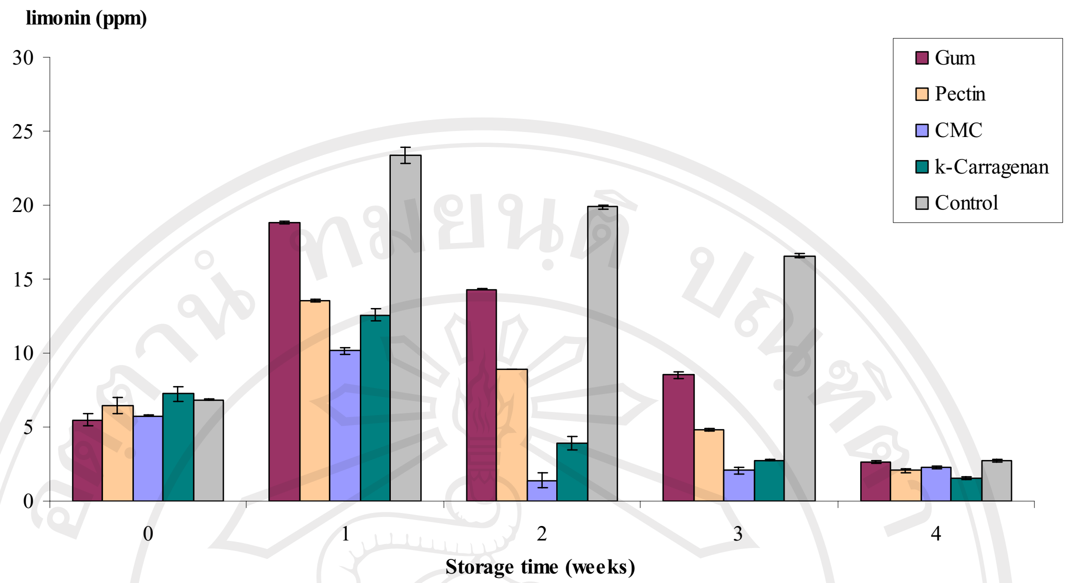

Figure 4.9 The d-limonin content of lime juices added with 0.5 g/l (w/v) hydrocolloids during one month storage at ambient temperature

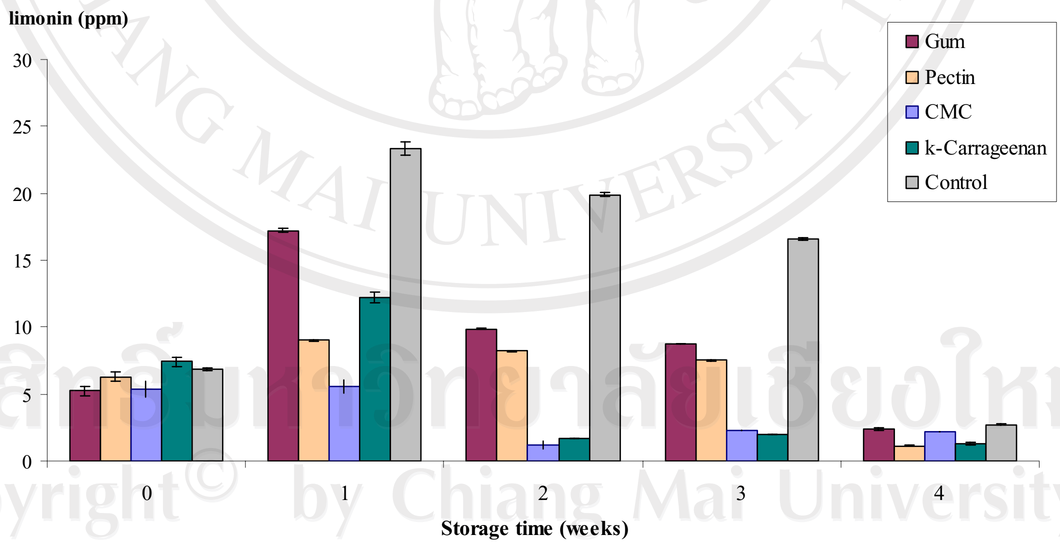

Figure 4.10 The d-limonin content of lime juices added with 1.0 g/l (w/v) hydrocolloids during one month storage at ambient temperature

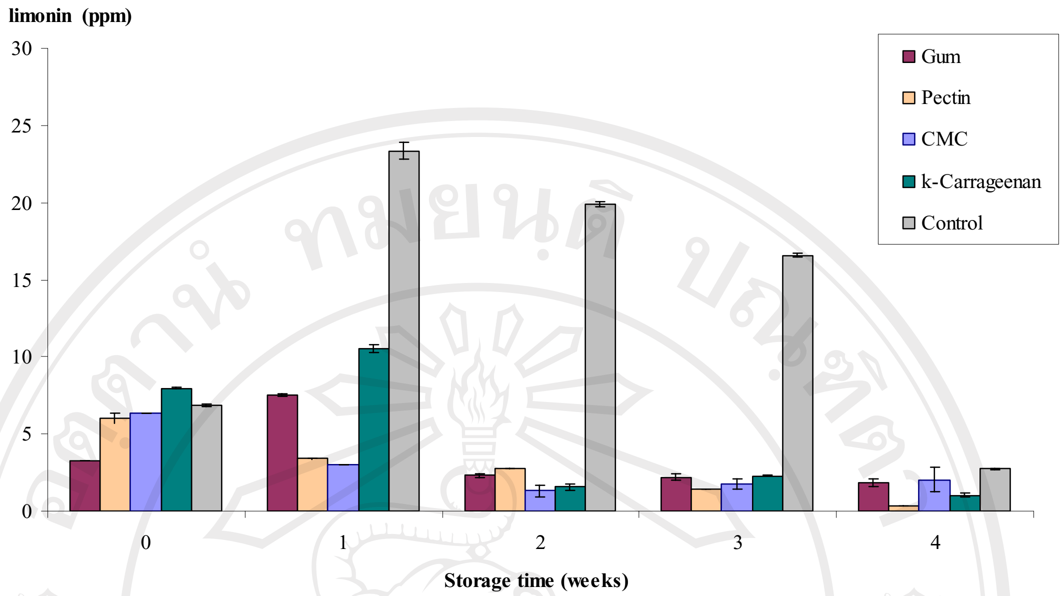

Figure 4.11 The d-limonin content of lime juices added with 1.5 g/l (w/v) hydrocolloids during one month storage at ambient temperature

Other mechanism that could reduce the level of d-limonin by hydrocolloids was the possibility of hydrocolloids absorption properties. Hydrocolloids, such as cellulose esters, including cellulose acetate, cellulose triacetate and cellulose acetate butyrate in either gel or powder form could adsorb limonin from citrus juices (Chandler and Johnson, 1977). Raksaphort and Soontarapa (2005) displayed that d-limonin content in orange juice was reduced and absorbed by chitosan and the mechanism of debittering was due to forming hydrogen bond between amino group of chitosan and lactone group of d-limonin.

Finding in this research clearly shown that the presence of different hydrocolloids could significantly reduce the level of d-limonin in the lime juice directly after the hydrocolloid addition and/or mainly during the storage period at ambient temperature. The presence of CMC was better than those of the other hydrocolloid in reducing the d-limonin level of d-limonin after one week storage, the concentration of 1.5 g/l (w/v) CMC did not significantly exhibit a lower level of d-limonin compared to that of the 1.0 g/l (w/v) CMC at the beginning and at the end of the storage (Figure 4.10-4.11). Therefore, the concentration of 1.0 g/l (w/v) CMC was chosen to be studied further in the next experimental section.

4.5.2 Chemical characteristics of hydrocolloids treated-lime juice during one month storage at ambient temperature

Chemical properties of hydrocolloid treated-lime juice based on total titratable acidity, total soluble solids and pH could be observed in Figures 4.12, 4.13 and 4.14, respectively. In general, the statistical analysis showed that different types and levels of hydrocolloid did not significantly affect the chemical properties of the lime juices at the beginning of the storage period. However, during the storage period, the presence of different types and levels of hydrocolloids significantly produced different effects on the lime juice chemical characteristics.

Total acidity (%citric acid), pH value and Total Soluble Solid

Additions of gum acasia, pectin and CMC into the lime juices produced lime juice acidities within the acidity of the control treatment in the first two weeks of storage followed by more significant changes in the juice acidities after this period. At the same time, these three types of hydrocolloids significantly reduced the total soluble solids of the juice samples to be lower than that of the control treatment during the storage period.

On the other hand, the presence of κ-carrageenan in the lime juice samples significantly caused the lime juice acidities and total soluble solids to be higher than those of the control and other hydrocolloid treated-lime juices after one week storage at ambient temperature.

The effect of different hydrocolloid types on the total acidity and total soluble solids of the lime juice was not significantly be affected by the addition levels of the hydrocolloids, except for the κ-carrageenan.

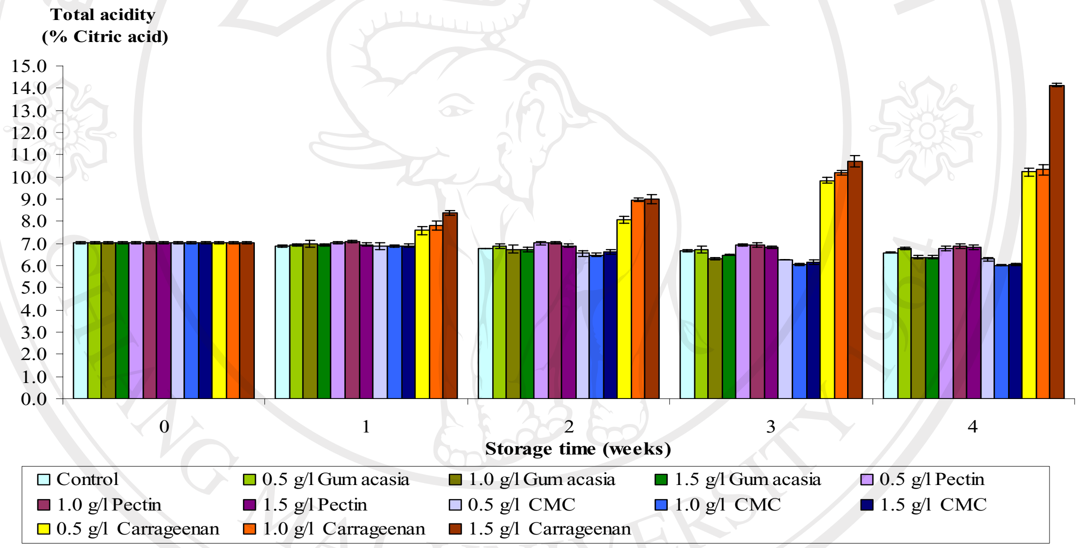

Figure 4.12 Total acidity (% citric acid) of lime juices added with different types and levels of hydrocolloids during one month storage at ambient temperature

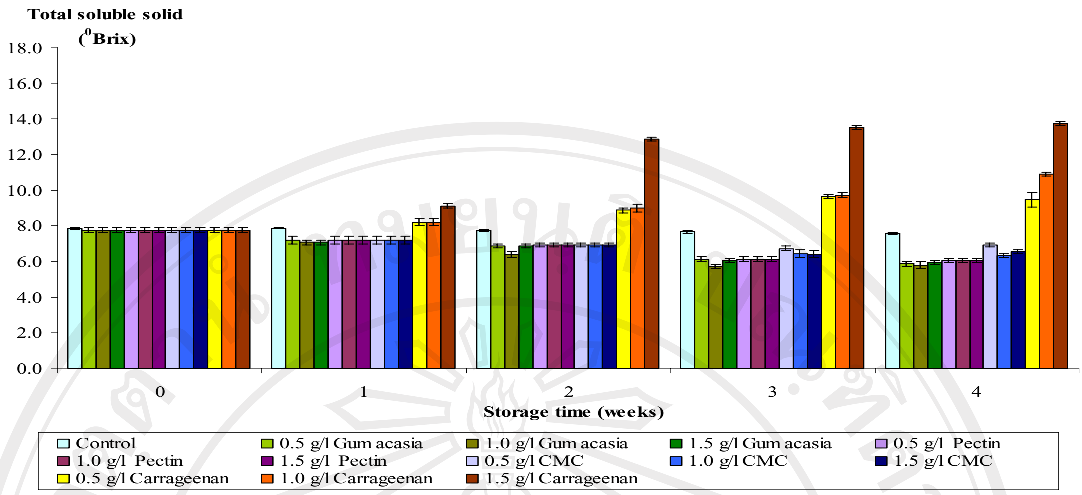

Figure 4.13 Total soluble solid (^{0}Brix) of lime juices added with different types and levels of hydrocolloids during one month storage at ambient temperature

For the pH of the lime juice, Figure 4.14 clearly illustrated that keeping the lime juices at ambient temperature significantly increased the pH of the juice samples during the storage period. In general, the hydrocolloid treated-lime juices had higher pH values than that of the control treatment after 2 weeks storage time. This finding was not significantly be affected by the hydrocolloid levels.

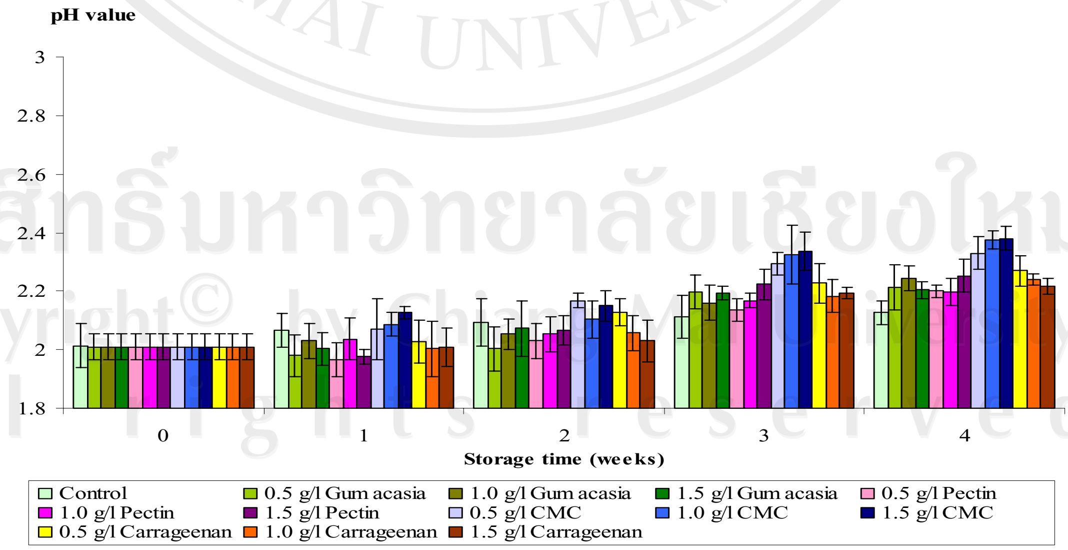

Figure 4.14 pH value of lime juices added with different types and levels of hydrocolloids during one month storage at ambient temperature

A significant different effect of κ-carrageenan on the total acidity and total soluble solids of the lime juices might be affected by the specific properties of compound, including binding water, promoting gel formation at low concentration, swelling and acting a thickener (Nussinovitch, 1997). Different types of hydrocolloids and use levels have an effect on the properties of the solutions (Phillips and Williams, 2000). In addition, the concentration of κ-carrageenan used in this study was higher than that normally applied in the food industry, which was a concentration of lower or up to 0.03% (Blanshard and Mitchell, 1979). This higher concentration of κ-carrageenan could also responsible for changing in the lime juice chemical characteristics. The significant decrease in the total soluble solid of the lime juice control treatment and hydrocolloids treated-lime juices, expect κ-carrageenan, could be affected by the growth of microorganisms that used the materials in the juices as their food sources as was found in the section 4.2.1. This section also explained the results of total acidity and pH value found in this section.

4.5.3 Nutritive values of hydrocolloids treated-lime juice of during one month storage at ambient temperature

As was found in the sections 4.2.2, the nutritional value measured as ascorbic acid of the lime juices was significantly reduced during the storage period for all of the hydrocolloid treated-lime juices (Figure 4.15). A very high reduction rate of the ascorbic acid was found in the first week of the storage followed by a lower reduction rate due to a low level of the ascorbic acid in the samples.

Ascorbic acid is a heat sensitive nutrient (Saguy *et al*., 1978). The compound is affected by the oxygen in the headspace of a package and the oxygen permeated through package that can limit shelf life some food products (Braddock, 1999). Sizer *et al*. (1988) suggested that minimizing the oxygen content in a package is essential in obtaining minimal oxidative degradations of ascorbic acid, flavor and color of food products.

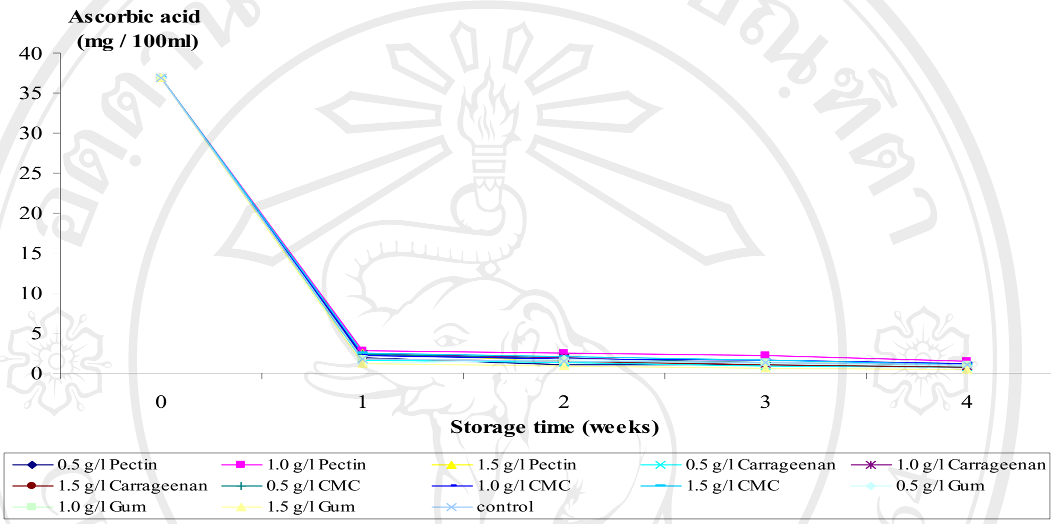

Figure 4.15 Ascorbic acid of lime juices added with different types and levels of hydrocolloids during one month storage at ambient temperature

4.5.4. Physical characteristics of hydrocolloids treated-lime juice during one month storage at ambient temperature

4.5.4.1 L*-value of lime juices

The measurement results of L*-value of lime juice color during storage were displayed Tables 4.4, 4.5 and 4.6 for different hydrocolloids of 0.5, 1.0 and 1.5 g/l (w/v), respectively. In general, during the storage period, the control lime juice treatment (no hydrocolloid addition) had a significant change in the L*-values causing the juice to become into darker, loss its cloudiness and precipitation of the colloidal juice suspension.

On the other hand, when gum acasia, pectin and CMC were present in the lime juices in any addition levels, the color of the juice samples was stabilized. These hydrocolloid treated-lime juices had a lighter color during the storage period with a brighter appearance and less precipitation of the colloidal suspension. This effect was not being observed in the κ-carrageenan treated-lime juices.

The reduction of L*-value and the increase in a*-value (section 4.5.4.2) of the lime juices in the presence of κ-carrageenan colud be affected by the functional properties of κ-carrageenan as was mentioned in section 4.5.2 (Nussinovitch, 1997) that caused the lime juice samples to be more concentrated than other lime juice treatments. Klim and Nagy (1988) also mentioned detrimental changes in the color of citrus juices were primarily caused by the non-enzymatic browning reaction.

Among different types of hydrocolloids, the CMC treated-lime juices significantly demonstrated higher L*-values than those of the other hydrocolloid treatments, including the control treatment, throughout the storage period. This effect was not significantly be affected by the addition levels of the hydrocolloid.

Table 4.4 L*-value of lime juices added with different hydrocolloid types at a concentration of 0.5 g/l (w/v) during one month storage at ambient temperature

Hydrocolloid types	**Storage period (weeks)**				
	0^{NS}	**1**	**2**	**3**	**4**
Control	54.57 ± 2.63^{a}	53.62 ± 0.12^{a}	46.66 ± 0.60^{c}	45.88 ± 1.50^{c}	52.71 ± 0.87^{b}
Gum acasia	54.57 ± 2.63^{a}	55.84 ± 0.13^{c}	51.23 ± 0.37^{b}	57.65 ± 1.11^{b}	53.50 ± 1.12^{b}
Pectin	54.57 ± 2.63^{a}	54.72 ± 0.16^{b}	59.82 ± 0.37^{a}	56.09 ± 0.66^{b}	59.38 ± 0.32^{a}
CMC	54.57 ± 2.63^{a}	54.47 ± 1.16^{ab}	59.36 ± 0.36^{a}	59.60 ± 0.32^{a}	58.64 ± 0.90^{a}
κ-Carrageenan	54.57 ± 2.63^{a}	49.94 ± 0.26^{d}	47.29 ± 0.62^{c}	45.25 ± 0.20^{c}	45.12 ± 0.34^{c}

Values within a column followed by different letters were significantly different ($p<0.05$)
Mean ± SD and NS = not significant different

Table 4.5 L*-value of lime juices added with different hydrocolloid types at a concentration of 1.0 g/l (w/v) during one month storage at ambient temperature

Hydrocolloid types	Storage period (weeks)				
	0^{NS}	1	2	3	4
Control	54.57 ± 2.63[a]	53.62 ± 0.12[b]	46.66 ± 0.60[c]	45.88 ± 1.50[c]	52.71 ± 0.87[c]
Gum acasia	54.57 ± 2.63[a]	55.08 ± 0.35[a]	59.26 ± 0.36[a]	57.45 ± 0.73 [b]	55.14 ± 0.31[b]
Pectin	54.57 ± 2.63[a]	54.66 ± 0.41[a]	54.14 ± 0.22 [b]	55.81 ± 0.17[a]	53.83 ±1.24[b]
CMC	54.57 ± 2.63[a]	55.07 ± 0.71[a]	59.01 ± 0.17[a]	59.09 ± 0.20[b]	57.87 ± 0.71[a]
κ-Carrageenan	54.57 ± 2.63[a]	50.82 ± 0.29[c]	44.46 ± 0.29 [d]	43.51 ± 1.00 [d]	48.06 ± 1.15 [d]

Values within a column followed by different letters were significantly different ($p<0.05$)
Mean ± SD and NS = not significant different

Table 4.6 L*-value of lime juices added with different hydrocolloid types at a concentration of 1.5 g/l (w/v) during one month storage at ambient temperature

Hydrocolloid types	Storage period (weeks)				
	0^{NS}	1	2	3	4
Control	54.57 ± 2.63[a]	53.62 ± 0.12[c]	46.66 ± 0.60[d]	45.88 ± 1.50[d]	52.71 ± 0.87[c]
Gum acasia	54.57 ± 2.63[a]	53.48 ± 0.08[c]	58.22 ± 0.23[b]	57.12 ± 0.42 [b]	57.99 ± 0.05[b]
Pectin	54.57 ± 2.63[a]	54.47 ± 0.22[b]	55.83 ± 0.46 [c]	50.15 ± 0.58[c]	58.63 ± 0.23[b]
CMC	54.57 ± 2.63[a]	55.40 ± 0.33[a]	58.93 ± 0.22[a]	59.09 ± 0.10[a]	59.96 ± 0.09[a]
κ-Carrageenan	54.57 ± 2.63[a]	51.72 ± 0.28[d]	45.17 ± 0.13[e]	42.97 ± 0.28[e]	40.88 ± 0.81[d]

Values within a column followed by different letters were significantly different ($p<0.05$)
Mean ± SD and NS = not significant different

4.5.4.2 a* and b*-values of lime juice

Changes in the a* and b* values of lime juices added with different hydrocolloid types were corresponded to changing in the L-value of the juice samples (section 4.5.4.1). The a*-value of different hydrocolloid treated-lime juices displayed in Tables 4.7-4.9 showed clearly that all the lime juice treatments significantly had reduction in the negative a*-value or reduction in the green color with the control and κ-carrageenan treated-lime juice samples developed positive a*-value or red color at the end of the storage period.

Whereas, the b*-value of the lime juice treatments illustrated in Table 4.10-4.12 significantly demonstrated on increase in the positive b*-value or yellow color throughout the storage period. Therefore, all of the lime juice samples looked to be more red and brownish appearance at the end of the storage period.

The κ-carrageenan treated-lime juices significantly developed higher a*-value or more red color compared to those of the control and other hydrocolloid treated-lime juice samples at the end of the storage period. At the concentration of 1.5 g/l (w/v) κ-carrageenan, the lime juice treatment significantly had the highest a*-value compared to those of the other κ-carrageenan addition levels. At the same time, the b*-value of the κ-carrageenan treated-lime juices significantly had more yellow color than those of the other hydrocolloid treated-lime juices but was lower or similar to that of the control treatment.

Changing in the b*-value of the κ-carrageenan treated-lime juice was significantly be affected by the hydrocolloid concentrations in the juices. Compared to the κ-carrageenan treated-lime juices, the CMC treated-lime juices experienced the less changes in the a* and b*-values throughout the storage period. The a* and b*-values of the CMC treated-lime juices at the end of the storage period had the closest values with the a* and b*-values of the fresh lime juices compared to those of the control and other hydrocolloid treated-lime juices, especially for the concentration of 0.5 g/l (w/v) CMC.

Lee (1992) reported that browning in citrus is unique to a typical Maillard-type browning. Since the citrus fruits have significant amounts of ascorbic acid, the ascorbic acid would mainly be oxidized due to the compounds formed in the Maillard reactions. This report supported the finding in this experiment that the color of the lime juice were became darker (a reduction in the L*-value) with a development in the red brownish color (an increase in the positive a* and b*-value) during the storage period. At the same time, the content of the ascorbic acid in the juices was significantly decreased (Figure 4.15, section 4.5.3). Further explanation for the changing in the lime juice color could be seen in the section 4.2.3.

Table 4.7 a*-value of lime juices added with different hydrocolloid types at a concentration of 0.5 g/l (w/v) during one month storage at ambient temperature

Hydrocolloid types	**Storage period (weeks)**				
	0^{NS}	**1**	**2**	**3**	**4**
Control	- 2.58 $\pm$ 0.13^{a}	-2.33 $\pm$ 0.14^{b}	-0.19 $\pm$ 0.14^{d}	0.51 $\pm$ 1.50^{d}	0.65 $\pm$ 0.44^{c}
Gum acasia	- 2.58 $\pm$ 0.13^{a}	-2.35 $\pm$ 0.06^{b}	-1.16 $\pm$ 0.05^{b}	-1.95 $\pm$ 0.16^{ab}	-0.33 $\pm$ 0.68^{b}
Pectin	- 2.58 $\pm$ 0.13^{a}	-2.68 $\pm$ 0.08^{a}	-2.05 $\pm$ 0.02^{a}	-1.59 $\pm$ 0.08^{c}	-1.63 $\pm$ 0.09^{a}
CMC	- 2.58 $\pm$ 0.13^{a}	-2.08 $\pm$ 0.14^{c}	-2.04 $\pm$ 0.04^{a}	-2.00 $\pm$ 0.19^{a}	-1.60 $\pm$ 0.06^{a}
κ-Carrageenan	- 2.58 $\pm$ 0.13^{a}	-1.81 $\pm$ 0.04^{d}	-0.43 $\pm$ 0.06^{c}	1.23 $\pm$ 0.03^{e}	2.61 $\pm$ 0.11^{d}

Values within a column followed by different letters were significantly different ($p<0.05$)
Mean $\pm$ SD and NS = not significant different

Table 4.8 a*-value of lime juices added with different hydrocolloid types at a concentration of 1.0 g/l (w/v) during one month storage at ambient temperature

Hydrocolloid types	**Storage period (weeks)**				
	0^{NS}	**1**	**2**	**3**	**4**
Control	- 2.58 ± 0.13^{a}	-2.33 ± 0.14^{b}	-0.19 ± 0.14^{e}	0.51 ± 1.50^{c}	0.65 ± 0.44^{c}
Gum acasia	- 2.58 ± 0.13^{a}	- 2.00 ± 0.04^{c}	-1.91 ± 0.02^{b}	-1.95 ± 0.08^{ab}	- 0.28 ± 0.11^{b}
Pectin	- 2.58 ± 0.13^{a}	- 2.69 ± 0.04^{a}	-1.47 ± 0.04^{c}	-1.72 ± 0.05^{b}	- 0.28 ± 0.28^{b}
CMC	- 2.58 ± 0.13^{a}	-2.34 ± 0.14^{b}	-2.09 ± 0.02^{a}	-2.23 ± 0.12^{a}	-1.74 ± 0.15^{a}
κ-Carrageenan	- 2.58 ± 0.13^{a}	-1.97 ± 0.05^{c}	-1.08 ± 0.10^{d}	1.48 ± 0.21^{d}	2.26 ± 0.20^{d}

Values within a column followed by different letters were significantly different ($p<0.05$)
Mean ± SD and NS = not significant different

Table 4.9 a*-value of lime juices added with different hydrocolloid types at a concentration of 1.5 g/l (w/v) during one month storage at ambient temperature

Hydrocolloid types	**Storage period (weeks)**				
	0^{NS}	**1**	**2**	**3**	**4**
Control	-2.58 ± 0.13^{a}	-2.33 ± 0.14^{a}	-0.19 ± 0.14^{d}	0.51 ± 1.50^{c}	0.65 ± 0.44^{c}
Gum acasia	-2.58 ± 0.13^{a}	-1.94 ± 0.85^{b}	-1.87 ± 0.02^{b}	-2.40 ± 0.05^{a}	-2.10-± 0.04^{ab}
Pectin	-2.58 ± 0.13^{a}	-2.47 ± 0.34^{a}	-1.62 ± 0.06^{c}	-0.70 ± 0.08^{b}	-1.63 ± 0.05^{b}
CMC	-2.58 ± 0.13^{a}	- 2.29 ± 0.06^{a}	- 2.10 ± 0.02^{a}	-2.34 ± 0.08^{a}	-2.61 ± 0.11^{a}
κ-Carrageenan	-2.58 ± 0.13^{a}	-1.79 ± 0.08^{b}	-1.67 ± 0.01^{c}	2.42 ± 0.02^{d}	4.80 ± 0.68^{d}

Values within a column followed by different letters were significantly different ($p<0.05$)
Mean ± SD and NS = not significant different

Table 4.10 b*-value of lime juices added with different hydrocolloid types at a concentration of 0.5 g/l (w/v) during one month storage at ambient temperature

Hydrocolloid types	**Storage period (weeks)**				
	0[NS]	**1**	**2**	**3**	**4**
Control	8.45 ± 0.27[a]	11.47 ± 0.19[c]	18.25 ± 0.48[d]	18.91 ± 0.47[c]	21.0 ± 0.72[d]
Gum acasia	8.45 ± 0.27[a]	8.65 ± 0.16[a]	9.91 ± 0.03[b]	11.51 ± 0.50[a]	17.31 ± 0.95[c]
Pectin	8.45 ± 0.27[a]	10.72 ± 0.06 [b]	9.33 ± 0.20[a]	11.39 ± 0.14[a]	10.6 ± 0.14[a]
CMC	8.45 ± 0.27[a]	8.65 ± 0.16[a]	9.81 ± 0.12[ab]	10.13 ± 0.38[a]	11.80 ± 0.52[b]
κ-Carrageenan	8.45 ± 0.27[a]	15.43 ± 0.47[d]	16.34 ± 0.38[c]	16.25 ± 0.15[b]	21.58 ± 0.05[d]

Values within a column followed by different letters were significantly different ($p<0.05$)
Mean ± SD and NS = not significant different

Table 4.11 b*-value of lime juices added with different hydrocolloid types at a concentration of 1.0 g/l (w/v) during one month storage at ambient temperature

Hydrocolloid types	**Storage period (weeks)**				
	0[NS]	**1**	**2**	**3**	**4**
Control	8.45 ± 0.27[a]	11.47 ± 0.19[c]	18.25 ± 0.48[d]	18.91 ± 0.47[d]	21.0 ± 0.72[d]
Gum acasia	8.45 ± 0.27[a]	10.45 ± 0.15[a]	9.57 ± 0.13[b]	12.38 ± 0.19[b]	13.56 ± 0.26[a]
Pectin	8.45 ± 0.27[a]	10.72 ± 0.11[b]	13.58 ± 0.15[a]	12.48 ± 0.22[a]	13.60 ± 0.23[a]
CMC	8.45 ± 0.27[a]	9.22 ± 0.01[a]	8.49 ± 0.11[ab]	10.75 ± 0.34[a]	14.83 ± 0.48[b]
κ-Carrageenan	8.45 ± 0.27[a]	16.13 ± 0.67[d]	17.83 ± 0.09[c]	15.27 ± 0.65[c]	18.12 ± 0.53[c]

Values within a column followed by different letters were significantly different ($p<0.05$)
Mean ± SD and NS = not significant different

Table 4.12 b*-value of lime juices added with different hydrocolloid types at a concentration of 1.5 g/l (w/v) during one month storage at ambient temperature

Hydrocolloid types	**Storage period (weeks)**				
	0[NS]	**1**	**2**	**3**	**4**
Control	8.45 ± 0.27[a]	11.47 ± 0.19[c]	18.25 ± 0.48[e]	18.91 ± 0.47[c]	21.00 ± 0.72[e]
Gum acasia	8.45 ± 0.27[a]	8.88 ± 0.86[a]	9.76 ± 0.02[a]	14.51 ± 0.22[b]	19.46 ± 0.02[a]
Pectin	8.45 ± 0.27[a]	10.55 ± 0.12[b]	11.05 ± 0.12[c]	13.54 ± 0.11[b]	13.22 ± 0.04[c]
CMC	8.45 ± 0.27[a]	8.68 ± 0.05[a]	10.73 ± 0.02[b]	11.82 ± 1.04[a]	14.83 ± 0.48[b]
κ-Carrageenan	8.45 ± 0.27[a]	17.48 ± 0.21[d]	15.69 ± 0.18[d]	14.53 ± 0.22[b]	17.15 ± 0.50[d]

Values within a column followed by different letters were significantly different ($p<0.05$)
Mean ± SD and NS = not significant different

The presence of hydrocolloids in lime juice was indirectly, but significantly affected the changes in the lime juice color due to the ability of the chemical compounds to stabilize the cloudiness of the lime juice during the storage period. It could be directly observed that the colloidal suspension in the hydrocolloid treated-lime juices experienced less changes compared to the control sample throughout the studied storage time. The L*-value of hydrocolloid treated-lime juices was not reduced as the control treatment, except for the κ-carrageenan treated-lime juices, and there was less formation of the pulp sedimentation in these samples. The loss in the juice cloudiness is generally perceived as a juice with a poor quality standard by the consumers.

Other research studies by Liang *et al.* (2006) and Genovese and Lozano (2001) also demonstrated that hydrocolloids used in food suspensions could lead to a stabilization of insoluble particles because of their functional properties that could be ionized in aqueous solutions.

In addition, the addition of hydrocolloids would help in retaining and improving or stabilizing the colloidal particles in fruit juices in prolonged storage periods.

In terms of ionization, food gums may not have electric charge. Turbidity in fruit juices is caused by particles with a positively charged nucleus of carbohydrates and proteins, surrounded by negatively charged pectins (Yamasaki *et al*., 1964). Since, juice particles are negatively charge, addition of food gums with negative charge is expected to increase electrostatic repulsive forces between particles. Yamasaki *et al.* (1964) found that negatively charged colloids (like CMC, gum acasia and sodium aliginate) in concentrations as low as 0.05%, completely inhibit apple juice clarification.

4.6 The effects of High Pressure Processing (HPP) in the presence and absence of CMC on the physical, chemical, nutritional, microbial qualities and d-limonin content of lime juice during one month storage

In this section, a CMC concentration of 1.0 g/l (w/v) was used together with a HPP treatment at 400, 500 or 600 MPa for 15 minutes to study changes in the qualities of lime juice during storage for one month at refrigerator and ambient temperatures. The HPP treatments were conducted at a processing temperature of 25 ± 2^{o}C.

4.6.1 The effect of HPP in the presence and absence of CMC on the d-limonin content of lime juices during one month storage

The measurement for the d-limonin content of different lime juice treatments shown in Figures 4.16 and 4.17 clearly demonstrated that the HPP treated-lime juice samples both in the presence and absence of CMC were significantly contained lower amount of d-limonin compared to the control treatment (not being processed by HPP) directly after the processing and throughout the storage period. The presence of CMC could only produced a further significant reduction in the d-limonin content of the lime juices when the juices were treated at lower pressure (e.g. 400 MPa for 15 minutes of holding time) or kept at higher storage temperature.

The HPP treated-lime juice samples could produce lime juice with a d-limonin concentration of less than 6 ppm, which was the limit of the human threshold to detect the chemical compound (Nienaber and Shellhammer, 2001). This finding indicated that HPP could stabilize the d-limionin compound directly after the processing and during the storage period.

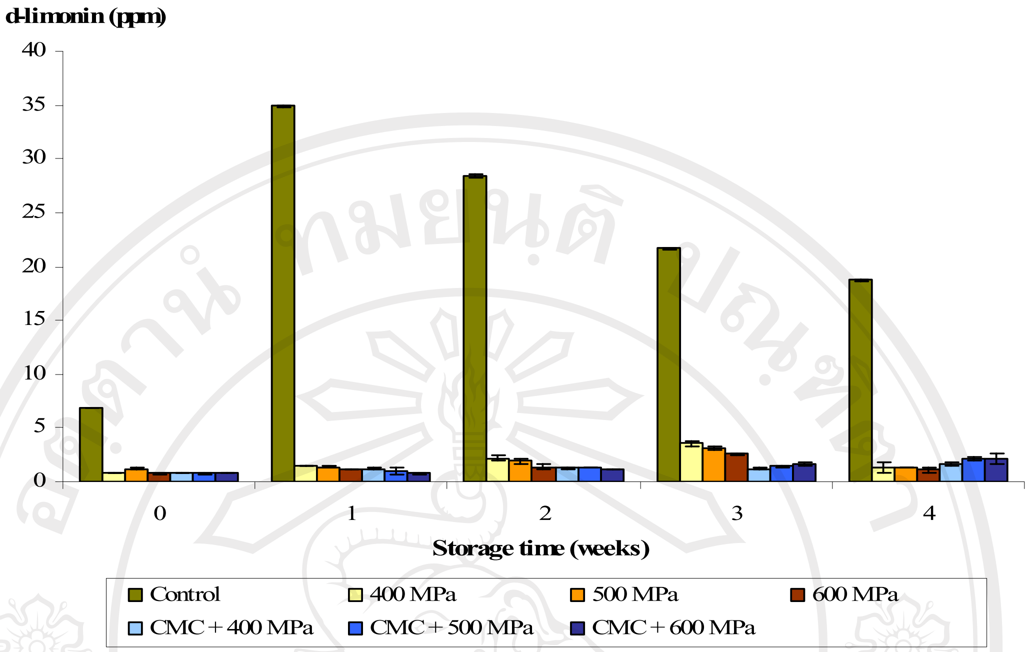

Figure 4.16 The d-limonin content (ppm) of HPP treated-lime juices in the presence and absence of 1.0 g/l (w/v) CMC during one month storage at 4-6^{o}C

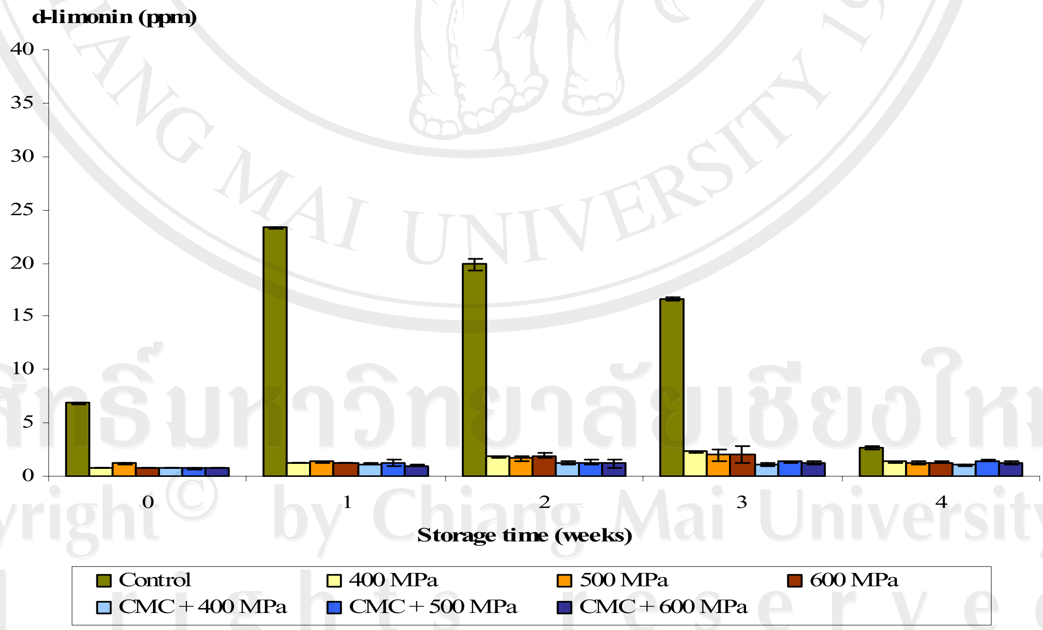

Figure 4.17 The d-limonin content (ppm) of HPP treated-lime juices in the presence and absence of 1.0 g/l (w/v) CMC during one month storage at ambient temperature

Increasing the pressure of the HPP treatment did not significantly produce higher reduction in the d-limonin content, especially for the lime juices kept at ambient temperature. Processing the lime juices at 400 MPa for 15 minutes could effectively maintained the d-limonin content of the lime juices to be lower than 3.56 ± 0.46 ppm throughout the storage period at any storage temperatures. A further significant reduction was achieved when 1.0 g/l (w/v) CMC was present in the lime juices.

The effectiveness of the HPP treatment in reducing the d-limonin content could be due to changing in the structures of the d-limonin and enzyme that was responsible to cause the bitterness in citrus juices, which was limonoid D-ring lactone hydrolase (Hasegawa, 1995). Seyderhelim *et al*. (1996) and Morild (1981) stated that the effects of HPP include protein denaturation and enzyme inactivation. The effect of HPP on enzyme protein structure could either be reversible or irreversible changes (Cheftel, 1992). In general, the HP effect on the enzyme catalytic activity depends on the type of enzyme, temperature, nature of substrates, length of processing and applied pressure (Vila Real *et al*., 2007 and Thumthanaruk, 2002). The degree of enzyme inactivation could be varied depending on the type of fruit and vegetable products studied (Knorr *et al*., 2002)

Findings in this section could be a result from two possible mechanisms. The first one was unfavorable conditions for conversion of limonoate-A-ring lactone (a precursor) to d-limonin in the presence of CMC. The other mechanism was a possibility of a complete inactivation without reversible mechanism of the limonoid D-ring lactone hydrolase in the lime juices after treated by HPP at 400-600 MPa for 15 minutes. The inactivation of the enzyme might not be reversible, since there was only an increase for up to 2.55 ± 0.25 ppm of the d-limonin content in the lime juices throughout the studied storage period at any storage temperatures.

4.6.2 The effects of HPP on the microbial quality of lime juices during one month storage

The microbiological qualities of the HPP treated-lime juices monitored from the presence of the total plate count (TPC) and the count of yeast and mold during one month storage at refrigerator and ambient temperatures was displayed in Tables 4.13 to 4.16. Before doing the HPP treatments, the fresh lime juices contained TPC and a count of yeasts and molds of <30 and <15 CFU/ml, respectively. During one month storage, the number of TPC and the count of yeast and mold were maintained to be the same as the initial microbial counts in the HPP treated-lime juices irrespectively to the applied pressure or storage temperatures to keep the juice samples. On the other hand, the control lime juices (not being processed by HPP) had a significant increase for more than 2 log CFU/ml for its microbial count during the same storage period.

The finding clearly demonstrated that the HPP could significantly reduce the growth of bacteria, yeast and mold in the lime juice samples during storage, especially storage at elevated temperature. The effectiveness of the HPP treatment in inhibiting the microbial growth in the lime juice during storage suggested that the processing method could be useful technique in extending the shelf-life of lime juices. The result was also being supported with the fact that at the low pH of the lime juice (pH 2.3), the growth of pathogenic bacteria would be suppressed. The microorganisms that are responsible for the spoilage in citrus juice will include yeast, molds and lactic acid bacteria (Parish, 1998a and Zook *et al*., 1999).

Table 4.13 Total Plate Count (CFU/ml) of High Pressure Processing treated-lime juices during one month storage at 4-6^{o}C

Process Condition	Storage period (weeks)					
	Before Processing	0	1	2	3	4
Control	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}
400 MPa	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}
CMC + 400 MPa	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}
500 MPa	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}
CMC + 500 MPa	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}
600 MPa	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}
CMC + 600 MPa	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}

Values within a row followed by different letters were significantly different ($p<0.05$)

Table 4.14 Total Plate Count (CFU/ml) of High Pressure Processing treated-lime juices during one month storage at ambient temperature

Process Condition	Storage period (weeks)					
	Before Processing	0	1	2	3	4
Control	< 25^{a}	< 25^{a}	45^{b}	68^{b}	400^{c}	660^{d}
400 MPa	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}
CMC + 400 MPa	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}
500 MPa	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}
CMC + 500 MPa	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}
600 MPa	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}
CMC + 600 MPa	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}	< 25^{a}

Values within a row followed by different letters were significantly different ($p<0.05$)

Table 4.15 Yeast and Mold (CFU/ml) of High Pressure Processing treated-lime juices during one month storage at 4-6^{o}C

Process Condition	Storage period (weeks)					
	Before Processing	**0**	**1**	**2**	**3**	**4**
Control	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]
400 MPa	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]
CMC + 400 MPa	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]
500 MPa	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]
CMC + 500 MPa	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]
600 MPa	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]
CMC + 600 MPa	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]

Values within a row followed by different letters were significantly different ($p<0.05$)

Table 4.16 Yeast and Mold (CFU/ml) of High Pressure Processing treated-lime juices during one month storage at ambient temperature

Process Condition	Storage period (weeks)					
	Before Processing	**0**	**1**	**2**	**3**	**4**
Control	< 15[a]	< 15[a]	30[b]	39[b]	47[b]	108[c]
400 MPa	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]
CMC + 400 MPa	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]
500 MPa	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]
CMC + 500 MPa	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]
600 MPa	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]
CMC + 600 MPa	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]	< 15[a]

Values within a row followed by different letters were significantly different ($p<0.05$)

Results in this study also clearly illustrated that the major spoilage microorganism in the lime juices were yeast, mold and some lactic acid bacteria as they were able to survive and grow at low pH environments of the lime juices and also able to use of sugar and vitamins in the juice (Deak and Beuchat, 1996). At the same time, yeasts and molds are more easily to be inactivated by pressure than bacteria. Among bacteria, vegetative forms are more susceptible than spores, which are extremely resistant to pressure action (Bayindirli *et al*., 2006). Reports have shown that pressures between 300 and 600 MPa can inactivate food spoilage and pathogenic microorganisms (Bayindirli *et al*., 2006 and Palou *et al*., 1999). The ability of high pressure to inhibit microorganisms depends on the type of microorganisms and on the composition of food (Houska *et al*., 2006).

4.6.3 The effect of HPP in presence and absence of CMC on the ascorbic acid content of lime juices during one month storage

A decrease in the vitamin C concentration to a level that is unacceptable by legislation or industrial practice often defines citrus juice shelf life. This fact also indicated the importance of the ascorbic acid level as an indicator quality of citrus juice. In this study, the ascorbic acid content of the HPP treated-lime juice stored for one month could be seen in Figures 4.18 and 4.19.

The results clearly showed that the HPP treatment did not significantly reduce the ascorbic acid content of the lime juices directly after the processing. In fact, the HPP processing significantly affected the retention of the vitamin during the storage period, particularly when the juice samples were kept at refrigerator temperature. Applying different pressure levels did not significantly affect the retention of the ascorbic acid content in the lime juices. However, the presence of 1.0 g/l (w/v) CMC could significantly improve the ascorbic acid retention in the juice samples, which could be due to encapsulation of the vitamin by hydrocolloid (Gibbs *et al*., 1999).

The highest ascorbic acid retention in the lime juices was achieved in the juices treated at 600 MPa, added with 1.0 g/l (w/v) CMC and stored at 4-6^{o}C.

Monitoring the ascorbic acid level during the storage time demonstrated a gradual, but significant reduction in the level of the acid. The highest reduction rate was shown in the control treatment followed by the lime juice samples kept at ambient temperature. The ascorbic acid level of lime juices kept at chilled temperatures experienced the lowest reduction rate which indicated that storage temperature was one of the important factors in maintaining the level of ascorbic acid during storage. During storage, the rate of vitamin C content of citrus juice gradually decreased could due to many factor such as light, storage temperature, storage time and packaging (Polydera *et al*., 2003).

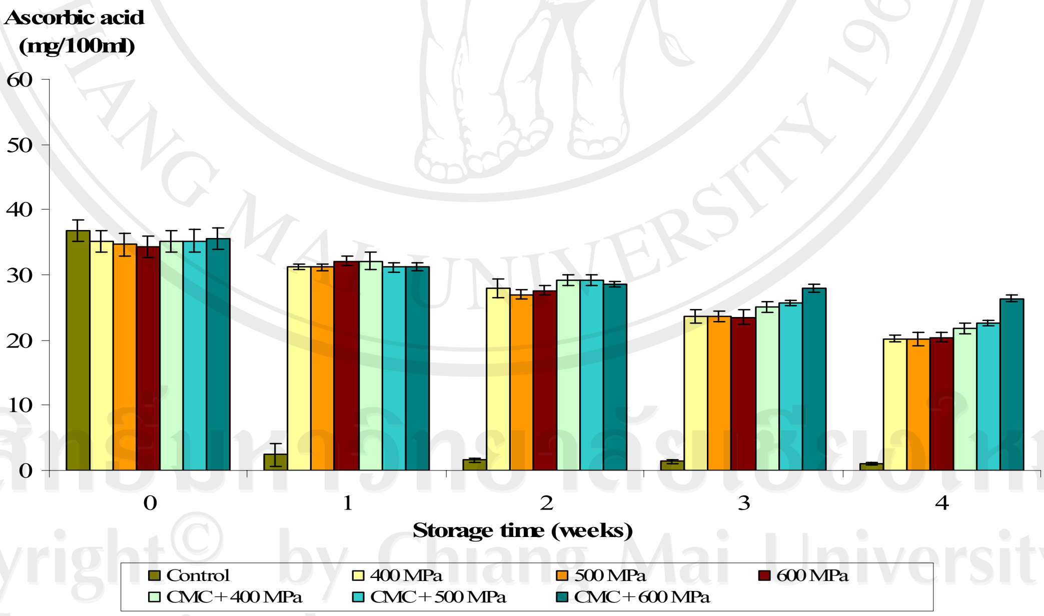

Figure 4.18 The ascorbic acid (mg/100 ml) of High Pressure Processing treated-lime juices in the presence and absence of 1.0 g/l (w/v) CMC during one month storage at 4-6°C

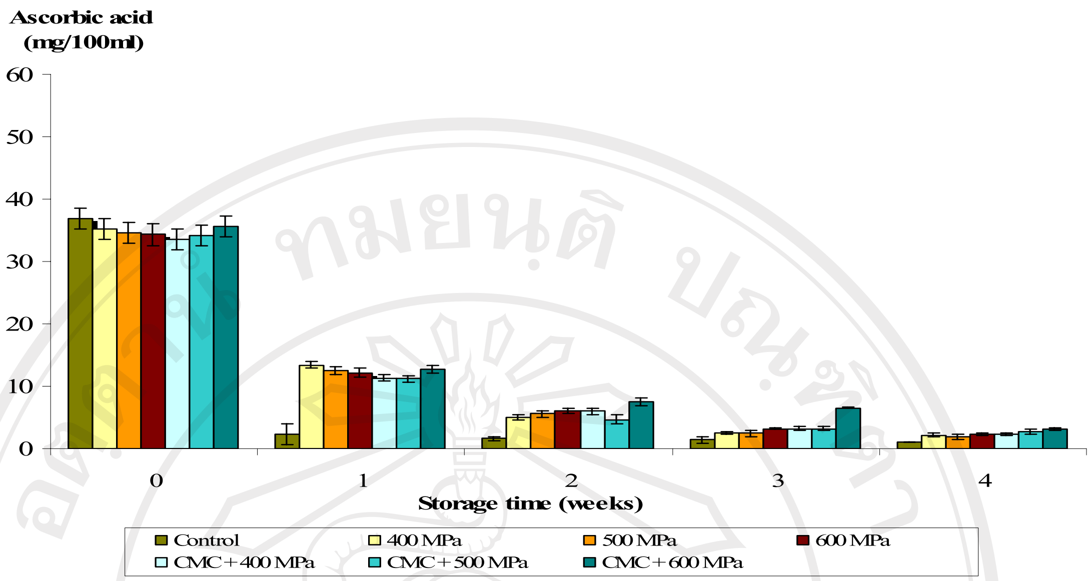

Figure 4.19 The ascorbic acid (mg/100 ml) of High Pressure Processing treated-lime juices in the presence and absence of 1.0 g/l (w/v) CMC during one month storage at ambient temperature

Degradation of vitamin C proceeds both in aerobic and anaerobic pathways (Johnson et al., 1995). Oxidation of ascorbic acid occurs mainly during the processing of citrus juices (Gordon and Samaniego-Esguerra, 1990). Oxidation of ascorbic acid takes place as either two one-electron transfer processes or as a single two-electron reaction without detection of the semihydroascorbate intermediate. In one-electron oxidation, the first step involves transfer of an electron to form the free radical semidehydroascorbic acid. Loss of an additional electron yields semidehydroascorbic acid, which is highly unstable because of the susceptibility to hydrolysis of the lactone bridge. Such hydrolysis, which irreversibly form 2,3-diketogulonic acid, is responsible for less of vitamin C activity (Fennema, 1996).

Burdurlu *et al*. (2006) and Bull et al. (2004) reported that the loss of ascorbic acid in citrus juice concentrates at all storage temperatures was described as a first-order reaction and decomposed easily in acid solutions and the amount of oxygen dissolved and light into the juice. In addition, better vitamin C retention could be achieved in the presence of higher concentrations of citric acid (Nagy, 1980).

4.6.4 The effect of HPP in the presence and absence of CMC on the pH value of lime juice during one month storage

Changing in the pH value of the HPP treated-lime juices in the presence and absence of 1.0 g/l (w/v) CMC during one month storage at chilled and ambient temperature was displayed in Figures 4.20 and 4.21, respectively.

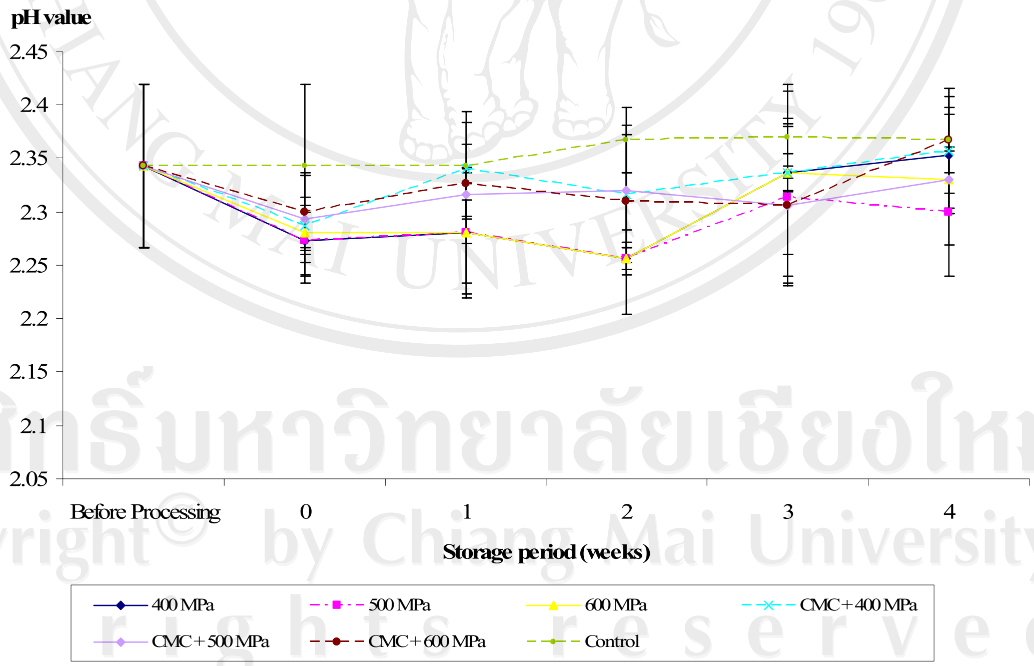

Figure 4.20 pH of High Pressure Processing treated-lime juices in the presence and absence of 1.0 g/l (w/v) CMC during one month storage at 4-6^{o}C

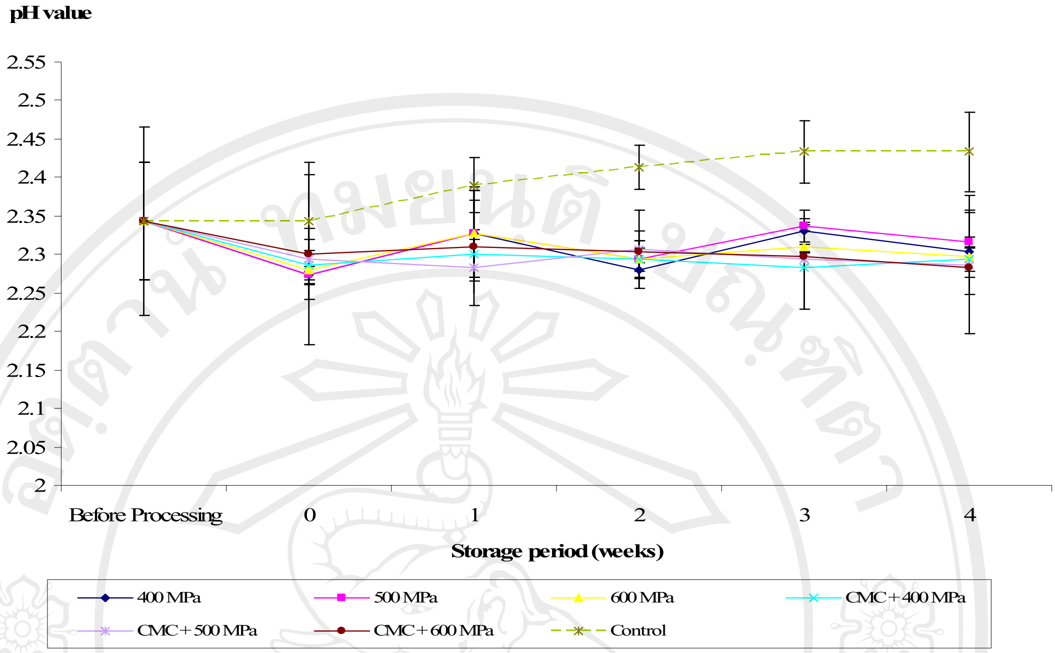

Figure 4.21 pH of High Pressure Processing treated-lime juices in the presence and absence of 1.0 g/l (w/v) CMC during one month storage at ambient temperature

The Figures showed that processing the lime juice by HPP treatments caused reduction in the lime juice pH values for up to 0.24 units directly after the pressurization process. This result could be due to the formation of ions in aqueous solution, which is favored by pressure because it involved a volume decrease due to the electrostrictive effect i.e the columbic field of the charged groups produce a compact alignment of water around themselves. Therefore, the electrostatic interactions easily break under pressure (Cheftel and Culioli, 1997). Heremans and Smeller (1998) also reported that under pressure (depending on temperature) water dissociate to its ionic product $[H^+]$ x $[OH^-]$, therefore, pH of water decreased by 0.2 - 0.5 pH units per 100 MPa. The separation of positive and negative charges under pressure is driven by a water electrostriction phenomenon: water molecules rearrange in a more compact manner with a smaller total volume around electric charges, due to dipole-dipole interactions and hydrogen bonding (Riahi, 2003).

HPP compression of foods may shift the pH of solution or food during treatment. Finding in this study was similar to Kolakowaki *et al*. (2001) and Stipple *et al*. (2002). For fruit juices, which are in general quite acid, a treatment of 500 MPa would cause pH shift of about one unit to acid side (Hereman and Smeller, 1998).

There was not any significant difference in the changing of the pH value of the HPP treated-lime juices and the control treatment during storage.

4.6.5 The effect of HPP in the presence and absence of CMC on the total acidity and total soluble solid of lime juices during one month storage

Measurement of total acidity and total soluble solid of the HPP treated-lime juices in the presence and absence of 1.0 g/l (w/v) CMC during one month storage at refrigerator and ambient temperatures was demonstrated in Figures 4.22 and 4.23, respectively.

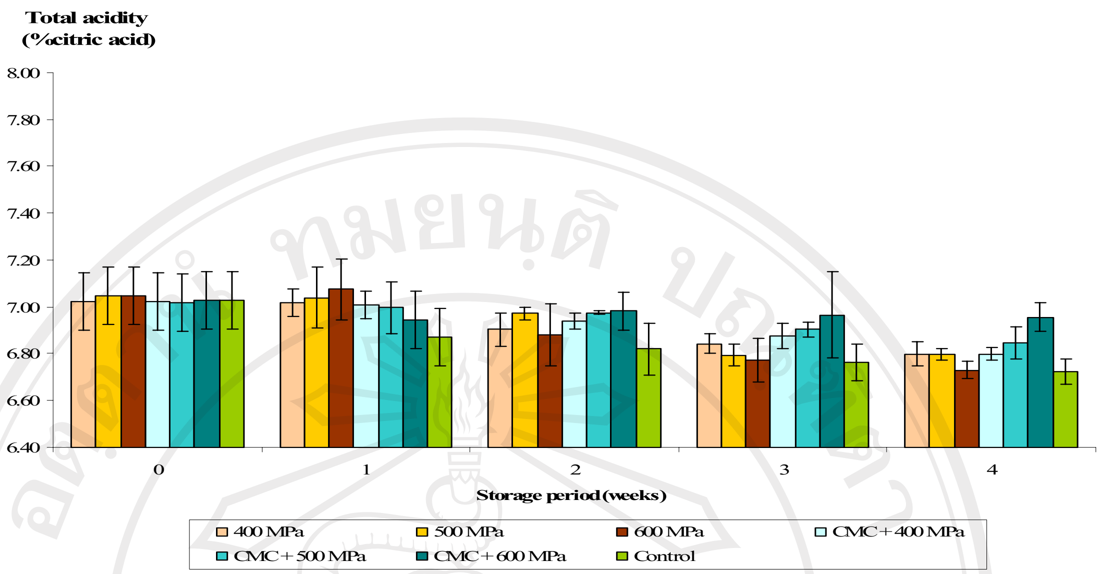

Figure 4.22 Total acidity (%citric acid) of High Pressure Processing treated-lime juices in the presence and absence of 1.0 g/l (w/v) CMC during one month storage at 4-6°C

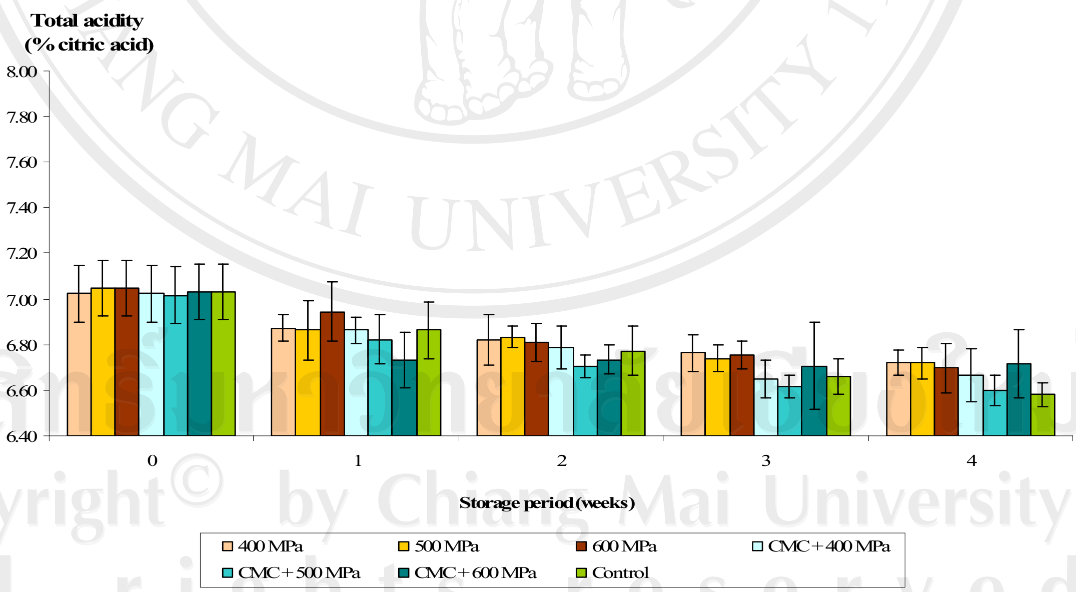

Figure 4.23 Total acidity (%citric acid) of High Pressure Processing treated-lime juices in the presence and absence of 1.0 g/l (w/v) CMC during one month storage at ambient temperature

The results clearly displayed that the total acidity of the lime juices was significantly decreased during the storage period, except for the lime juice samples treated at 600 MPa, added with 1.0 g/l (w/v) CMC and kept at 4-6^{o}C. Higher reduction rate in the total acidity of the lime juices was occurred when the juice samples were stored at higher storage temperature. Reduction in the total acidity could be correlated with the chemical reactions happened in the juice during storage, such as retention of ascorbic acid (sections 4.2.2, 4.5.3 and 4.6.3). Nagy (1980) stated that better vitamin C retention could be achieved in the presence of higher citric acid concentrations.

The results of total soluble solid measurement were correspondent to the results of total acidity that during storage, the total soluble solid of the lime juices was significantly decreased, except for the lime juice samples treated at 600 MPa, added with 1.0 g/l (w/v) CMC and kept at 4-6^{o}C. The decrease in the total soluble solid could be affected by the chemical reactions occurred in the lime juices as was explained for the total acidity, since most of the soluble solids in citrus juice was organic acids, not in carbohydrates (Kimball, 1991).

4.6.6 The effect of HPP in the presence and absence of CMC on the color (L*, a* and b*-value) of lime juice during one month storage

The effect of HPP on the color of lime juices, including L*, a* and b*-values could be seen in Tables 4.17 to 4.22. The results showed that the HPP treatment had a little effect on the color of the lime juices directly after the pressurization treatment. There was not any significant different in the L*-value of the lime juice after processing the juices at different pressure levels. During the storage period, the L*-value of the lime juice was significantly reduced.

For the a* and b*-values, the collected data displayed that the HPP treated-lime juices at 400 and 600 MPa with the presence of CMC experienced less changes in the a* and b*-values. Tables 4.19 to 4.22 clearly illustrated that during the storage period, the control treatment had a significant reduction in the green color (negative a*-value) or development in the red color and a significant increase in the yellow color (positive b*-value). Changes in the a* and b*-values of the HPP treated-lime juices were occurred at a slower rate compared to those of the control treatment.

A higher decrease in lightness was found when the lime juices were treated at lower pressure level or higher pH or stored at higher temperature (Lopez-malo *et al.,* 1998). Changes in the a* and b*-values could be affected by the result of non-enzymatic browning reactions and loss ascorbic acid in the lime juices (Polydera, 2003).

The color measurement of the lime juices indicated that the HPP treatment could preserve and reduce changes in the lime juice color. This could be partly due to the retention of the ascorbic acid in the HPP treated-lime juices (section 4.6.3) that affected the juice color. The brown color in citrus juice was mainly because of non-enzymatic browning reactions in which ascorbic acid was oxidized (Clegg, 1966). At the same time, the packaging of citrus juice products should prevent the juice from light and oxidizer in the environment to retain the ascorbic acid content of the juice.

Table 4.17 The effect of HPP on color (L*-value) of CMC-treated and non-treated lime juice during storage at 4-6°C

Process Condition	Storage period (weeks)					
	Before Processing[NS]	0[NS]	1	2	3	4
Control	54.90 ± 0.76[a]	54.57 ± 2.63[a]	55.38 ± 0.55[a]	46.82 ± 0.78[e]	47.79 ± 0.91[e]	56.0 ± 0.07[ab]
400 MPa	54.90 ± 0.76[a]	55.38 ± 0.55[a]	55.53 ± 0.93[a]	53.95 ± 0.62[d]	58.58 ± 0.41[a]	48.34 ± 0.37[e]
CMC 1.0 g/L + 400 MPa	54.90 ± 0.76[a]	55.46 ± 0.08[a]	55.15 ± 0.18[a]	55.84 ± 0.39[ab]	56.65 ± 0.22[b]	50.05 ± 0.79[d]
500 MPa	54.90 ± 0.76[a]	55.05 ± 0.74[a]	52.62 ± 0.30[b]	54.13 ± 0.37[cd]	56.10 ± 0.67[b]	55.01 ± 0.24[b]
CMC 1.0 g/L + 500 MPa	54.90 ± 0.76[a]	55.57 ± 0.34[a]	55.05 ± 0.14[a]	56.29 ± 0.52[a]	48.72 ± 0.49[e]	47.17 ± 0.75[e]
600 MPa	54.90 ± 0.76[a]	55.36 ± 0.32[a]	55.57 ± 0.94[a]	53.72 ± 0.49[d]	52.36 ± 0.39[d]	57.24 ± 1.72[a]
CMC 1.0 g/L + 600 MPa	54.90 ± 0.76[a]	55.46 ± 0.08[a]	54.69 ± 0.32[a]	54.98 ± 0.02[bc]	56.65 ± 0.22[c]	52.51 ± 0.35[c]

Values within a column followed by different letters were significantly different ($p<0.05$)
Mean ± SD
NS = Not significant different

Table 4.18 The effect of HPP on color (L*-value) of CMC-treated and non-treated lime juice during storage at ambient temperature

Process Condition	Storage period (weeks)					
	Before Processing^{NS}	**0^{NS}**	**1**	**2**	**3**	**4**
Control	54.90 ± 0.76^{a}	54.57 ± 2.63^{a}	53.62 ± 0.12^{bc}	53.62 ± 0.12^{d}	53.62 ± 0.12^{f}	56.00 ± 0.07^{a}
400 MPa	54.90 ± 0.76^{a}	55.38 ± 0.55^{a}	52.85 ± 0.56^{c}	53.99 ± 0.27^{c}	48.97 ± 0.50^{de}	44.82 ± 0.35^{d}
CMC 1.0 g/L + 400 MPa	54.90 ± 0.76^{a}	55.46 ± 0.08^{a}	56.51 ± 0.50^{a}	57.84 ± 0.70^{a}	48.09 ± 0.21^{e}	52.22 ± 0.84^{a}
500 MPa	54.90 ± 0.76^{a}	55.05 ± 0.74^{a}	54.54 ± 0.52^{b}	56.45 ± 0.92^{b}	51.63 ± 0.93^{c}	49.96 ± 0.66^{b}
CMC 1.0 g/L + 500 MPa	54.90 ± 0.76^{a}	55.57 ± 0.34^{a}	56.05 ± 0.88^{a}	57.61 ± 0.37^{a}	58.07 ± 0.52^{a}	46.17 ± 0.75^{c}
600 MPa	54.90 ± 0.76^{a}	55.36 ± 0.32^{a}	54.33 ± 0.44^{b}	57.58 ± 0.25^{a}	49.93 ± 0.61^{d}	44.64 ± 0.15^{d}
CMC 1.0 g/L + 600 MPa	54.90 ± 0.76^{a}	55.46 ± 0.08^{a}	56.02 ± 0.80^{a}	42.66 ± 0.15^{e}	54.03 ± 0.86^{b}	49.50 ± 0.66^{b}

Values within a column followed by different letters were significantly different ($p<0.05$)
Mean ± SD
NS = Not significant different

Table 4.19 The effect of HPP on color (a*-value) of CMC-treated and non-treated lime juice during storage at 4-6^{o}C

Process Condition	Storage period (weeks)					
	Before Processing^{NS}	0	1	2	3	4
Control	- 2.58 ± 0.13	-2.58 ± 0.13^{a}	-2.36 ± 0.08^{ab}	-1.87 ± 0.22^{c}	-1.68 ± 0.14^{b}	-2.01 ± 0.07^{a}
400 MPa	- 2.58 ± 0.13	-2.36 ± 0.08^{b}	-2.25 ± 0.09^{bc}	-1.39 ± 0.23^{d}	-1.80 ± 0.09^{b}	-0.66 ± 0.29^{c}
CMC 1.0 g/L + 400 MPa	- 2.58 ± 0.13	-2.59 ± 0.08^{a}	-2.54 ± 0.10^{a}	-2.41 ± 0.16^{ab}	-2.25 ± 0.16^{a}	-2.15 ± 0.22^{a}
500 MPa	- 2.58 ± 0.13	-2.10 ± 0.04^{c}	-2.00 ± 0.04^{d}	-2.16 ± 0.04^{bc}	-1.31 ± 0.07^{c}	-1.86 ± 0.04^{a}
CMC 1.0 g/L + 500 MPa	- 2.58 ± 0.13	-2.34 ± 0.07^{b}	-2.15 ± 0.13^{bcd}	-1.96 ± 0.27^{c}	-1.00 ± 0.08^{d}	- 0.39 ± 0.62^{c}
600 MPa	- 2.58 ± 0.13	-2.10 ± 0.06^{c}	-2.08 ± 0.06^{cd}	-2.52 ± 0.07^{a}	-2.14 ± 0.24^{a}	-0.85 ± 0.10^{b}
CMC 1.0 g/L + 600 MPa	- 2.58 ± 0.13	-2.56 ± 0.11^{a}	-2.54 ± 0.22^{a}	-2.40 ± 0.12^{ab}	-2.27 ± 0.13^{a}	-2.16 ± 0.04^{a}

Values within a column followed by different letters were significantly different ($p<0.05$)
Mean ± SD
NS = Not significant different

Table 4.20 The effect of HPP on color (a*-value) of CMC-treated and non-treated lime juice during storage at ambient temperature

Process Condition	Storage period (weeks)					
	Before Processing[NS]	**0**	**1**	**2**	**3**	**4**
Control	- 2.58 ± 0.13	-2.58 ± 0.13[a]	-2.33 ± 0.14[a]	-0.19 ± 0.14[e]	0.51 ± 1.50[d]	0.65 ± 0.44[c]
400 MPa	- 2.58 ± 0.13	-2.36 ± 0.08[b]	-1.67 ± 0.06[c]	-1.48 ± 0.06[d]	-1.36 ± 0.06[c]	-0.06 ± 0.06[b]
CMC 1.0 g/L + 400 MPa	- 2.58 ± 0.13	-2.59 ± 0.08[a]	-2.40 ± 0.11[a]	-2.08 ± 0.09[c]	-1.01 ± 0.06[d]	-0.26 ± 0.55[b]
500 MPa	- 2.58 ± 0.13	-2.10 ± 0.04[c]	-1.96 ± 0.02[b]	-2.06 ± 0.10[c]	-1.35 ± 0.17[c]	-0.41 ± 0.08[b]
CMC 1.0 g/L + 500 MPa	- 2.58 ± 0.13	-2.34 ± 0.07[b]	-2.45 ± 0.18[a]	-2.51 ± 0.05[a]	-2.44 ± 0.04[a]	-1.13 ± 0.06[a]
600 MPa	- 2.58 ± 0.13	-2.10 ± 0.06[c]	-1.94 ± 0.10[b]	-2.20 ± 0.04[bc]	-1.42 ± 0.10[c]	-0.07 ± 0.02[b]
CMC 1.0 g/L + 600 MPa	- 2.58 ± 0.13	-2.56 ± 0.11[a]	-2.42 ± 0.11[a]	-2.40 ± 0.26[ab]	-1.80 ± 0.21[b]	-0.49 ± 0.53[b]

Values within a column followed by different letters were significantly different ($p<0.05$)
Mean ± SD
NS = Not significant different

Table 4.21 The effect of HPP on color (b*-value) of CMC-treated and non-treated lime juice during storage at 4-6^{o}C

Process Condition	Storage period (weeks)					
	Before Processing^{NS}	0	1	2	3	4
Control	8.45 ± 0.27	8.45 ± 0.27^{a}	8.96 ± 0.30^{ab}	15.77 ± 0.24^{d}	16.30 ± 0.80^{e}	18.65 ± 0.06^{d}
400 MPa	8.45 ± 0.27	8.96 ± 0.30^{b}	9.64 ± 0.23^{d}	9.84 ± 0.55^{b}	10.79 ± 0.21^{b}	10.01 ± 0.37^{ab}
CMC 1.0 g/L + 400 MPa	8.45 ± 0.27	8.44 ± 0.20^{a}	9.11 ± 0.12^{bc}	9.45 ± 0.12^{ab}	9.75 ± 0.34^{a}	9.81 ± 0.44^{a}
500 MPa	8.45 ± 0.27	8.06 ± 0.36^{a}	9.50 ± 0.02^{cd}	9.62 ± 0.07^{b}	11.82 ± 0.18^{c}	11.47 ± 0.12^{c}
CMC 1.0 g/L + 500 MPa	8.45 ± 0.27	8.38 ± 0.25^{a}	8.70 ± 0.15^{ab}	9.47 ± 0.41^{ab}	10.22 ± 0.19^{ab}	10.71 ± 0.46^{ab}
600 MPa	8.45 ± 0.27	8.96 + 0.30^{a}	8.53 ± 0.30^{a}	10.51 ± 0.26^{c}	12.62 ± 0.06^{d}	10.61 ± 0.58^{b}
CMC 1.0 g/L + 600 MPa	8.45 ± 0.27	8.34 ± 0.14^{a}	8.77 ± 0.30^{ab}	8.91 ± 0.24^{a}	9.76 ± 0.10^{a}	9.45 ± 0.24^{a}

Values within a column followed by different letters were significantly different ($p<0.05$)
Mean ± SD
NS = Not significant different

Table 4.22 The effect of HPP on color (b*-value) of CMC-treated and non-treated lime juice during storage at ambient temperature

Process Condition	Storage period (weeks)					
	Before Processing[NS]	**0**	**1**	**2**	**3**	**4**
Control	8.45 $\pm$ 0.27	8.45 $\pm$ 0.27^{a}	11.47 $\pm$ 0.19^{d}	18.25 $\pm$ 0.48^{d}	18.91 $\pm$ 0.47^{f}	21.0 $\pm$ 0.72^{d}
400 MPa	8.45 $\pm$ 0.27	8.96 $\pm$ 0.30^{b}	11.12 $\pm$ 0.09^{d}	11.69 $\pm$ 0.10^{b}	11.70 $\pm$ 0.33^{b}	11.84 $\pm$ 0.23^{a}
CMC 1.0 g/L + 400 MPa	8.45 $\pm$ 0.27	8.44 $\pm$ 0.20^{a}	10.04 $\pm$ 0.17^{c}	12.68 $\pm$ 0.76^{c}	14.48 $\pm$ 0.17^{d}	16.70 $\pm$ 0.75^{c}
500 MPa	8.45 $\pm$ 0.27	8.06 $\pm$ 0.36^{a}	9.41 $\pm$ 0.17^{b}	11.35 $\pm$ 0.05^{a}	12.03 $\pm$ 0.08^{b}	13.10 $\pm$ 0.24^{b}
CMC 1.0 g/L + 500 MPa	8.45 $\pm$ 0.27	8.38 $\pm$ 0.25^{a}	9.67 $\pm$ 0.34^{b}	11.54 $\pm$ 0.23^{b}	13.31 $\pm$ 0.15^{c}	16.71 $\pm$ 0.55^{c}
600 MPa	8.45 $\pm$ 0.27	8.96 + 0.30^{a}	11.11 $\pm$ 0.17^{d}	10.71 $\pm$ 0.47^{ab}	11.18 $\pm$ 0.17^{a}	11.56 $\pm$ 0.13^{a}
CMC 1.0 g/L + 600 MPa	8.45 $\pm$ 0.27	8.34 $\pm$ 0.14^{a}	9.03 $\pm$ 0.17^{a}	11.72 $\pm$ 0.34^{b}	15.77 $\pm$ 0.30^{e}	17.06 $\pm$ 0.23^{c}

Values within a column followed by different letters were significantly different ($p<0.05$)
Mean ± SD
NS = Not significant different

4.7 The effect of High Pressure Processing in the presence and absence of CMC on the sensory quality of lime juice during one month storage

The sensory characteristics of lime juice samples including bitterness, color, sourness, aroma and overall acceptability were conducted for lime juice samples that were not treated with HPP (control), treated with HPP at 400-600 MPa for 15 minutes and treated with HPP with an addition of 1.0 g/l (w/v) CMC. The sensory evaluation of the juices was conducted using 9-point hedonic scale and the results were displayed in Tables 4.23 to 4.32.

In general, the collected data displayed that the HPP could improve the sensory quality of the lime juice samples by having higher acceptance for bitterness, color, sourness, aroma and overall acceptances compared to those of the control samples during the storage period. The presence of CMC was only improved the bitterness of the lime juice samples compared to that of the HPP treated-lime juices.

Thermal treatment has been widely used to inactivate spoilage and pathogenic microorganisms and enzymes to extend the shelf life of juice products. However, thermal treatment can lower the sensory and nutritional qualities of juices (Chen *et al*., 1993). On the other hand, HPP has been intensively studied as a non-thermal agent to preserve and inactivate microorganisms in foods while reducing the loss of flavor, color, and nutrients of juices from heat (Deliza *et al*., 2005).

4.7.1 Bitterness characteristic

Tables 4.23 and 4.24 displayed the bitterness characteristic of the lime juice samples stored at chilled and ambient temperatures, respectively. Guadagni *et al*., (1974) reported that the least sensitive individual had a d-limonin threshold detection limit of 5-6 ppm. The lime juices processed of 500 and 600 MPa together with the presence of 0.1 g/l (w/v) CMC produced the highest bitterness acceptance compared to the other treatments, especially at refrigerator temperature. The better acceptance of the HPP treated-lime juices compared to the control could be due to the interruption of the limonoid D-ring lactone hydrolase enzyme that was able to convert limonoate-A-ring lactone to d-limonin, which was the cause of the bitterness taste, by the HPP. Since the development of the bitter taste was reduced, the acceptance of the juice was increased. This finding was also consistent with the measurement of the d-limonin content (section 4.6.1).

4.7.2 Color characteristic

Color attributes of the lime juice samples shown in Tables 4.25 and 4.26 demonstrated that the HPP treated-lime juices and the HPP CMC added-lime juices were not significantly different in their color values at the end of the storage period at 4-6^{o}C. However, higher storage temperature caused a significant reduction in the color value of the lime juice irrespectively to the treatments received by the juice samples.

4.7.3 Aroma and sourness characteristic

Aroma and sourness characteristics of the lime juice samples, which could be seen in Tables 4.27 to 4.30 showed that there was not any significant differences in the term for sourness between the HPP treated-lime juices and the HPP CMC added-lime juices at the end of the storage period at chilled temperatures. However, the two treatments were significantly scored to be different for their aroma properties during the storage period. All of the HP lime juice treatments had better aroma and sourness properties than those of the control treatments. Higher storage temperature also reduced the acceptance of the aroma and sourness of the juice samples. In citrus fruit, terpenes and sesquiterpenes are found in the juice oils, which are responsible for off-flavor of lime juice during storage (Wood, 1988). Freshness is likely to be determined by consumers with their perceptions. Aroma and color are important elements in consumers' perceptions for the freshness of juice products (IFT, 2001). Results in this study indicated that the HP processing could help to extend the shelf life of lime juice by slowing the physical and chemical changes in the lime juice.

4.7.4 Overall acceptability of lime juices

The overall acceptance of the lime juice displayed in Tables 4.31 and 4.32 demonstrated that the HPP CMC-added lime juices treated at 500 and 600 MPa at the end of their storage period at 4-6^{o}C were not significantly different than those of the fresh lime juices. The overall acceptance of foods may be mainly determined by freshness. Higher scores of the HPP treated-lime juices in the term of bitterness, color, sourness, aroma and overall acceptance than control treatments may be associated with higher freshness of the juice.

Eventhough the storage was significantly affected the overall acceptance of the HPP treated-lime juices, these samples were still being ranked between very much liked to moderately liked during one month storage.

Table 4.23 Effect of HPP on bitterness characteristic of CMC-treated and non-treated lime juice during storage at 4-6^{0}C

Process Condition	Storage period (weeks)					
	Before processing	0	1	2	3	4
Control	7.69 ± 0.85^a	7.69 ± 0.85^a	2.54 ± 1.05^b	2.69 ± 0.85^b	2.46 ± 0.97^b	2.00 ± 1.00^b
400 MPa	7.69 ± 0.85^a	7.56 ± 0.73^a	5.22 ± 1.20^b	4.67 ± 1.32^b	4.22 ± 1.30^b	4.00 ± 0.87^b
CMC 1.0 g/L + 400 MPa	7.69 ± 0.85^a	7.67 ± 0.87^a	6.44 ± 0.88^b	5.78 ± 1.09^b	5.56 ± 1.24^b	5.67 ± 2.00^b
500 MPa	7.69 ± 0.85^a	7.67 ± 0.71^a	6.00 ± 1.47^b	5.56 ± 1.59^{bc}	5.20 ± 1.93^{bc}	4.67 ± 1.50^c
CMC 1.0 g/L + 500 MPa	7.69 ± 0.85^{ab}	7.89 ± 0.78^a	7.11 ± 0.78^{abc}	7.00 ± 1.12^{bc}	6.89 ± 1.05^{bc}	6.78 ± 0.67^c
600 MPa	7.69 ± 0.85^a	7.56 ± 1.13^a	5.00 ± 1.66^b	4.33 ± 0.71^b	4.22 ± 1.09^b	4.11 ± 0.78^b
CMC 1.0 g/L + 600 MPa	7.69 ± 0.85^a	7.78 ± 0.67^a	6.78 ± 1.09^b	6.67 ± 1.00^b	6.44 ± 1.01^b	6.33 ± 1.00^b

Values within a row followed by different letters were significantly different ($p<0.05$)
Mean ± SD

Table 4.24 Effect of HPP on bitterness characteristic of CMC-treated and non-treated lime juice during storage at ambient temperature

Process Condition	Storage period (weeks)					
	Before processing	0	1	2	3	4
Control	7.69 ± 0.85^{a}	7.69 ± 0.85^{a}	3.77 ± 1.17^{b}	3.38 ± 1.12^{b}	3.54 ± 1.05^{b}	3.38 ± 0.87^{b}
400 MPa	7.69 ± 0.85^{a}	7.56 ± 0.73^{a}	5.78 ± 1.20^{b}	4.00 ± 1.12^{c}	4.33 ± 1.32^{c}	3.67 ± 1.41^{c}
CMC 1.0 g/L + 400 MPa	7.69 ± 0.85^{a}	7.67 ± 0.87^{a}	6.11 ± 1.05^{b}	5.56 ± 1.42^{bc}	5.33 ± 1.22^{bc}	4.67 ± 1.22^{c}
500 MPa	7.69 ± 0.85^{a}	7.67 ± 0.71^{a}	6.78 ± 0.97^{a}	4.33 ± 1.41^{bc}	5.00 ± 1.12^{b}	3.78 ± 1.30^{c}
CMC 1.0 g/L + 500 MPa	7.69 ± 0.85^{a}	7.89 ± 0.78^{a}	7.11 ± 0.78^{a}	5.44 ± 1.24^{b}	5.55 ± 1.42^{b}	4.67 ± 1.50^{b}
600 MPa	7.69 ± 0.85^{a}	7.56 ± 1.13^{a}	5.44 ± 1.67^{b}	4.89 ± 1.54^{b}	4.33 ± 1.73^{b}	4.56 ± 1.88^{b}
CMC 1.0 g/L + 600 MPa	7.69 ± 0.85^{a}	7.78 ± 0.67^{a}	6.44 ± 1.13^{b}	5.78 ± 0.83^{b}	5.56 ± 0.88^{b}	5.89 ± 1.17^{b}

Values within a row followed by different letters were significantly different ($p<0.05$)
Mean ± SD

Table 4.25 Effect of HPP on color appearance of CMC-treated and non-treated lime juice during storage at 4-6^{0}C

Process Condition	**Storage period (weeks)**					
	Before processing	**0**	**1**	**2**	**3**	**4**
Control	8.31 ± 0.75[a]	8.31 ± 0.75[a]	5.15 ± 1.68[a]	5.15 ± 0.99[a]	4.85 ± 1.14[a]	4.85 ± 1.07[a]
400 MPa	8.31 ± 0.75[a]	8.00 ± 0.71[a]	7.67 ± 0.87[a]	7.67 ± 1.00[a]	7.56 ± 0.88[a]	7.44 ± 0.73[a]
CMC 1.0 g/L + 400 MPa	8.31 ± 0.75[a]	8.44 ± 1.00[a]	7.56 ± 1.24[a]	7.56 ± 1.24[a]	7.33 ± 1.58[a]	7.33 ± 1.00[a]
500 MPa	8.31 ± 0.75[a]	8.11 ± 0.60[a]	7.78 ± 0.83[a]	7.89 ± 0.78[a]	7.78 ± 0.83[a]	8.00 ± 0.87[a]
CMC 1.0 g/L + 500 MPa	8.31 ± 0.75[a]	8.33 ± 1.00[a]	7.22 ± 0.67[a]	7.22 ± 0.67[a]	7.44 ± 0.88[a]	7.44 ± 0.73[a]
600 MPa	8.31 ± 0.75[a]	8.33 ± 0.71[a]	8.11 ± 0.78[a]	7.78 ± 0.97[a]	7.67 ± 1.12[a]	7.44 ± 1.01[a]
CMC 1.0 g/L + 600 MPa	8.31 ± 0.75[a]	8.44 ± 0.73[a]	7.44 ± 1.51[ab]	7.44 ± 0.73[ab]	7.33 ± 1.00[b]	7.33 ± 1.00[b]

Values within a row followed by different letters were significantly different ($p<0.05$)
Mean ± SD

Table 4.26 Effect of HPP on color appearance of CMC-treated and non-treated lime juice during storage at ambient temperature

Process Condition	Storage period (weeks)					
	Before processing	**0**	**1**	**2**	**3**	**4**
Control	8.31 ± 0.75^{a}	8.31 ± 0.75^{a}	3.46 ± 1.20^{b}	2.77 ± 1.01^{b}	1.46 ± 0.88^{c}	1.23 ± 0.44^{d}
400 MPa	8.31 ± 0.75^{a}	8.00 ± 0.71^{a}	5.44 ± 0.73^{b}	2.89 ± 0.93^{c}	1.89 ± 0.60^{d}	1.22 ± 0.44^{e}
CMC 1.0 g/L + 400 MPa	8.31 ± 0.75^{a}	8.44 ± 1.00^{a}	5.67 ± 1.32^{b}	3.78 ± 1.79^{c}	2.11 ± 0.93^{d}	1.78 ± 1.09^{d}
500 MPa	8.31 ± 0.75^{a}	8.11 ± 0.60^{a}	5.56 ± 1.42^{a}	3.78 ± 1.20^{b}	3.11 ± 1.76^{b}	1.56 ± 0.73^{c}
CMC 1.0 g/L + 500 MPa	8.31 ± 0.75^{a}	8.33 ± 1.00^{a}	5.78 ± 1.64^{b}	4.11 ± 1.36^{c}	3.33 ± 1.87^{c}	1.67 ± 0.87^{d}
600 MPa	8.31 ± 0.75^{a}	8.33 ± 0.71^{a}	5.22 ± 1.56^{b}	3.11 ± 1.62^{c}	3.00 ± 1.22^{c}	1.11 ± 0.33^{d}
CMC 1.0 g/L + 600 MPa	8.31 ± 0.75^{a}	8.44 ± 0.73^{a}	5.89 ± 0.93^{b}	3.67 ± 2.00^{c}	3.11 ± 1.27^{c}	1.22 ± 0.67^{d}

Values within a row followed by different letters were significantly different ($p<0.05$)
Mean ± SD

Table 4.27 Effect of HPP on aroma characteristic of CMC-treated and non-treated lime juice during storage at 4-6^{0}C

Process Condition	Storage period (weeks)					
	Before processing	0	1	2	3	4
Control	8.08 ± 0.76^{a}	8.08 ± 0.76^{a}	4.85 ± 2.44^{b}	4.85 ± 1.34^{b}	4.38 ± 1.56^{b}	4.31 ± 1.70^{b}
400 MPa	8.08 ± 0.76^{a}	8.00 ± 0.71^{a}	7.67 ± 0.87^{ab}	7.44 ± 0.53^{ab}	7.33 ± 0.71^{ab}	7.22 ± 0.83^{b}
CMC 1.0 g/L + 400 MPa	8.08 ± 0.76^{a}	8.11 ± 0.93^{a}	7.00 ± 1.22^{b}	6.44 ± 1.13^{b}	6.44 ± 0.88^{b}	6.44 ± 1.13^{b}
500 MPa^{NS}	8.08 ± 0.76	8.00 ± 0.70	7.44 ± 0.73	7.33 ± 0.71	7.44 ± 0.73	7.33 ± 0.71
CMC 1.0 g/L + 500 MPa	8.08 ± 0.76^{a}	8.11 ± 0.93^{a}	6.89 ± 1.17^{b}	6.78 ± 0.83^{b}	6.44 ± 0.73^{b}	6.67 ± 1.22^{b}
600 MPa	8.08 ± 0.76^{a}	8.11 ± 1.05^{a}	7.11 ± 1.17^{b}	6.67 ± 0.87^{b}	6.44 ± 1.01^{b}	6.33 ± 0.87^{b}
CMC 1.0 g/L + 600 MPa	8.08 ± 0.76^{a}	8.00 ± 0.87^{a}	6.89 ± 0.60^{a}	6.78 ± 1.09^{b}	6.22 ± 1.56^{b}	5.89 ± 1.45^{b}

Values within a row followed by different letters were significantly different ($p<0.05$)
Mean ± SD
NS = Not significant different

Table 4.28 Effect of HPP on aroma characteristic of CMC-treated and non-treated lime juice during storage at ambient temperature

Process Condition	Storage period (weeks)					
	Before processing	**0**	**1**	**2**	**3**	**4**
Control	8.08 ± 0.76^{a}	8.08 ± 0.76^{a}	2.92 ± 0.95^{b}	1.62 ± 0.65^{c}	1.46 ± 0.78^{c}	1.31 ± 0.63^{c}
400 MPa	8.08 ± 0.76^{a}	8.00 ± 0.71^{a}	6.00 ± 0.87^{b}	4.78 ± 1.09^{c}	3.56 ± 1.13^{d}	2.33 ± 0.87^{e}
CMC 1.0 g/L + 400 MPa	8.08 ± 0.76^{a}	8.11 ± 0.93^{a}	5.89 ± 0.93^{b}	4.89 ± 1.36^{b}	3.67 ± 1.32^{c}	2.44 ± 0.88^{d}
500 MPa	8.08 ± 0.76^{a}	8.00 ± 0.70^{a}	5.67 ± 1.41^{b}	4.67 ± 1.32^{bc}	4.22 ± 1.48^{bc}	3.22 ± 1.30^{c}
CMC 1.0 g/L + 500 MPa	8.08 ± 0.76^{a}	8.11 ± 0.93^{a}	5.78 ± 1.48^{b}	4.89 ± 1.17^{bc}	4.44 ± 1.42^{cd}	3.56 ± 1.01^{d}
600 MPa	8.08 ± 0.76^{a}	8.11 ± 1.05^{a}	5.78 ± 0.67^{b}	4.44 ± 1.24^{c}	3.22 ± 0.67^{d}	2.89 ± 0.93^{d}
CMC 1.0 g/L + 600 MPa	8.08 ± 0.76^{a}	8.00 ± 0.87^{a}	5.89 ± 0.78^{b}	4.67 ± 1.32^{c}	3.44 ± 0.88^{d}	3.22 ± 1.09^{d}

Values within a row followed by different letters were significantly different ($p<0.05$)
Mean ± SD

Table 4.29 Effect of HPP on sour taste of CMC-treated and non-treated lime juice during storage at 4-6^{0}C

Process Condition	Storage period (weeks)					
	Before processing	0	1	2	3	4
Control	8.15 ± 0.99^a	8.15 ± 0.99^a	5.08 ± 2.25^b	5.08 ± 1.19^b	4.92 ± 1.32^b	4.77 ± 1.36^b
400 MPa^{NS}	8.15 ± 0.99	8.11 ± 0.93	7.56 ± 1.13	7.44 ± 0.53	7.56 ± 0.53	7.56 ± 0.53
CMC 1.0 g/L + 400 MPa	8.15 ± 0.99^a	8.00 ± 0.87^a	7.11 ± 0.78^a	7.11 ± 1.17^a	7.00 ± 1.22^a	6.56 ± 1.51^b
500 MPa^{NS}	8.15 ± 0.99	8.00 ± 0.87	7.44 ± 0.88	7.56 ± 0.53	7.44 ± 0.88	7.56 ± 0.53
CMC 1.0 g/L + 500 MPa^{NS}	8.15 ± 0.99	8.11 ± 0.78	7.22 ± 0.67	7.22 ± 0.67	7.33 ± 0.71	7.44 ± 0.88
600 MPa^{NS}	8.15 ± 0.99	8.11 ± 0.78	7.11 ± 1.62	7.22 ± 0.67	7.11 ± 1.62	6.89 ± 0.93
CMC 1.0 g/L + 600 MPa	8.15 ± 0.99^a	8.02 ± 1.12^a	6.89 ± 1.05^b	7.00 ± 0.71^b	6.56 ± 0.73^b	6.33 ± 0.87^b

Values within a row followed by different letters were significantly different ($p<0.05$)
Mean ± SD
NS = Not significant different

Table 4.30 Effect of HPP on sour taste of CMC-treated and non-treated lime juice during storage at ambient temperature

Process Condition	**Storage period (weeks)**					
	Before processing	**0**	**1**	**2**	**3**	**4**
Control	8.15 ± 0.99^{a}	8.15 ± 0.99^{a}	5.54 ± 1.61^{b}	4.77 ± 0.93^{bc}	4.46 ± 1.51^{c}	3.69 ± 1.89^{d}
400 MPa	8.15 ± 0.99^{a}	8.11 ± 0.93^{a}	6.78 ± 1.09^{b}	6.67 ± 1.00^{b}	6.00 ± 1.12^{b}	5.33 ± 1.73^{bc}
CMC 1.0 g/L + 400 MPa	8.15 ± 0.99^{a}	8.00 ± 0.87^{a}	6.89 ± 1.05^{b}	6.67 ± 1.00^{b}	5.89 ± 0.93^{bc}	5.00 ± 1.66^{c}
500 MPa	8.15 ± 0.99^{a}	8.00 ± 0.87^{a}	6.89 ± 1.05^{b}	6.67 ± 0.71^{b}	6.00 ± 1.58^{b}	5.56 ± 0.88^{c}
CMC 1.0 g/L + 500 MPa	8.15 ± 0.99^{a}	8.11 ± 0.78^{a}	6.89 ± 1.05^{b}	6.56 ± 0.88^{bc}	5.89 ± 1.45^{c}	5.44 ± 1.42^{c}
600 MPa	8.15 ± 0.99^{a}	8.11 ± 0.78^{a}	6.89 ± 1.27^{b}	6.33 ± 1.00^{bc}	5.67 ± 0.87^{c}	5.78 ± 0.83^{c}
CMC 1.0 g/L + 600 MPa	8.15 ± 0.99^{a}	8.02 ± 1.12^{a}	6.89 ± 1.27^{b}	6.11 ± 0.93^{b}	6.00 ± 0.71^{bc}	5.56 ± 0.73^{c}

Values within a row followed by different letters were significantly different ($p<0.05$)
Mean ± SD

Table 4.31 Effect of HPP on the overall acceptance of CMC-treated and non-treated lime juice during storage at 4-6^{0}C

Process Condition	**Storage period (weeks)**					
	Before processing	**0**	**1**	**2**	**3**	**4**
Control	8.15 ± 0.69^a	8.15 ± 0.69^a	4.54 ± 2.76^b	4.31 ± 1.55^b	3.69 ± 2.32^{bc}	4.15 ± 1.34^b
400 MPa	8.15 ± 0.69^a	8.00 ± 0.50^a	6.67 ± 1.32^b	6.44 ± 1.01^b	6.22 ± 0.97^b	6.00 ± 1.12^b
CMC 1.0 g/L + 400 MPa	8.15 ± 0.69^a	8.22 ± 0.83^a	7.22 ± 0.67^a	7.00 ± 1.00^{ab}	7.00 ± 1.00^{ab}	6.44 ± 1.13^b
500 MPa	8.15 ± 0.69^a	8.00 ± 0.87^a	6.89 ± 0.93^b	6.56 ± 0.73^b	6.89 ± 0.93^b	6.56 ± 0.73^b
CMC 1.0 g/L + 500 MPa^{NS}	8.15 ± 0.69	8.22 ± 0.67	7.44 ± 0.53	7.33 ± 0.71	7.22 ± 0.67	7.11 ± 0.60
600 MPa	8.15 ± 0.69^a	7.89 ± 1.05^a	6.67 ± 0.71^b	6.00 ± 0.87^{bc}	6.11 ± 0.78^{bc}	5.89 ± 1.05^{bc}
CMC 1.0 g/L + 600 MPa	8.15 ± 0.69^a	8.00 ± 0.71^a	6.44 ± 1.01^b	6.56 ± 1.24^b	7.00 ± 0.71^{ab}	7.22 ± 0.83^a

Values within a row followed by different letters were significantly different ($p<0.05$)
Mean ± SD
NS = Not significant different

Table 4.32 Effect of HPP on the overall acceptance of CMC-treated and non-treated lime juice during storage at ambient temperature

Process Condition	Storage period (weeks)					
	Before processing	0	1	2	3	4
Control	8.15 ± 0.69^{a}	8.15 ± 0.69^{a}	4.62 ± 1.26^{b}	3.08 ± 1.12^{c}	1.85 ± 1.21^{d}	1.38 ± 0.51^{e}
400 MPa	8.15 ± 0.69^{a}	8.00 ± 0.50^{a}	6.00 ± 1.32^{b}	5.22 ± 0.83^{c}	4.00 ± 0.87^{d}	2.56 ± 1.33^{e}
CMC 1.0 g/L + 400 MPa	8.15 ± 0.69^{a}	8.22 ± 0.83^{a}	6.67 ± 1.12^{b}	5.56 ± 0.88^{cd}	4.78 ± 1.30^{d}	4.11 ± 1.69^{d}
500 MPa	8.15 ± 0.69^{a}	8.00 ± 0.87^{a}	6.00 ± 1.00^{b}	5.11 ± 1.45^{c}	1.33 ± 1.32^{d}	2.00 ± 1.12^{d}
CMC 1.0 g/L + 500 MPa	8.15 ± 0.69^{a}	8.22 ± 0.67^{a}	6.78 ± 0.97^{b}	6.22 ± 1.09^{b}	4.89 ± 1.83^{c}	2.56 ± 2.01^{d}
600 MPa	8.15 ± 0.69^{a}	7.89 ± 1.05^{a}	6.11 ± 1.17^{b}	5.67 ± 1.00^{c}	3.22 ± 1.20^{d}	2.00 ± 1.00^{e}
CMC 1.0 g/L + 600 MPa	8.15 ± 0.69^{a}	8.00 ± 0.71^{a}	6.89 ± 1.05^{b}	6.00 ± 1.12^{b}	4.11 ± 1.54^{c}	3.56 ± 1.88^{c}

Values within a row followed by different letters were significantly different ($p<0.05$)
Mean ± SD