Chapter 4

Result and discussion

4.1 Finding a good quality orange juice from orange fruit produced locally in Chiang Mai

Orange varieties that were studied in this research were Keaw Wan Prae and Sai Namphung. After producing orange juices, the two orange varieties were different in their taste and color based on a direct observation. The Keaw Wan Prae orange juice had only sour taste and the juice had a yellow to orange color, whereas the Sai Namphung orange juice had sour and sweet taste with more orange color than the other orange juice.

4.1.1 Physical measurement of orange juices from Keaw Wan Prae and Sai Namphung varieties

The results of physical measurement of different varieties of orange juices were displayed in Table 4.1. From this Table it showed that both orange varieties did not significantly produce different orange juice yields after extraction processes. However, these orange juices were significantly different in other physical properties, including b* value, cloudiness of the juice and viscosity parameter.

For color measurement, Table 4.1 displayed that the L and a* values of Keaw Wan Prae and Sai Namphung orange juices were not significantly different, which meant that the lightness and redness of both orange juices were similar. However, the b* value for the 2 orange juices was significantly different. The b* value of Sai Namphung orange juice was higher than that of Keaw Wan Prae orange juice. This result was confirmed the result of the direct observation for the orange juices (please, see above explanation) and it could be seen from the picture in Appendix A Fig. A3.

At the same time, the result of the color measurement was affected by the carotenoid content in the orange juices. Carotenoids are yellow, orange and red pigments that present in many commonly eaten fruits and vegetables (Holden *et al.*, 1999). As the content of carotenoid was higher, the intensity of the color would also be higher. However, the result of this section found that the Keaw Wan Prae orange juice that had lower b* value, had a higher carotenoid content than that of the Sai Namphung orange juice (Table 4.6). The contradiction effect of carotenoid that was found in this section showed that other factors might have higher contribution to the result of the color measurement. These factor would include the ripeness period and climate conditions during growing of the orange fruit (Ingallinera *et al.*, 2004).

Table 4.1 The physical characteristics of Keaw Wan Prae and Sai Namphung orange juices

Physical measurement	**Orange varieties**	
	Keaw Wan Prae	**Sai Namphung**
1. Color		
- L value	41.82±1.20[a]	41.04±1.20[a]
- a* value	5.81±0.98[a]	7.09±0.58[a]
- b* value	29.43±0.31[a]	31.03±0.56[b]
2. Cloudiness (%T)	15.44±2.16[a]	21.72±0.35[b]
3. Viscosity (cps)	2.11±0.03[a]	4.92±0.06[b]
4. Extraction (%)	32.95±3.95[a]	31.62±1.66[a]

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

Cloud stability is an important quality parameter for orange juice. Loss of this parameter can be due to the activity of pectinesterase (PE) enzyme (Ingallinera *et al.*, 2005). In this study, the cloud stability was measured based on light transmission (%T)

using a spectrophotometer at 640 nm. The result showed that the Sai Namphung orange juice was significantly higher in the cloudiness measurement compared to that of the Keaw Wan Prae orange juice. At the same time, a measurement in the PE enzyme showed that the Keaw Wan Prae orange juice was significantly contained higher concentration of the enzyme than that of the Sai Namphung orange juice (please, refer to Table 4.2). Since the measurement of cloudiness of the orange juices was carried out for fresh orange juices, the cloudiness values in this section might not be directly correlated with the amount of the PE enzyme. There was a possibility that the cloudiness measurement was more correlated with the sugar content of the orange juice, since the reducing sugar of the Sai Namphung orange juice was significantly lower than that of the Keaw Wan Prae orange juice (Table 4.2). Moreover, the Keaw Wan Prae orange juice had higher values of Total Soluble Solid, Total Solid, invert sugar and total sugar compared to those of the Sai Namphung orange juice (Table 4.2).

Viscosity is an important parameter for orange juice because this property has a potential impact on the sensory quality of the juice, such as mouthful. The viscosity of an orange juice is related to the quantity and consistency of the juice pulp (Farnworth *et al.*, 2001). In this study, the result showed that the viscosity of Sai Namphung orange juice was significantly higher than that of the Keaw Wan Prae orange juice (Table 4.1). This result might be correlated with the result of fiber measurement (please, refer to Table 4.2), in which the Sai Namphung orange juice contained higher fiber content than that of the Keaw Wan Prae orange juice.

4.1.2 Chemical measurement of orange juices from Keaw Wan Prae and Sai Namphung varieties

The chemical measurement of Keaw Wan Prae and Sai Namphung orange juices can be seen in Table 4.2. From 14 different analyses, it showed that both orange juices were not significantly different in the term of pH, Total Soluble Solid (TSS),

moisture content, total solid, invert sugar, total sugar, protein and fiber values. Whereas the values of total titrable acidity, reducing sugar, fat, ash, carbohydrate and PE enzyme were significantly different for these orange juices.

Table 4.2 The chemical properties of Keaw Wan Prae and Sai Namphung orange juices

Chemical measurement	Orange varieties	
	Keaw Wan Prae	Sai Namphung
1. pH	4.41±0.07[a*)]	4.46±0.04[a]
2. Total Soluble Solid (TSS) (^{0}Brix)	12.67±0.64[a]	11.93±0.07[a]
3. Total titrable acidity (% citric acid)	0.63±0.06[a]	0.49±0.05[b]
4. Moisture (%)	87.61±0.65[a]	88.22±0.19[a]
5. Total Solid (TS) (%)	12.39±0.65[a]	11.78±0.19[a]
6. Reducing sugar (%)	5.56±0.23[a]	3.92±0.36[b]
7. Invert sugar (%)	9.17±0.47[a]	9.05±0.53[a]
8. Total sugar (%)	8.99±0.46[a]	8.79±0.53[a]
9. Protein content (%)	0.74±0.08[a]	0.77±0.05[a]
10. Fat content (%)	0.63±0.06[a]	0.36±0.08[b]
11. Ash content (%)	0.31±0.02[a]	0.98±0.05[b]
12. Carbohydrate (%)	10.62±0.60[a]	9.42±0.31[b]
13. PE enzyme (PMEu/g.s.s)	1.17±0.14[a]	0.65±0.12[b]
14. Fiber	0.09±0.04[a]	0.24±0.10[a]

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

The results of total titrable acidity showed that Keaw Wan Prae orange juice was significantly contained more citric acid than that of Sai Namphung orange juice.

This result was correlated with the taste of the juice, in which a sour taste could be detected in the Keaw Wan Prae orange juice more than that in the Sai Namphung orange juice. For the contents of reducing sugar, fat, carbohydrate and PE enzyme, the Keaw Wan Prae orange juice also had higher quantity than those in the Sai Namphung orange juice. In the other hand, the Sai Namphung orange juice significantly contained more ash content compared to that in the Keaw Wan Prae orange juice.

Beside these analyses, the orange juice samples of Keaw Wan Prae and Sai Namphung were also analysed for their flavour composition using a combination of Gas Chromatography and Mass Spectophotometer (GC-MS). The results for this measurement were displayed in Tables 4.3 and 4.4 for Keaw Wan Prae and Sai Namphung orange juices, respectively. The overall results showed that the Keaw Wan Prae orange juice had 21 flavour components, including 4 alcohols, 1 ester, 1 aldehyde, 13 terpene hydrocarbons and 2 unknown compounds (Table 4.3). Whereas the Sai Namphung orange juice was richer with flavour compounds, that had 26 identified chemical compounds, including 6 alcohols, 5 esters, 14 terpene hydrocarbons and 1 unknown compound (Table 4.4).

From the alcohol group, the important flavour components in the Keaw Wan Prae and Sai Namphung orange juices were ethanol, linalool and α-terpineol. The presence of ethanol gave an alcoholic note in both orange juices. The ethanol content in the Keaw Wan Prae and Sai Namphung orange juices were 0.69 and 4.02%, respectively. This ethanol content rises in ripening fruit as it is augmented by the action of microorganisms which use sugars present in fruit as a source of energy (Sánchez, 2004). Therefore, the result of the flavour measurement was related to the levels of reducing sugar and the numbers of microorganism in the orange juice samples. The analysis results showed that the Sai Namphung orange juice significantly had lower reducing sugar content and higher number of microorganisms compared to those in the Keaw Wan Prae orange juice (Table 4.2 and 4.5). Therefore, it could be

concluded that the presence of microorganisms in the Sai Namphung orange juice played an important role in reducing the level of reducing sugar and increasing the amount of ethanol in the juice. For another flavour component, linalool, it makes a positive contribution to orange flavour in combination with several other orange volatiles. Beside that, the component also has a floral–woody odour with a faintly

Table 4.3 Flavour components of Keaw Wan Prae orange juice

Component	Retention time (min)	% of total
Alcohols		
Ethanol	1.61	0.69
Linalool	9.46	0.70
Terpinene-4-ol	11.44	0.03
α-Terpineol	11.72	0.05
Esters		
Ethyl acetate	2.01	0.07
Aldehyde		
Hexanal	3.41	0.05
Terpene hydrocarbons		
Ethylallene or isoprene	1.72	0.10
α-Pinene	5.89	1.32
Camphene	6.15	0.03
Sabinene	6.63	0.07

Table 4.3 (continued) Flavour components of Keaw Wan Prae orange juice

Component	Retention time (min)	% of total
β-Pinene	6.72	0.76
β-Myrcene	7.00	3.02
1-Phellandrene	7.32	0.08
α-Terpinene	7.61	0.07
Camphogen	7.68	0.08
d-Limonene	7.96	92.00
Trans- β-Ocimene or 1,3,7-Octatrine	8.30	0.14
γ-Terpinene	8.57	0.40
α-Terpinolene	9.31	0.13
Unknown		
no.1	2.40	0.20
no.2	4.00	0.02

Table 4.4 Flavour components in Sai Namphung orange juice

Component	Retention time (min)	% of total
Alcohols		
Ethanol	1.62	4.02
3-Methyl-1-butanol	2.80	0.11
2-Methyl-1-butanol	2.84	0.05
Linalool	9.46	0.56
Terpinene-4-ol	11.44	0.04
α-Terpineol	11.72	0.05

Table 4.4 (Continued) Flavour components in Sai Namphung orange juice

Component	Retention time (min)	% of total
Esters		
Ethyl acetate	2.01	0.36
Ethyl propionate	2.60	0.16
Ethyl isobutyrate or Ethyl isobutanate	3.05	0.05
Ethyl butyrate or Ethyl butanoate	3.49	0.06
Ethyl 2-methyl butanoate	4.20	0.17
Terpene hydrocarbons		
Ethylallene or isoprene	1.72	0.19
α-Thujene	5.74	0.06
α-Pinene	5.89	0.97
Camphene	6.15	0.02
Sabinene	6.63	0.30
β-Pinene	6.72	2.61
β-Myrcene	7.00	3.59
1-Phellandrene	7.31	0.08
α-Terpinene	7.60	0.09
Camphogen	7.68	0.05
d-Limonene	7.98	85.58
Trans- β-Ocimene or 1,3,7-Octatrine	8.30	0.24
γ-Terpinene	8.57	0.34
α-Terpinolene	9.31	0.11
Unknown		
no.1	2.40	0.15

citrusy note (Selli *et al.*, 2004). The GC-MS results showed that the Keaw Wan Prae orange juice had a higher content of linalool compared to the Sai Namphung orange juice (Tables 4.3 and 4.4). Another flavour compound of α-terpineol is generally considered to make a negative contribution to orange flavour and the increase in its amount is one of the more consistent findings in aged and heat-abused orange juice products (Selli *et al.*, 2004). Since the Keaw Wan Prae and Sai Namphung orange juices that were analyzed by the GC-MS in this section were prepared from fresh orange juices, the amount of α-terpineol in both orange juices were low and closed to each other (Tables 4.3 and 4.4).

Ethyl acetate was the main ester component that was present in the Keaw Wan Prae and Sai Namphung orange juices. Beside this compound, the Sai Namphung orange juice also had other 4 ester components that were presence at lower amounts than ethyl acetate (Table 4.4). The presence of ethyl acetate was positively correlated with off-flavour and the presence of an after taste in the juice (Namutebi, 1998). Since the amount of ethyl acetate was 5 times higher in the Sai Namphung orange juice compared to that in the Keaw Wan Prae orange juice, there was a possibility that off-flavour and an after taste could be detected easier in the Sai Namphung orange juice compared to those in the Keaw Wan Prae orange juice.

The variety of terpene hydrocarbon components in the Sai Namphung orange juice was higher by 1 compound compared to the terpene hydrocarbons in the Keaw Wan Prae orange juice, which had 13 compounds. However, the main terpene hydrocarbons that were present in both orange juices were β-Myrcene, d-limonene and γ-Terpinene. The amount of d-limonene in the orange juice samples represented as the main flavour compound with a contribution of 92.0 and 85.6% in the Keaw Wan Prae and Sai Namphung orange juices, respectively. D-Limonene has a weak, citrus-like aroma and is considered as one of the major contributors to orange flavour (Selli *et al.*, 2004). According to Jia *et al.* (1999), d-limonene is the most important flavour for

orange juice quality. The compound β-Myrcene is the second most abundant terpene hydrocarbons in both orange juices. This finding was similar to the report for other orange juices (Selli *et al.*, 2004). The compound had an "almost citrusy" and a "sweet-balsamin-herbaceous" taste at levels below 10 ppm (Selli *et al.*, 2004). For γ-Terpinene that was present at 0.34-0.40% in both orange juices, the flavour compound is a component of orange peel oil and is slightly bitter-herbaceous, but becomes pleasant and citrusy at concentrations below 40 ppm (Selli *et al.*, 2004).

Besides alcohol, ester and terpene hydrocarbon compounds, the Keaw Wan Prae orange juice contained an aldehyde compound that was identified as hexanal. Hexanal has a strong, penetrating, fatty-green and grassy unripe fruit odour (Leffingwell, 1999).

4.1.3 Microbiological measurement of orange juices from Keaw Wan Prae and Sai Namphung varieties

The number of microorganisms in orange juices is an important quality parameter for orange juices because the presence of these organisms can significantly affect the shelf life of the juices, particularly for the fresh orange juice. The result for the microbiological analyses of Keaw Wan Prae and Sai Namphung orange juices was arranged in Table 4.5. The collected data showed that the Sai Namphung orange juice significantly contained higher amounts of microbial population compared to that in the Keaw Wan Prae orange juice. The presence of a high number of microorganisms in the Sai Namphung orange juice affected the chemical and flavour characteristics of the juice. Data from chemical analyses showed that the Sai Namphung orange juice contained higher levels of ethanol flavour and lower levels of reducing sugar content compared to those in the Keaw Wan Prae orange juice. There was a high possibility that the microorganisms in the Sai Namphung orange juice used the sugar in the orange juice and produced ethanol flavour as suggested by Sánchez *et al.* (2004). In

addition, the chemical analyses showed that the Keaw Wan Prae orange juice had a higher titrable acidity compared to that of the Sai Namphung orange juice (Table 4.2). The higher level of citric acid or lower pH values might responsible to a lower number of microorganisms in the Keaw Wan Prae orange juice, since the low pH or high acidity inhibits microorganism growth (Pandell, 1999).

Table 4.5 Microbiological analyses of Keaw Wan Prae and Sai Namphung orange juices

Microbiological analysis	Orange varieties	
	Keaw Wan Prae	Sai Namphung
1. TPC (log CFU/ml)	2.83±0.18[a*)]	6.31±0.38[b]
2. Yeast and Mold (log CFU/ml)	3.20±0.13[a]	3.62±0.22[b]

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

4.1.4 Nutritional measurement of different orange juices from Keaw Wan Prae and Sai Namphung varieties

The nutritional analysis of orange juice samples was carried out for vitamin C and carotenoid contents. The result for the analyses was exhibited in Table 4.6.

Vitamin C or ascorbic acid is one of the most important vitamins found in citrus juices, including orange juice. Although different varieties of orange will present different levels of vitamin C content, other factors, such as ripening processes and levels of citric acid can also affect the content of this vitamin in orange juices (Nagy, 1980). From Table 4.6, it was shown clearly that the Keaw Wan Prae orange juice was significantly contained more vitamin C compared to that in the Sai Namphung orange juice. The Keaw Wan Prae orange juice had a vitamin C content of 30.71 ± 8.13 mg/100 ml orange juice, which was almost 3 times higher than that in the Sai Namphung orange juice. Since Nagy (1980) reported that the content of vitamin C

was stabilized by a higher level of citric acid, a measurement of total titrable acidity of the Keaw Wan Prae orange juice supported this report. The Keaw Wan Prae orange juice had a citric acid level of 0.63 ± 0.06%, which was significantly higher than that in the Sai Namphung orange juice (Table 4.2).

Table 4.6 Vitamin C and carotenoid contents of Keaw Wan Prae and Sai Namphung orange juices

Nutritional measurement	**Orange varieties**	
	Keaw Wan Prae	**Sai Namphung**
1. Vitamin C (mg/100ml)	30.71±8.13[a*)]	10.83±1.67[b]
2. Carotenoid content (μg/ml)	6.61±0.61[a]	4.08±0.39[b]

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

The presence of carotenoids in orange juices affects the color and the nutritional value of the juices (Bull *et al.*, 2004). From Table 4.6, it could be seen that the Keaw Wan Prae orange juice was significantly contained more carotenoids than that in the Sai Namphung orange juice. The carotenoid contents of the Keaw Wan Prae and Sai Namphung orange juices were 6.61 ± 0.61 and 4.08 ± 0.39 μg/ml, respectively. Another report showed that the carotenoid content of Florida orange juice was 3.0 ± 1.4 μg/ml (Gardner *et al.*, 2000).

From different analyses that were carried out for the orange juice samples of Keaw Wan Prae and Sai Namphung, it could be concluded that both orange juices were not significantly different in the terms of pH, moisture content, invert sugar, total sugar, protein, L value (lightness), a* value (redness), total soluble solid, total solid and percent extraction. The Keaw Wan Prae orange juice were significantly contained higher citric acid, reducing sugar, fat, carbohydrate, PE enzyme, vitamin C and

carotenoid compared to those in the Sai Namphung orange juice. At the same time the Sai Namphung orange juice significantly had higher b* value (yellowness), cloudiness value, viscosity value, ash content, fiber content and microbial counts, including bacteria, yeast and mold, compared to those in the Keaw Wan Prae orange juice. Identification of flavor components in the Sai Namphung orange juice also showed that the juice contained a higher number of flavour compounds that those in the Keaw Wan Prae orange juice. Since the Sai Namphung orange juice contained higher flavour components and the production of the fresh orange in the market is increasing, there is a possibility to produce a fresh Sai Namphung orange juice with an addition of a food grade acid or other antimicrobial compounds to decrease the microbial load in the juice and a fortification of vitamin C to increase the nutritional value of the final product.

Although the Sai Namphung orange juice was superior in the term of flavour compound compared to the Keaw Wan Prae orange juice, the later orange juice had better nutritional and microbial values. Therefore, the Keaw Wan Prae orange juice was chosen to be further studied in the next experiment.

4.2 The effects of sugar and salt additions on the quality of fresh orange juice during chilled storage

In this section, Keaw Wan Prae orange was used as a raw material source because of its nutritional and microbiological qualities. The orange was purchased from a local market and processed as orange juices. Into these orange juices, 3 different sugar concentrations, including 0, 2.5 and 5% ($^{w}/_{v}$), and 3 levels of salt additions, which were 0, 0.05 and 0.1% ($^{w}/_{v}$), were added. The purpose of this addition was to evaluated the effects of sugar and salt on the quality of fresh orange juice during storage at refrigerated/chilled temperature (4-6^{o}C). The addition of sugar and

salt was done by some orange juice seller/consumers to improve/adjust the taste of the orange juice.

4.2.1 Physical characteristics of Keaw Wan Prae orange juice during chilled storage as affected by sugar and salt additions

4.2.1.1 Color of the orange juice

Orange juice samples were measured for their color based on L, a* and b* values. The results of this measurement were exhibited in Fig. 4.1 – 4.3. In general, these figures showed that these color values of orange juice samples were changed during storage at refrigerated temperature. The L and b* values of the orange juice samples were found to be increased during the storage time, while the a* value was decreased (Appendix E, Tables 1.1-1.3). This finding indicated that the lightness and yellowness of orange juice samples were increased, whereas the redness of the juice was decreased during storage at refrigerated temperature. This result was similar to the observation of Choi *et al.* (2002).

Comparing different orange juice treatments with the control orange juice (no addition of salt and sugar) at different storage times, the result did not show any specific pattern for each color value (Appendix E, Tables 1.1-1.3). However, when these treatments were grouping depending on the concentrations of salt or sugar only (Tables 4.7 and 4.8), some general discussion could be made. For example, the b* value was more affected by the presence of sugar more than by the presence of salt. The orange juice samples added with sugar both at 2.5 and 5.0% ($^{w}/_{v}$) would have higher b* value compared to those in the no-added sugar orange juice samples (Table 4.7). The L and a* values were not significantly affected by the presence of salt and sugar (Appendix E, Tables 3.1 and 3.2).

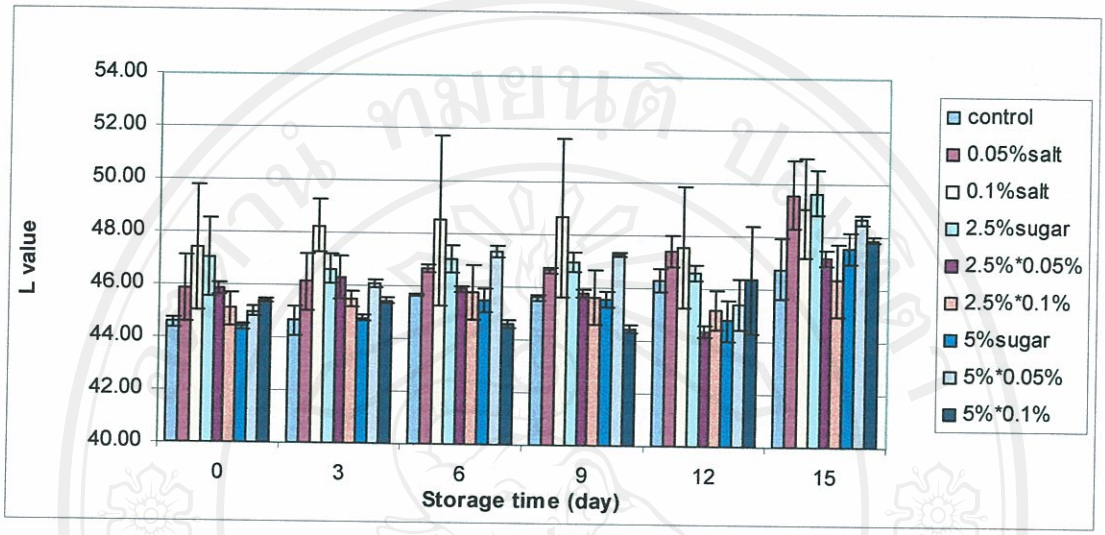


Fig. 4.1 The result of L value of orange juice during chilled storage as affected by sugar and salt additions

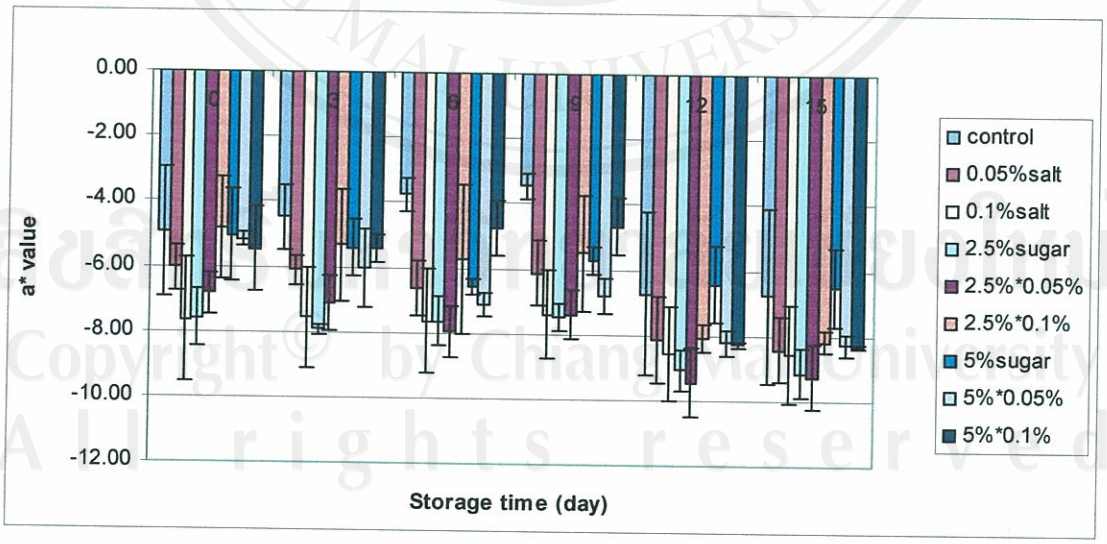


Fig. 4.2 The result of a* value of orange juice during chilled storage as affected by sugar and salt additions

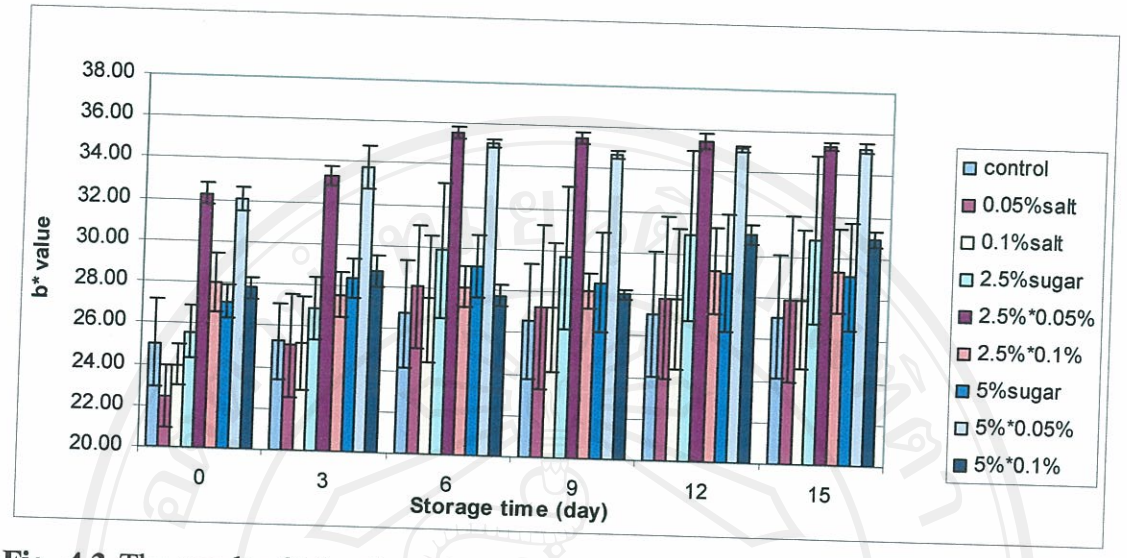


Fig. 4.3 The result of b* value of orange juice during chilled storage as affected by sugar and salt additions

Good color of orange juice means that the juice has a yellow–orange color that is bright and typical of the freshly extracted juice of oranges and is free from browning due to scorching, oxidation, caramelization or other causes (USDA, 1972).

The results of the color measurement were calculated further as a browning value to have a more understanding about the effect of sugar and salt additions on the color of orange juice. The calculation results for the browning value were displayed in Fig. 4.4, Tables 4.9 and 4.10. Fig. 4.4 showed that the browning value of orange juice was increased during the storage time, which was a result that was similar to the report of Koca *et al.* (2003). Since the control was used as a reference and had a zero browning value, the addition of sugar in orange juices was found to significantly increase the browning value of the juice compared to that of the no-added sugar orange juices (Table 4.9). Koca *et al.* (2003) reported that non-enzymatic browning could happen in citrus juices due to the reactions of sugar and amino acids. In this research, data also showed that an addition of 0.05% ($^{w}/_{v}$) salt was significantly increased the browning value compared to the 0.1% ($^{w}/_{v}$) salt (Table 4.10).

Table 4.7 The color result of orange juice during chilled storage as affected by sugar addition

Storage time (day)	(No added sugar)			2.5 % ($^{w}/_{v}$) sugar			5 % ($^{w}/_{v}$) sugar		
	L value	a* value	b* value	L value	a* value	b* value	L value	a* value	b* value
0	45.95±1.81$^{a*)}$	-6.21±1.84^{a}	23.82±1.79^{a}	45.99±1.19^{a}	-6.41±1.55^{a}	28.64±3.10^{b}	44.95±0.46^{a}	-5.22±0.98^{a}	28.99±2.41^{b}
3	46.34±1.75^{a}	-6.06±1.64^{a}	25.17±1.91^{a}	46.12±0.73^{a}	-6.79±1.49^{a}	29.25±3.21^{b}	45.43±0.59^{b}	-5.62±0.83^{a}	30.30±2.73^{c}
6	46.94±2.04^{a}	-6.01±1.97^{a}	27.45±2.56^{a}	46.23±0.84^{a}	-7.10±1.64^{a}	31.18±3.76^{b}	45.79±1.24^{a}	-6.14±1.16^{a}	30.66±3.45^{b}
9	46.96±2.01^{a}	-5.66±1.95^{a}	27.03±2.90^{a}	46.14±0.84^{a}	-6.79±1.38^{b}	31.08±3.80^{b}	45.75±1.23^{a}	-5.74±1.08^{a}	30.40±3.45^{b}
12	47.09±1.36^{a}	-7.80±1.79^{a}	27.59±3.03^{a}	45.38±1.04^{b}	-8.86±0.90^{a}	31.85±3.62^{b}	45.52±1.37^{b}	-7.64±1.10^{a}	31.75±0.03^{b}
15	48.46±1.83^{a}	-7.93±1.84^{a}	27.66±3.03^{a}	47.72±1.71^{a}	-8.87±0.83^{a}	31.87±3.53^{b}	48.03±0.57^{a}	-7.70±1.08^{a}	31.79±3.03^{b}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

Table 4.8 The color results of orange juice during chilled storage as affected by salt addition

Storage time (day)	(No added salt)			0.05 % ($^{w}/_{v}$) salt			0.10 % ($^{w}/_{v}$) salt		
	L value	a* value	b* value	L value	a* value	b* value	L value	a* value	b* value
0	45.35±1.48$^{a*)}$	-5.85±1.83^{a}	25.89±1.89^{a}	45.57±0.77^{a}	-6.02±0.86^{a}	28.94±4.94^{b}	45.97±1.63^{a}	-5.97±1.87^{a}	26.61±2.16^{a}
3	45.34±1.03^{a}	-5.94±1.68^{a}	26.81±1.85^{a}	46.18±0.69^{b}	-6.42±0.92^{a}	30.72±4.45^{b}	46.38±1.49^{b}	-6.12±1.61^{a}	27.18±2.05^{a}
6	46.05±0.82^{a}	-5.98±1.79^{a}	28.59±2.63^{a}	46.63±0.64^{a}	-7.23±0.83^{b}	32.89±3.91^{b}	46.28±2.42^{a}	-6.04±1.93^{a}	27.81±1.66^{a}
9	46.04±0.75^{a}	-5.55±1.78^{a}	28.25±2.86^{a}	46.56±0.65^{a}	-6.78±0.88^{b}	32.47±4.40^{b}	46.25±2.45^{a}	-5.86±1.71^{a,b}	27.78±1.66^{a}
12	45.89±0.96^{a}	-7.41±1.89^{a}	29.03±3.36^{a}	45.74±1.45^{a}	-8.60±1.07^{a}	32.81±4.21^{b}	46.36±1.89^{a}	-8.29±0.78^{a}	29.36±2.43^{a}
15	47.99±1.51^{a}	-7.48±1.97^{a}	29.03±3.27^{a}	48.47±1.24^{a}	-8.66±0.86^{a}	32.87±4.19^{b}	47.76±1.67^{a}	-8.35±0.80^{a}	29.43±2.37^{a}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

According to Lee (1992), he reported that citrus browning is unique to the typical Maillard-type browning. However, since citrus fruits have significant amounts of ascorbic acid, the ascorbic acid would be mainly oxidized due to the Maillard reaction compounds. The results of this experiment supported this report because as the browning value of orange juice increased (Fig. 4.4), the content of vitamin C in the juice was reduced (Fig. 4.13).

Another color parameter that was calculated from the L, a* and b* values was total color difference (ΔE*) value, which could be seen in Fig. 4.5, Tables 4.11 and 4.12. The ΔE* value used the L, a* and b* values of the control orange juice at 0 day storage as a reference value. The result of the ΔE* value calculation showed that this value was increased during refrigerated storage. This result was similar to the observation of Choi *et al.* (2002). At 0 day, the presence of sugar and/or salt increased the ΔE* value of orange juice treatments (Fig. 4.5, Appendix E, Table 3.5). However during the storage time, it was only the addition of sugar and 0.05% ($^{w}/_{v}$) salt that significantly affected the ΔE* value of orange juice (Table 4.11 and 4.12).

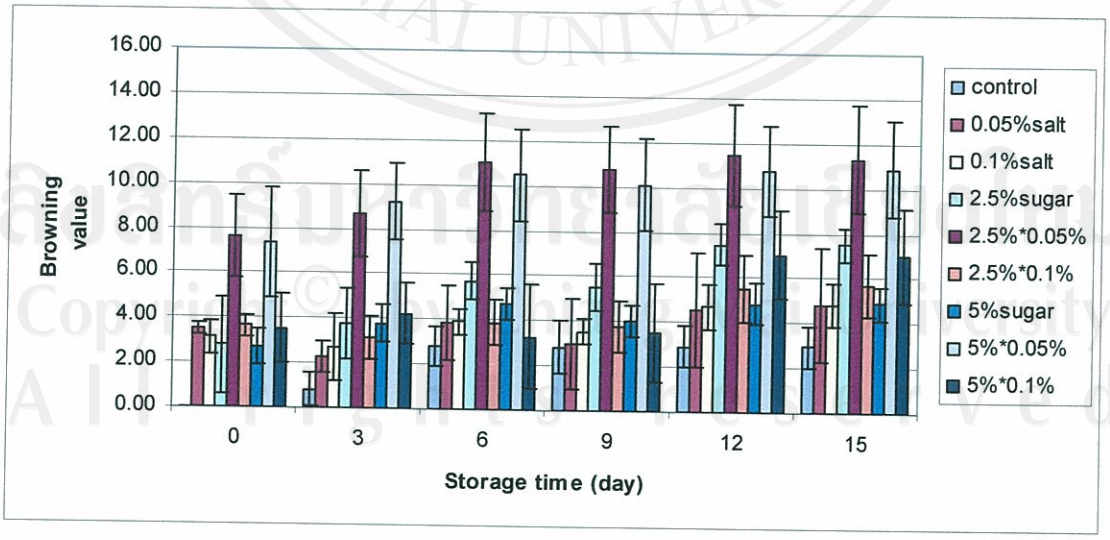


Fig. 4.4 The result of browning value of orange juice during chilled storage as affected by sugar and salt additions

Table 4.9 The browning value of orange juice during chilled storage as affected by sugar addition

Storage time (day)	no added sugar	2.5% ($^{w}/_{v}$) sugar	5% ($^{w}/_{v}$) sugar
0	2.21±1.92$^{a*)}$	4.66±2.58^{b}	4.51±2.47^{b}
3	1.93±0.99^{a}	5.17±3.01^{b}	5.75±3.00^{b}
6	3.50±0.63^{a}	6.86±3.70^{b}	6.15±3.80^{b}
9	3.11±0.38^{a}	6.67±3.67^{b}	5.86±3.66^{b}
12	4.04±0.99^{a}	8.13±3.02^{b}	7.52±2.98^{b}
15	4.19±1.07^{a}	8.15±2.88^{b}	7.58±3.05^{b}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

Table 4.10 The browning value of orange juice during chilled storage as affected by salt addition

Storage time (day)	no added salt	0.05% ($^{w}/_{v}$) salt	0.10% ($^{w}/_{v}$) salt
0	1.82±1.58$^{a*)}$	6.15±2.28^{c}	3.42±0.28^{b}
3	2.78±1.71^{a}	6.72±3.83^{b}	3.35±0.80^{a}
6	4.40±1.50^{a}	8.43±3.99^{b}	3.68±0.35^{a}
9	4.12±1.34^{a}	7.94±4.32^{b}	3.58±0.14^{a}
12	5.07±2.82^{a}	8.90±3.79^{b}	5.73±1.18^{a}
15	5.11±2.28^{a}	9.00±3.62^{c}	5.81±1.12^{b}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

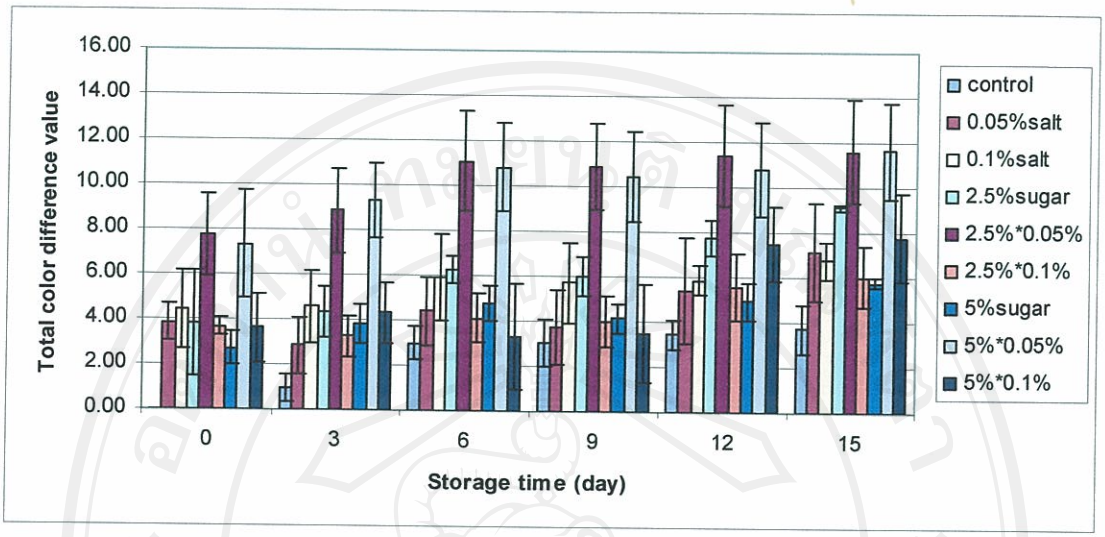


Fig. 4.5 The result of total color difference (ΔE*) value of orange juice during chilled storage as affected by sugar and salt additions

Table 4.11 The total color difference (ΔE*) value of orange juice during chilled storage as affected by sugar addition

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

Storage time (day)	no added sugar	2.5% ($^{w}/_{v}$) sugar	5% ($^{w}/_{v}$) sugar
0 Day	$2.77\pm2.42^{a*)}$	5.07 ± 2.28^{b}	4.56 ± 2.45^{b}
3 Day	2.79 ± 1.82^{a}	5.49 ± 2.94^{b}	5.82 ± 3.05^{b}
6 Day	4.44 ± 1.45^{a}	7.15 ± 3.57^{b}	6.31 ± 3.98^{b}
9 Day	4.17 ± 1.39^{a}	6.95 ± 3.53^{b}	6.02 ± 3.83^{b}
12 Day	4.91 ± 1.28^{a}	8.25 ± 2.96^{b}	7.72 ± 2.94^{b}
15 Day	5.86 ± 1.87^{a}	8.91 ± 2.80^{b}	8.39 ± 2.97^{b}

Table 4.12 The total color difference (ΔE*) value of orange juice during chilled storage as affected by salt addition

Storage time (day)	no added salt	0.05% ($^{w}/_{v}$) salt	0.1% ($^{w}/_{v}$) salt
0	2.18±1.97$^{a*)}$	6.31±2.12^{b}	3.92±0.46^{c}
3	3.03±1.83^{a}	7.00±3.61^{b}	4.06±0.69^{c}
6	4.69±1.63^{a}	8.77±3.77^{b}	4.43±1.34^{a}
9	4.40±1.51^{a}	8.34±3.99^{b}	4.39±1.17^{a}
12	5.37±2.18^{a}	9.21±3.29^{b}	6.29±1.01^{a}
15	6.20±2.72^{a}	10.12±2.59^{b}	6.85±0.87^{a}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

4.2.1.2 Cloud stability

Cloud stability has traditionally been considered as an important quality parameter for citrus juices (Parish, 1998). In this study, measurement of cloud stability was based on light transmission (%T) using a spectrophotometer at a wavelength of 640 nm. If the result measurement of %T was high, it meant that an orange juice loss its cloud stability. The results for the cloud measurement of different orange juice treatments showed that %T values of the orange juice samples were increased during storage (Appendix E, Table 1.6 and Fig. 4.6). This result indicated that all the orange juice samples decreased in their cloud stabilities during storage. Bayindirli *et al.* (2006) reported that fresh orange juice usually losses its cloud within a few days under refrigerated storage. The cloud loss is mainly due to demethylated pectin interaction with calcium ions, causing a precipitation and as a result a clear serum layer is formed. Since demethylated pectin was correlated with an enzyme activity, a measurement of PE enzyme in this study showed that the PE enzyme activity in the orange juice

samples was increased during the storage (Fig. 4.8). The cloud stability result found in this research was similar to the findings of Parish (1998) and Bayindirli *et al.* (2006).

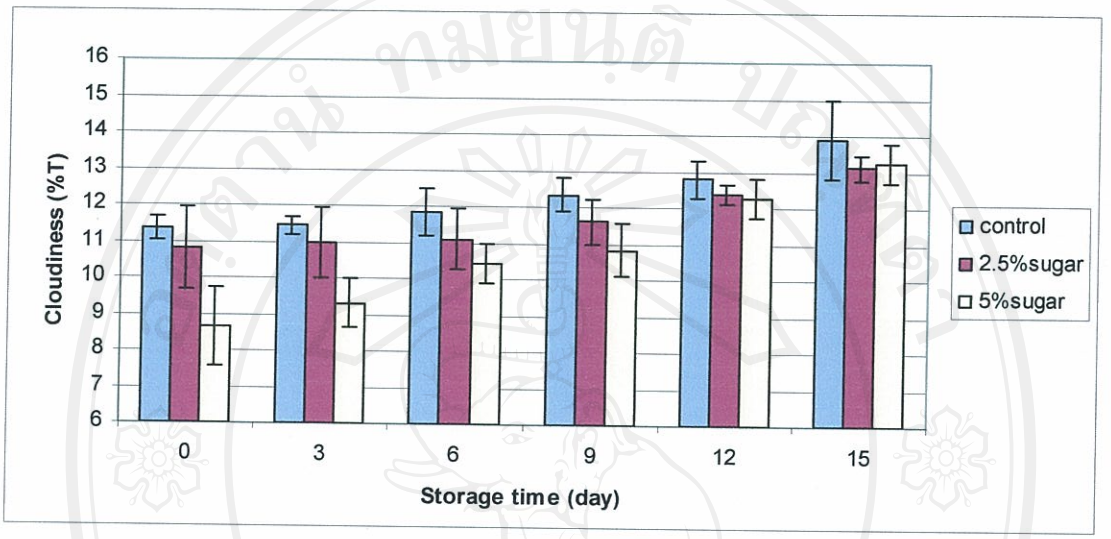


Fig 4.6 The result of cloud measurement (%T) for orange juice during chilled storage as affected by sugar addition

Looking more details on the effect of sugar and salt additions on the cloud measurement of the orange juice samples revealed that only the sugar addition that had a significant effect on the orange juice cloudiness (Appendix E, Table 3.6 and Fig. 4.6). An addition of 5% ($^{w}/_{v}$) sugar was found to be significantly reduced the %T or increased the cloudiness of the orange juice samples at the beginning of the storage period (Fig. 4.6). There was a possibility that at high sugar concentration, the sugar was not fully dissolved in orange juices, especially when the sugar was added at room temperature and stirred manually. The undissolved sugar formed particle suspension that reduced the transmission of the spectrophotometer light. However, this effect was disappered on the 12th day of storage, which indicated that the particle suspension formed by the sugar was reduced due to more dissolved sugar in the solution.

4.2.1.3 Viscosity

The measurement of viscosity of orange juice samples during chilled storage was displayed in Fig. 4.7. In general, the viscosity of the juice samples was reduced during storage. Factors that affect the viscosity values are temperature and soluble solids content (Leslaw and Teresa, 2003). The amount of soluble solid content in the orange juice samples was increased as different sugar and/or salt levels were added (Fig. 4.7). However, it was only orange juice samples with sugar addition that showed significantly different viscosity values compared to the no-added sugar orange juice at the beginning of the storage time (Table 4.13). During the storage time, especially at the end of this period, the addition of different sugar concentrations did not affect the viscosity measurement anymore. This result might correlate with the amount of reducing sugar. Although the amount of total soluble solids in the orange juice samples did not change significantly during the storage time (Appendix E, Table 1.9), the amount of reducing sugar was reduced at the end of 15 days storage (Appendix E,

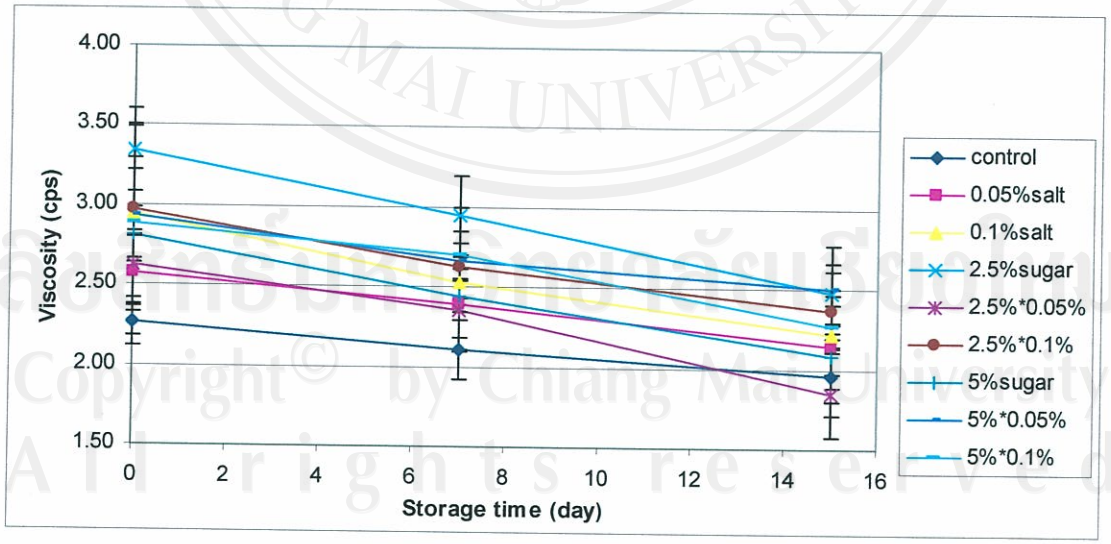


Fig. 4.7 The viscosity measurement of orange juice during chilled storage as affected by sugar and salt additions

Table 4.13 The result of viscosity measurement (cps) of orange juice during chilled storage as affected by sugar addition

Storage time (day)	no added sugar	2.5% sugar ($^{w}/_{v}$)	5% sugar ($^{w}/_{v}$)
0 Day	2.60±0.34$^{a*)}$	2.98±0.36^{b}	2.88±0.06^{a,b}
7 Day	2.34±0.21^{a}	2.64±0.30^{b}	2.60±0.14^{b}
15 Day	2.11±0.14^{a}	2.23±0.34^{a}	2.29±0.21^{a}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

Table 1.13 and Fig. 4.9). The low availability of reducing sugar at the end of the storage time might affect the measurement of viscosity to be not significantly different between different sugar treatments.

4.2.2 Chemical characteristics of Keaw Wan Prae orange juice during chilled storage as affected by sugar and salt additions

The chemical analyses that were carried out during storage of orange juice samples were moisture content, total solid, TSS, total titrable acidity, pH values, reducing sugar and PE enzymes. Among these analyses, it was only the measurement of pH values that showed a significant decrease during 15 days storage at refrigerated temperature (Appendix E, Table 1.8). For the other analyses, the statistical calculation showed that the storage time did not give a significant effect for different orange juice treatments (Appendix E, Table 1.9-1.14). Although the statistical results showed that the amount of reducing sugar and PE enzyme activity were not significantly different, the actual measurement of these parameters indicated that the activity of PE enzyme was increased during storage (Fig. 4.8), whereas a decrease in the amounts of reducing

sugar in orange juice samples was noted (Fig. 4.9). The reduction for the pH values and reducing sugar could be due to microorganism activities in the orange juice samples as the enumeration of these microorganisms was significantly increased (Section 4.2.3). At the same time the amount of reducing sugar was affected by the chemical reactions that occurred slowly in the orange juice samples, particularly Maillard reaction (Lee, 1992; Koca *et al.*, 2003).

In general, the addition of sugar, salt and their interactions did not significantly affect different chemical characteristics of orange juices (Appendix E, Table 3.8-3.14). However, some individual cases could be observed, for example a higher moisture content in the sugar added orange juice was found compared to those in the no-added orange juices (Appendix E, Table 3.11).

The analysis of PE enzyme also showed that the enzyme activities were significantly affected by the sugar addition, particularly at the end of 15 days storage at refrigerated temperature (Appendix E, Table 1.14 and Fig. 4.8). This data confirmed the report of Ingallinera *et al.* (2005) who wrote that PE enzyme is inhibited by high

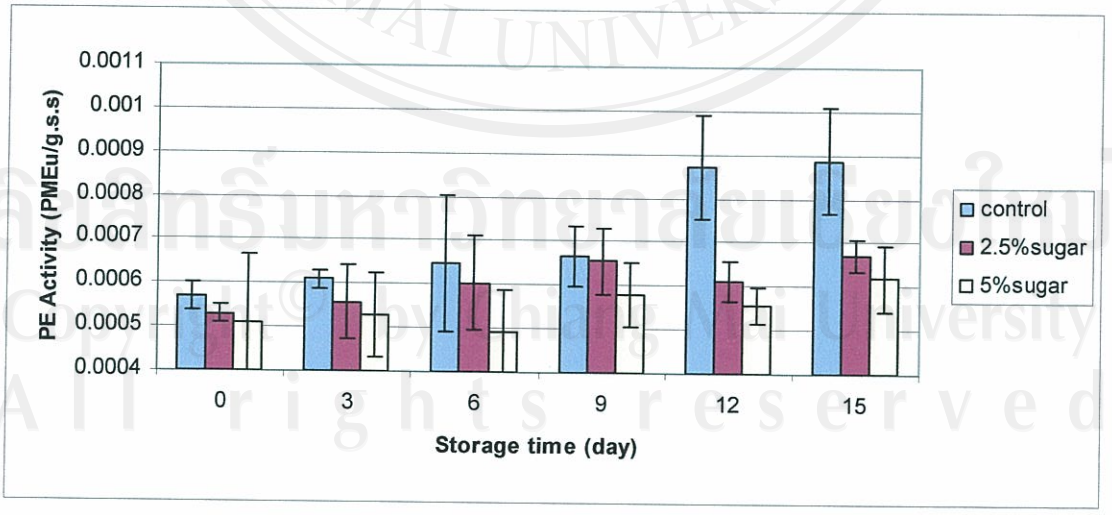


Fig. 4.8 The result of PE enzyme activity (PMEu/g.s.s) of orange juice during chilled storage as affected by sugar addition

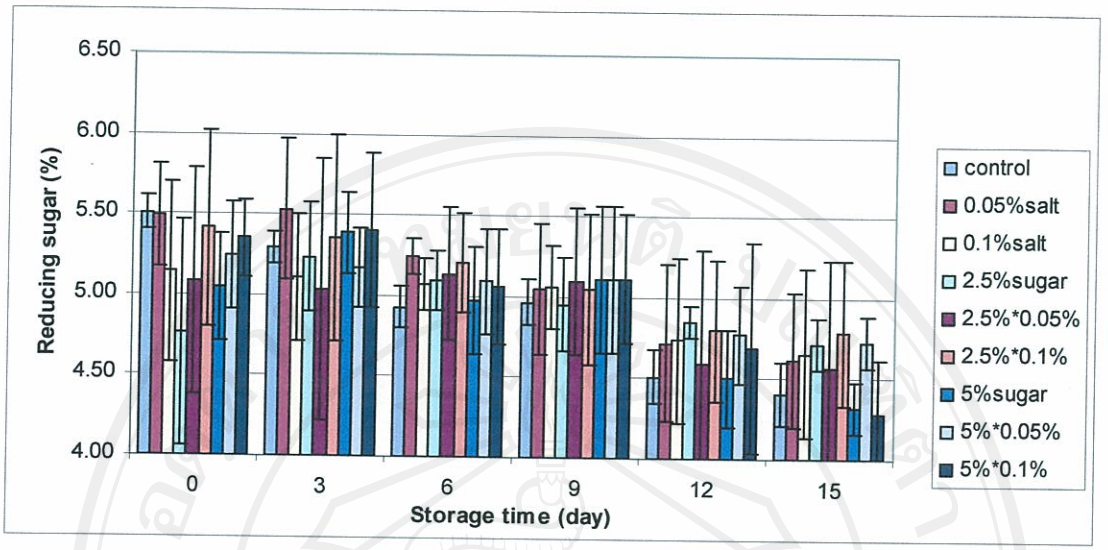


Fig. 4.9 The result of reducing sugar (%) of orange juice during chilled storage as affected by sugar and salt additions

sugar concentrations. At the beginning of the storage time, the activity of PE enzyme in orange juice samples were low, therefore the effect of sugar addition could not be significantly detected. However during the storage time, the PE enzyme in the control orange juices had higher activities, which showed its differences with the sugar–added orange juices. The addition of 5% ($^{w}/_{v}$) sugar in orange juices did not significantly displayed a lower enzyme activity compared to that of the 2.5% ($^{w}/_{v}$) sugar orange juices (Fig. 4.8).

4.2.3 Microbiological characteristics of Keaw Wan Prae orange juice during chilled storage as affected by sugar and salt additions

During storage at refrigerated temperature, orange juice samples were analyzed for their microorganism contents by doing Total Plate Count, enumeration of lactic acid bacteria and enumeration of yeast and mold. The analyses of Total Plate Count and lactic acid bacteria were done every 3 days during the storage time, while the

microbial load of yeasts and mold was monitored at the beginning and at the end of the storage time. The results of these enumerations were displayed in Fig. 4.10 – 4.12 and Tables 4.14 – 4.19. From the Fig. 4.10 – 4.12, it showed clearly that the number of microorganisms in the orange juice samples was significantly increased during the storage period. Although the study did not do a sensory evaluation, from a personal observation, it could be seen that some of the orange juice samples showed signs of spoilage after 12 days of storage. This observation meant that spoilage of orange juice could be detected when the total number of microorganisms in the juice was around 3.54 log CFU/ml.

Doing statistical analyses to the microbiological data revealed that the presence of sugar and salt in fresh orange juices could significantly affect the growth of microorganisms in the juices. Although the presence of different sugar concentrations did not significantly affected the results of Total Plate Count at the beginning of the storage time, the addition of 2.5% ($^{w}/_{v}$) sugar significantly caused more microorganisms to be detected in the orange juice samples after 15 days at chilled temperature (Table 4.14). Sugar affected the results of Total Plate Count because the presence of sugar increased the availability of disaccharide in the orange juice. At 0 day, the microorganisms in the orange juice were not in a growth state, therefore the presence of different sugar concentrations did not significantly affect the result of Total Plate Count. However, at the end of the storage time, the orange juice with 2.5% ($^{w}/_{v}$) sugar had the highest value of reducing sugar compared to those of the other treatments (Appendix E, Table 3.13). This higher sugar availability might give a better support for the growth of microorganisms in the orange juice, causing the orange juice treatment to have the highest value of Total Plate Count. In contradiction, the addition of salt in the orange juice was significantly decreased the number of total microorganisms at the commencement of the storage period. However, after 9 days storage at chilled temperature, the presence of 0.05 and 0.1% ($^{w}/_{v}$) salt was found to

significantly increase the number of total microorganisms in the orange juice samples (Table 4.15). The presence of salt was affected the Total Plate Count results at 0 day because salt has been well recognized to be an antimicrobial compound for general bacteria (Parish, 2006). However, some microorganisms, such as lactic acid bacteria, can tolerate the presence of salt. These microorganisms are normally used in fermented products, including sauerkraut, kimchi and pickled cucumber (Adam and Moss, 2000). Therefore, there was a high possibility that the finding of Total Plate Count was mainly affected by the growth of lactic acid bacteria in the salt-added orange juice. The results of lactic acid bacteria enumeration supported this possibility (Table 4.17).

For the interaction between salt and sugar, the statistical analysis showed that at the beginning of the storage period, the orange juice samples with 5% ($^{w}/_{v}$) sugar and 0.05% ($^{w}/_{v}$) salt was significantly contained a lower number of Total Plate Count compared to that of the control orange juice. However, at the end of the storage time, there was not any significant differences between different orange juice treatments in the term of Total Plate Count (Appendix E, Table 3.15).

The presence of sugar in orange juice did not significantly affect the number of lactic acid bacteria at 0 day storage. However, during storage period at chilled temperature, the presence of sugar significantly reduced the number of lactic acid bacteria, particularly after the orange juice samples were stored for 12 days (Table 4.16). This result indicated that the nutrient content of the no-added sugar orange juice was sufficient to support the growth of lactic acid bacteria causing the number of the bacteria in the no-added sugar orange juice samples to be higher than that in the sugar-added orange juices. The reduction in the number of lactic acid bacteria in the sugar-added orange juices did not correlate with the production of lactic acid by the microorganisms, since the pH values of the sugar-added orange juices were found to be not significantly different than those in the no-added sugar orange juices at the end of the storage period (Appendix E, Table 3.8).

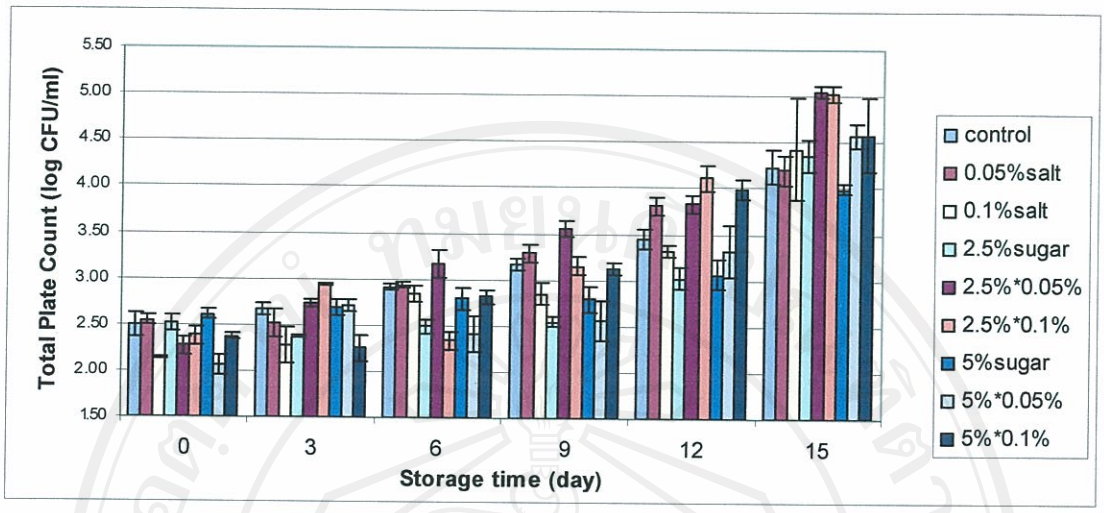


Fig. 4.10 The result of Total Plate Count of orange juice during chilled storage as affected by sugar and salt additions

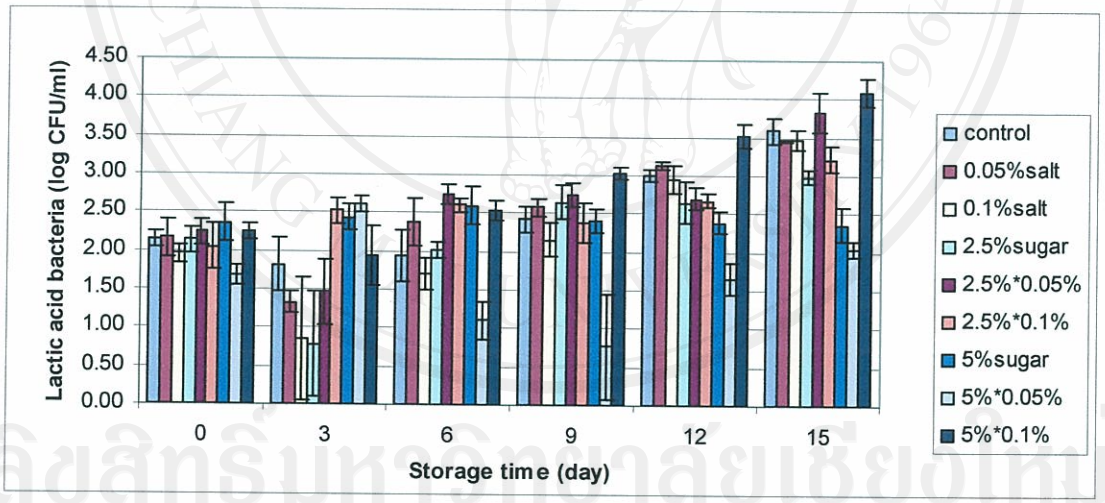


Fig. 4.11 The result of lactic acid bacteria measurement in orange juice during chilled storage as affected by sugar and salt additions

A slight different result to the sugar data was the result of the salt addition in orange juice samples against lactic acid bacteria. The salt addition in the orange juices was found to be significantly induced the growth of lactic acid bacteria during storage at chilled temperature, particularly for the orange juice samples with 0.1% ($^{w}/_{v}$) salt

(Table 4.17). This result was consistent with the data of the Total Plate Count (Table 4.15). The explanation for this finding could be seen in the discussion of Total Plate Count results.

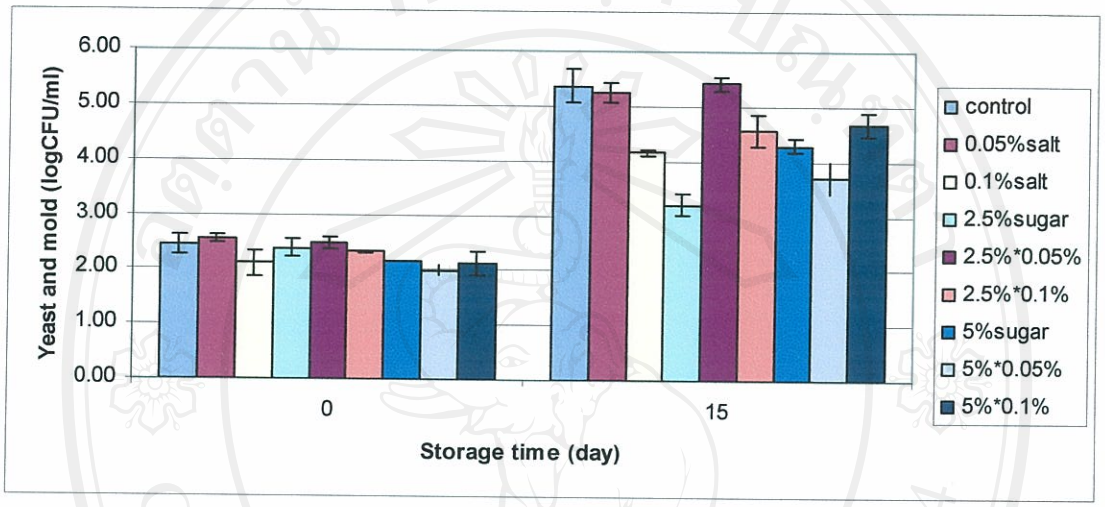


Fig. 4.12 The result of yeast and mold of orange juice during chilled storage as affected by sugar and salt additions

Table 4.14 The result of Total Plate Count (log CFU/ml) of orange juice during chilled storage as affected by sugar addition

Storage time (day)	no added sugar	2.5% ($^{w}/_{v}$) sugar	5% ($^{w}/_{v}$) sugar
0	2.40±0.22$^{a*)}$	2.39±0.12^{a}	2.34±0.27^{a}
3	2.48±0.20^{a}	2.68±0.29^{b}	2.55±0.26^{a}
6	2.90±0.05^{a}	2.66±0.44^{b}	2.67±0.23^{b}
9	3.10±0.23^{a}	3.08±0.50^{a}	2.83±0.28^{b}
12	3.53±0.25^{a,b}	3.65±0.56^{a}	3.45±0.48^{b}
15	4.28±0.12^{a}	4.81±0.39^{b}	4.38±0.34^{a}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

Table 4.15 The result of Total Plate Count (log CFU/ml) of orange juice during chilled storage as affected by salt addition

Storage time (day)	no added salt	0.05% ($^{w}/_{v}$) salt	0.1% ($^{w}/_{v}$) salt
0	2.54±0.06$^{a*)}$	2.29±0.24^{b}	2.30±0.14^{b}
3	2.58±0.18^{a,b}	2.65±0.12^{a}	2.49±0.39^{b}
6	2.73±0.22^{a,b}	2.84±0.39^{a}	2.66±0.28^{b}
9	2.83±0.31^{a}	3.13±0.51^{b}	3.04±0.16^{b}
12	3.18±0.24^{a}	3.65±0.29^{b}	3.80±0.42^{c}
15	4.19±0.18^{a}	4.61±0.43^{b}	4.68±0.31^{b}

*) Values within a row followed by different letters were significantly different (P ≤0.05)

Table 4.16 The result of lactic acid bacteria (log CFU/ml) of orange juice during chilled storage as affected by sugar addition

Storage time (day)	no added sugar	2.5% ($^{w}/_{v}$) sugar	5% ($^{w}/_{v}$) sugar
0	2.09±0.12$^{a*)}$	2.14±0.10^{a}	2.10±0.36^{a}
3	1.33±0.48^{a}	1.59±0.88^{a}	2.33±0.35^{b}
6	2.01±0.35^{a}	2.45±0.38^{b}	2.08±0.84^{a}
9	2.39±0.22^{a}	2.59±0.19^{a}	2.06±1.16^{b}
12	3.03±0.09^{a}	2.67±0.02^{b}	2.52±0.94^{b}
15	3.50±0.07^{a}	3.34±0.44^{b}	2.83±1.10^{c}

*) Values within a row followed by different letters were significantly different (P≤0.05)

Table 4.17 The result of lactic acid bacteria (log CFU/ml) of orange juice during chilled storage as affected by salt addition

Storage time (day)	no added salt	0.05% ($^{w}/_{v}$) salt	0.1% ($^{w}/_{v}$) salt
0	2.22±0.13$^{a*)}$	2.03±0.30^{a}	2.09±0.15^{a}
3	1.68±0.84^{a}	1.80±0.71^{a}	1.77±0.85^{a}
6	2.18±0.36^{a}	2.08±0.86^{a}	2.28±0.50^{a}
9	2.49±0.13^{a}	2.03±1.09^{b}	2.51±0.45^{a}
12	2.68±0.30^{a}	2.50±0.76^{b}	3.05±0.43^{c}
15	2.98±0.61^{a}	3.11±0.94^{a}	3.59±0.45^{b}

*) Values within a row followed by different letters were significantly different (P≤0.05)

Table 4.18 The result of yeast and mold (log CFU/ml) of orange juice during chilled storage as affected by sugar addition

Storage time (day)	no added sugar	2.5% ($^{w}/_{v}$) sugar	5% ($^{w}/_{v}$) sugar
0 Day	2.37±0.24$^{a*)}$	2.40±0.07^{a}	2.09±0.09^{b}
15 day	4.93±0.66^{a}	4.40±1.11^{b}	4.21±0.49^{b}

*) Values within a row followed by different letters were significantly different (P≤0.05)

Table 4.19 The result of yeast and mold (log CFU/ml) of orange juice during chilled storage as affected by salt addition

Storage time	no added salt	0.05% ($^{w}/_{v}$) salt	0.1% ($^{w}/_{v}$) salt
0 Day	2.33±0.15$^{a*)}$	2.34±0.31^{a}	2.19±0.12^{b}
15 day	4.28±1.08^{a}	4.79±0.95^{b}	4.46± 0.26^{a}

*) Values within a row followed by different letters were significantly different (P≤0.05)

For the presence of yeast and mold in orange juice samples, the data showed a similarity with the results of lactic acid bacteria. Tables 4.18 and 4.19 displayed that the presence of sugar was significantly reduced the number of yeast and mold in the orange juices, whereas the presence of salt was found to significantly increase the number of yeast and mold in the orange juices. Although the reduction in the number of lactic acid bacteria, yeast and mold in the presence of sugar could not be explained easily, the possibility of higher intensity of Maillard reaction in these juice samples could not be ruled out. Table 4.9 showed clearly that in the presence of sugar in the orange juice samples, the browning value of the samples was almost double than that in the no-added sugar orange juice. There was a possibility that chemical compounds formed during the Maillard reaction could give a negative effect in supporting the growth of some microorganism. A further study in this area would be needed to confirm this possibility.

4.2.4 Nutritive values of Keaw Wan Prae orange juice during chilled storage as affected by sugar and salt additions

4.2.4.1 Vitamin C content

The content of vitamin C in orange juice samples was monitored every 3 days during 15 days storage at refrigerated temperature and the results were exhibited in Fig. 4.13 and Tables 4.20 – 4.21. The Fig. 4.13 showed clearly that the content of vitamin C in the juice samples was significantly decreased during the storage period. This result was expected as the presence of vitamin C could be affected by the presence of oxygen and the method to handle and store the orange juice (Nagy, 1980). Reports from Bull *et al.* (2004), Choi *et al.* (2002) and Zanoni *et al.* (2004) also showed a similarity to the finding of this study.

Beside the storage time, the presence of salt and sugar was also significantly affected the vitamin C content in the orange juice samples (Tables 4.20 and 4.21). Sugar at concentrations of 2.5 and 5% ($^{w}/_{v}$) was found to reduce the content of vitamin C at the beginning of the storage time. However after 9 and 12 days of storage, the presence of 2.5 and 5% ($^{w}/_{v}$) sugar, respectively, in the orange juice samples could significantly maintain more vitamin C contents compared to the no-added sugar orange juice samples (Table 4.20). There was a possibility that a higher value of vitamin C in the sugar – added orange juice was due to the highest value of vitamin C found in the orange juice sample with 2.5% ($^{w}/_{v}$) sugar (Appendix E, Table 3.18). This result indicated that the presence of salt gave a more significant effect in the reduction of the vitamin C content compared to the presence of sugar. Therefore, the content of vitamin C was higher in the sugar-added orange juice samples compared to the control orange juice samples at the end of the storage time.

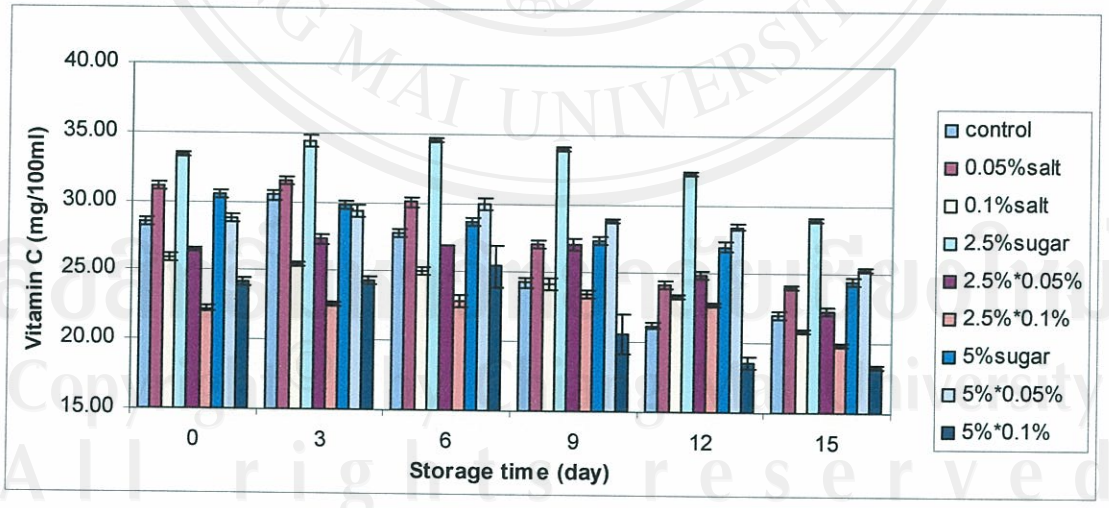


Fig. 4.13 The result of vitamin C content in orange juice during chilled storage as affected by sugar and salt additions

Table 4.20 The result of vitamin C content (mg/100ml) of orange juice during chilled storage as affected by sugar addition

Storage time (day)	no added sugar	2.5% ($^{w}/_{v}$) sugar	5% ($^{w}/_{v}$) sugar
0	28.48±2.55$^{a*)}$	27.38±5.65^{b}	27.85±3.28^{c}
3	29.16±3.20^{a}	28.12±5.92^{b}	27.85±3.02^{b}
6	27.63±2.52^{a}	28.12±5.86^{a}	27.98±2.29^{a}
9	25.19±1.64^{a}	28.15±5.31^{b}	25.54±4.36^{a}
12	22.94±1.53^{a}	26.64±4.98^{b}	24.66±5.30^{c}
15	22.33±1.58^{a}	23.73±4.69^{b}	22.74±3.82^{c}

*) Values within a row followed by different letters were significantly different (P≤0.05)

Table 4.21 The result of vitamin C content (mg/100ml) of orange juice during chilled storage as affected by salt addition

Storage time (day)	no added salt	0.05% ($^{w}/_{v}$) salt	0.1% ($^{w}/_{v}$) salt
0	30.81±2.48$^{a*)}$	28.78±2.26^{b}	24.12±1.85^{c}
3	31.55±2.49^{a}	29.41±2.10^{b}	24.17±1.45^{c}
6	30.32±3.63^{a}	28.94±1.76^{b}	24.47±1.32^{c}
9	28.50±4.92^{a}	27.63±0.95^{b}	22.74±1.91^{c}
12	26.83±5.52^{a}	25.82±2.26^{b}	21.59±2.61^{c}
15	25.19±3.50^{a}	23.90±1.53^{b}	19.73±1.29^{c}

*) Values within a row followed by different letters were significantly different (P≤0.05)

For the addition of salt in orange juice, this addition was shown to significantly bring a negative effect to the content of vitamin C in the orange juices (Table 4.21). Throughout the storage time, the salt-added orange juice samples had lower vitamin C contents compared to the no-added sugar control orange juice. There was a possibility that the vitamin C in the salt – added orange juice was more oxidized by the presence

of NaCl. The presence of NaCl could act as a catalyst as the presence of metal ions, such as Fe^{+++} and Cu^{++} (Gregory III, 1996).

4.2.4.2 Carotenoid content

Different to the vitamin C analysis, the analysis of carotenoid was carried out weekly during 15 days storage at chilled temperature. The result of this analysis could be seen in Table 4.22 and Appendix E, Table 1.19. In general, the content of carotenoid was shown to be increased during the storage period (Appendix E, Table 1.19). This result was contradicted to the previous results of Sánchez-Moreno *et al.* (2003) and Cortés *et al.* (2006). This contradiction could be due to the effect of storing the orange juice samples at frozen temperature. There was a possibility that keeping longer at freezing condition of the 0 day orange juice samples could damage the carotenoid compound in the samples and cause a lower measurement result than it should be.

Table 4.22 The result of carotenoid content (μg/ml) of orange juice during chilled storage as affected by sugar addition

Storage time (day)	no added sugar	2.5% ($^{w}/_{v}$) sugar	5% ($^{w}/_{v}$) sugar
0	4.55±0.07$^{a*)}$	5.25±0.21^{b}	5.89±0.09^{c}
7	5.96±0.08^{a}	5.69±0.12^{a}	5.57±0.11^{a}
15	6.23±0.11^{a}	5.84±0.07^{a}	5.91±0.05^{a}

*) Values within a row followed by different letters were significantly different (P≤0.05)

Analyzing the effect of salt and sugar additions to the orange juice samples, it was shown that only sugar that significantly affected the presence of carotenoid in the orange juices at the beginning of the storage time (Table 4.22). There was a possibility that this result was correlated with the viscosity of the orange juice samples, since the

addition of sugar caused a higher viscosity value of the sample compared to that of the no-added sugar orange juices (Table 4.13). The amount of carotenoid content in the sugar-added orange juice was lower than that in the no-added orange juice sample at the end of storage at chilled temperature. For the salt addition, the compound was found to be not significantly affected the content of carotenoid in the orange juice samples. At the same time, the interaction of salt and sugar was also not given a significant effect on the carotenoid content in the orange juice during 15 days storage.

From different analyses that were conducted to the Keaw Wan Prae orange juice samples, it could be concluded that the addition of salt and sugar affected the physical, chemical, microbiological and nutritional characteristics of the orange juice. However among these values, the important parameters that affected the quality of the orange juice during storage at refrigerated temperature were the microbiological and nutritive parameters of the juice. Since the microbiological results showed that the addition of 5% ($^{w}/_{v}$) sugar produced an orange juice with lower Total Plate Count and lactic acid bacteria together with higher amount of vitamin C compared to those in the no-added sugar orange juice, this concentration of sugar was selected to be studied further in the next section. Although the addition of 2.5% ($^{w}/_{v}$) sugar could significantly maintain a higher content of vitamin C compared to that in the 5% ($^{w}/_{v}$) sugar, this sugar concentration was not be chosen because at this concentration, the number of lactic acid bacteria and Total Plate Count was higher than those in the 5% ($^{w}/_{v}$) sugar. No salt addition was also selected because the present of salt caused more microorganisms to be detected in the orange juice samples and the content of the vitamin C was lower in the salt-added orange juices.

4.3 The effects of orange juice sac addition and pH values on the quality of fresh orange juice during chilled storage

In this section, all of the orange juice samples were added with 5% ($^{w}/_{v}$) sugar and varied for the content of orange juice sac, which was either 0 or 3% ($^{w}/_{v}$) orange juice sac, and pH values of 3.0, 3.5 and 4.0. The variation of orange juice sac was studied because some commercial orange juices were added with orange juice sac to improve their sensory characteristics and to represent juices similar to the fresh orange juice. To understand the effect of orange juice sac on the quality of fresh orange juice during storage at chilled temperature, orange juice sacs from a commercial orange juice were separated and added to the fresh orange juice variety Keaw Wan Prae prepared in the laboratorium. By doing this, the orange juice sac used during the experiment was not the orange juice sac of Keaw Wan Prae orange, but it was from a commercial orange juice. Beside the orange juice sac, pH values were also being investigated in this section. This factor was studied because the pH of orange juice can affect the number of microorganisms in the juice and changes in the orange juice quality during storage at refrigerated temperature. Working with orange also showed that the pH of orange juices was strongly affected by the production period of the orange. The initial pH value of orange juice samples in this section was 3.0. This initial pH value was differed from the orange juice pH values reported in the section 4.1 because of differences in the experimental times. In each of the experimental section, a batch of orange fruit was bought from a local market. There was a possibility that the sections 4.1 and 4.3 used different maturity stages of orange fruit, since the harvesting maturity stage affected titratable acidity and pH value of fruits (Chiesa, 1996). To alter the pH values of the juice sample, the samples were added with 0.5 N sodium hydroxide.

4.3.1 Physical characteristics of Keaw Wan Prae orange juice during chilled storage as affected by orange juice sacs and pH values

4.3.1.1 Color of orange juice

The color parameter of orange juice samples was evaluated from L value, a* value, b* value, total color difference (ΔE*) and browning value. In general, the values from all of these measurement and calculation were not significantly affected by the storage period (Appendix E, Tables 2.2-2.6).

Analyzing more details about the effect of orange juice sac and pH values on the color of orange juice, it was found that the addition of 3% ($^{w}/_{v}$) orange juice sac did not affect ΔE*, L, a*, b* and browning values (Appendix E, Table 4.2-4.6). However, the browning value was affected by pH values at the beginning of the storage time (Table 4.23). From Table 4.23, it could be seen that at 0 day, orange juices at a pH value of 3.0 significantly had a lower browning value than those of the orange juices with other pH values. This result might be affected by the presence of furfural because this compound was reported to be the main product degradation in juices with pH values below 4.0. Furfural was then passed a polymerization reaction to become an active aldehyde. The last compound might combine with amino acids and contribute to the browning reaction in the fruit juice (Koca *et al.*, 2003). In addition, Eren (1998) also reported that an increase in the browning reaction is highly occurred at higher pH values. This can happen because at lower pH values, the amounts of unpronated amino groups are less. Since a larger percentage of the amino acids is in the unpronated form at higher pH values, at this condition more moles of amino acid can react with reducing sugars.

Table 4.23 The result of browning value of orange juice during chilled storage as affected by different pH values

Storage time (day)	pH 3	pH 3.5	pH 4
0	$1.72 \pm 3.49^{a*)}$	4.6 ± 3.04^{b}	4.31 ± 3.46^{b}
3	4.53 ± 2.64^{a}	4.93 ± 2.10^{a}	6.10 ± 3.01^{a}
6	3.82 ± 2.72^{a}	4.66 ± 2.85^{a}	3.98 ± 2.44^{a}
9	5.16 ± 1.46^{a}	5.53 ± 2.58^{a}	4.95 ± 2.22^{a}
12	5.14 ± 2.01^{a}	4.82 ± 3.28^{a}	4.86 ± 3.63^{a}
15	4.16 ± 2.64^{a}	4.45 ± 2.29^{a}	3.83 ± 2.38^{a}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

4.3.1.2 Cloud stability of orange juice

The result of the cloud stability measurement of orange juice was displayed in Fig. 4.14 and Tables 4.24-4.25. From the Figure, it was showed clearly that the %T of the orange juice samples was significantly increased during storage at chilled temperature (Appendix E, Table 2.7). This result was similar to the previous result in the section 4.2.1.2. Both of these results showed that the cloudiness of orange juice would be reduced during refrigerated storage.

The presence of 3% ($^{w}/_{v}$) orange juice sac in orange juices was shown to significantly affect the cloudiness of the orange juices (Table 4.24). It could be seen from this Table that the cloudiness of orange juices with orange juice sacs was generally lower than those of the orange juices without orange juice sac. There was a possibility that the orange juice sac contained PE enzyme, causing the orange juice samples with orange juice sac contained more PE enzyme than of the orange juice without orange juice sac (Appendix E, Table 4.14. However, differences in the cloudiness parameter were only significant from the beginning of the storage period up to 9 days storage at refrigerated temperature. Physico–chemical and microbiological

changes in orange juices caused different orange juice sac treatments in the orange juice samples to be not significantly different at the end of the storage time.

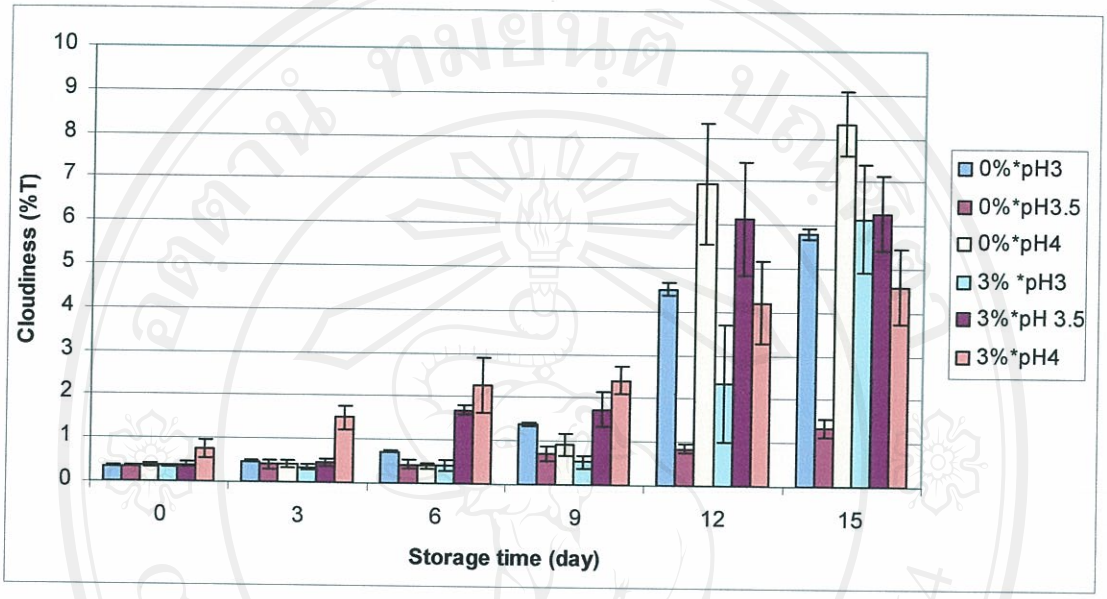


Fig. 4.14 The result of cloud stability of orange juice during chilled storage as affected by orange juice sac and pH levels

Table 4.24 The result of cloud stability (%T) of orange juice during chilled storage as affected by an addition of orange juice sac

Storage time (day)	No added orange juice sac	Added 3% ($^{w}/_{v}$) orange juice sac
0	$0.56 \pm 0.04^{a*)}$	0.51 ± 0.22^{b}
3	0.43 ± 0.07^{a}	0.77 ± 0.57^{b}
6	0.53 ± 0.17^{a}	1.47 ± 0.88^{b}
9	1.00 ± 0.33^{a}	1.56 ± 0.87^{b}
12	4.10 ± 2.74^{a}	4.23 ± 1.94^{a}
15	5.16 ± 3.09^{a}	5.67 ± 1.19^{a}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

Table 4.25 The result of cloud stability (%T) of orange juice during chilled storage as affected by different pH values

Storage time (day)	pH 3	pH 3.5	pH 4
0	$0.35 \pm 0.02^{a*)}$	0.37 ± 0.05^{a}	0.57 ± 0.25^{b}
3	0.41 ± 0.08^{a}	0.43 ± 0.09^{a}	0.96 ± 0.62^{b}
6	0.58 ± 0.18^{a}	1.07 ± 0.69^{b}	1.34 ± 1.10^{b}
9	0.95 ± 0.47^{a}	1.23 ± 0.63^{b}	1.67 ± 0.86^{c}
12	3.43 ± 2.82^{a}	3.49 ± 3.00^{a}	5.58 ± 1.82^{b}
15	5.97 ± 0.81^{a}	3.81 ± 2.77^{b}	6.46 ± 2.17^{a}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

For the effect of pH values on the cloudiness of orange juice samples (Table 4.25), it could be noticed that at pH 4.0, the orange juice samples would generally have lower cloud stability compared to those of the orange juices at pH values of 3.0 and 3.5. The reduction in the cloud stability was less occurred in the orange juice at a pH value of 3.5, eventhough the orange juice sample at a pH of 3.0 had significantly the highest cloud stability in the middle of the storage period (the 6th and 9th days). A higher cloud stability at lower pH values could be due to an inactivation of PE enzyme at lower temperatures at lower pH values as reported by Sun *et al.* (2006). The formation of cloud was significantly reduced in the orange juice at a pH value of 3.0 at the end of the storage time, causing the orange juice samples to be not significantly different in the term of cloud stability with the orange juice samples at a pH value of 4.0. The cloud formation that was occurred at low storage temperature for lower pH values was also reported by Alklint (2003). Therefore, the best orange juice sample in the term of cloud stability would be present in the orange juice samples at a pH value of 3.5 and without addition of orange juice sacs (Fig. 4.14).

4.3.1.3 Viscosity of orange juice

Viscosity of orange juice samples was mainly affected by different pH values and storage time (Fig. 4.15 and Table 4.26). The presence and absence of orange juice sac did not significantly affect the viscosity of the orange juice samples (Appendix E, Table 4.1). During the storage period at chilled temperature, the viscosity of orange juice samples was decreased (Appendix E, Table 2.1), as previously been showed in the section 4.2.1.3. There was a possibility that the viscosity reduction in this section was correlated with the amount of reducing sugar since the amount of reducing sugar was also decreased during the storage time (Fig. 4.18).

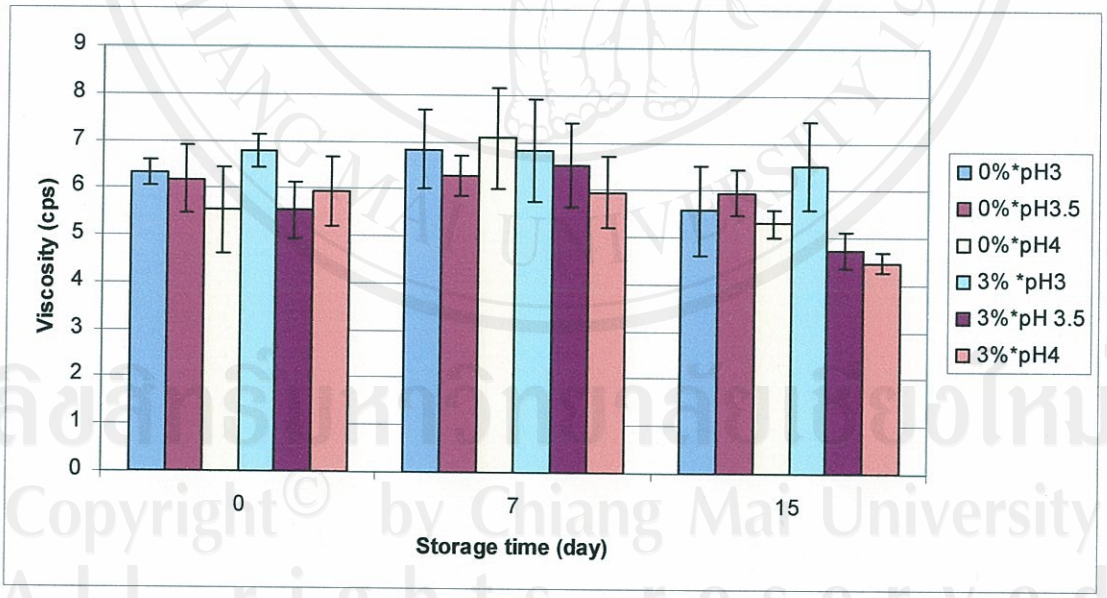


Fig. 4.15 The result of viscosity of orange juice during chilled storage as affected by orange juice sac and pH levels

Table 4.26 The result of viscosity (cps) of orange juice during chilled storage as affected by different pH values

Storage time (day)	pH 3	pH 3.5	pH 4
0	$6.56 \pm 0.38^{a*)}$	5.86 ± 0.69^{a}	5.74 ± 0.78^{a}
7	6.83 ± 0.86^{a}	6.40 ± 0.64^{a}	6.52 ± 1.03^{a}
15	6.05 ± 0.99^{a}	$5.34 \pm 0.78^{a,b}$	4.88 ± 0.50^{b}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

Significant reduction in the viscosity value was also occurred in the orange juice samples at a pH value of 4.0 at the end of the storage time (Table 4.26). There was not any apparent reason for this reduction, since the amount of reducing sugar in the orange juice samples at a pH value of 4.0 was significantly higher than that in the orange juice samples at a pH value of 3.0 at the end of the storage time (Table 4.32). A further investigation might be needed to have a better understanding about the relationship between pH values and viscosity of orange juices.

4.3.2 Chemical characteristics of Keaw Wan Prae orange juice during chilled storage as affected by orange juice sacs and pH values

The chemical characteristics of orange juice samples during storage at refrigerated temperature were monitored through the analyses of pH value, total titrable acidity, PE enzyme activity, moisture content, total solid, total soluble solid (TSS), reducing sugar and fiber content. From all of these analyses, it was only the pH values that were significantly be affected during the storage time (Fig. 4.16 and Appendix E, Table 2.8). Whereas, values of total titrable acidity and PE enzyme of the orange juice samples were not significantly be affected by the storage time (Appendix

E, Table 2.10-2.14). Values of moisture content and reducing sugar of the orange juice samples were found to be slightly reduced only at the end of the storage compared to the beginning of the storage period (Fig. 4.17–4.18 and Appendix E, Tables 2.11 and 2.13). At the same time, the measurements of total solid, TSS and fiber content of the orange juice samples showed increasing values at the end of the storage time (Fig. 4.19-4.20 and Appendix E, Tables 2.9, 1.12 and 2.15). The increasing values of the fiber content in the orange juice samples during storage could be affected from the cell wall of microorganisms because the microbial cell walls contain peptidoglycan, a polymer of N-acetyl glucosamine, N-acetyl muramic acid and amino acids (Anonymous, 2006b).

For the effect of orange juice sacs addition on different chemical measurements of orange juice samples, it was found that by the presence 3% ($^{w}/_{v}$) orange juice sacs, the values of moisture content and fiber content were increased compared to those of the orange juices without addition of orange juice sacs (Tables 4.27 and 4.28). On the other hand, the presence of 3% ($^{w}/_{v}$) orange juice sacs did not significantly affect the other chemical parameters of the orange juice (Appendix E, Tables 4.8 - 4.10, 4.12 - 4.14).

Beside the orange juice sac, chemical values of orange juice samples were also significantly being affected by different pH values of orange juices, particularly for the values of total titrable acidity, moisture content, total solid, TSS and reducing sugar (Tables 4.29 – 4.32). In general, orange juices at a pH value of 3.0 significantly had higher total titrable acidity and moisture content values compared to those of the orange juices at higher pH values (Tables 4.29 and 4.30). At the same time, the pH 3.0 orange juices had lower values of total solid, TSS (significant throughout the storage time) and reducing sugar (significant at the end of the storage time) compared to those of the other orange juices (Tables 4.31 and 4.32). The lower values of TSS and reducing sugar of the orange juices at a pH value of 3.0 could be due to a higher rate of

Maillard reaction occurred at higher pH values (section 4.3.1.1, Eren (1998) and Koca *et al.*, 2003).

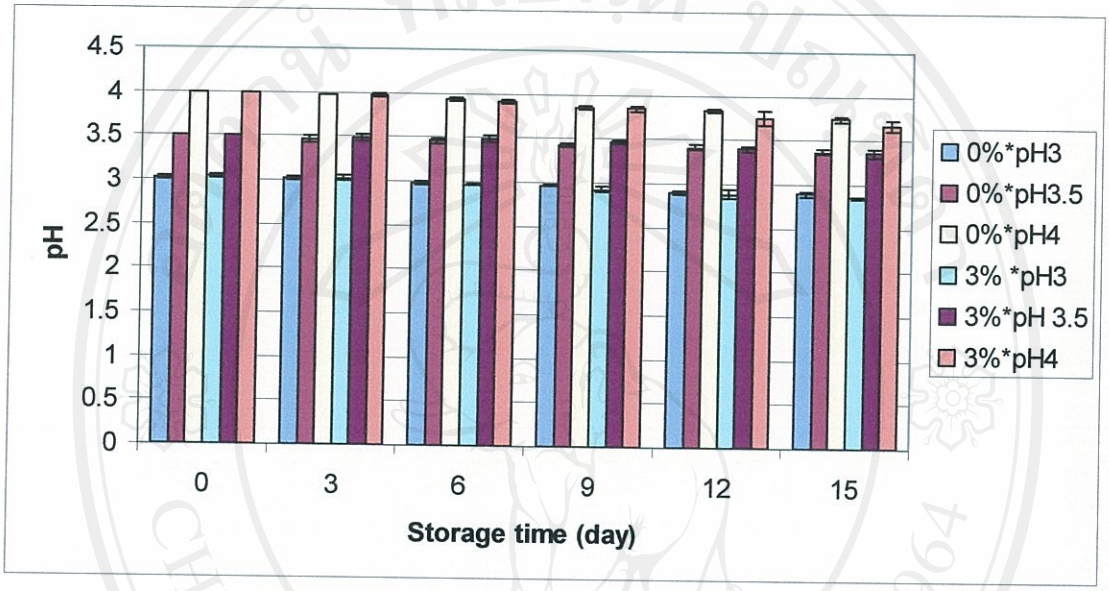


Fig. 4.16 The result of pH of orange juice during chilled storage as affected by orange juice sac and pH levels

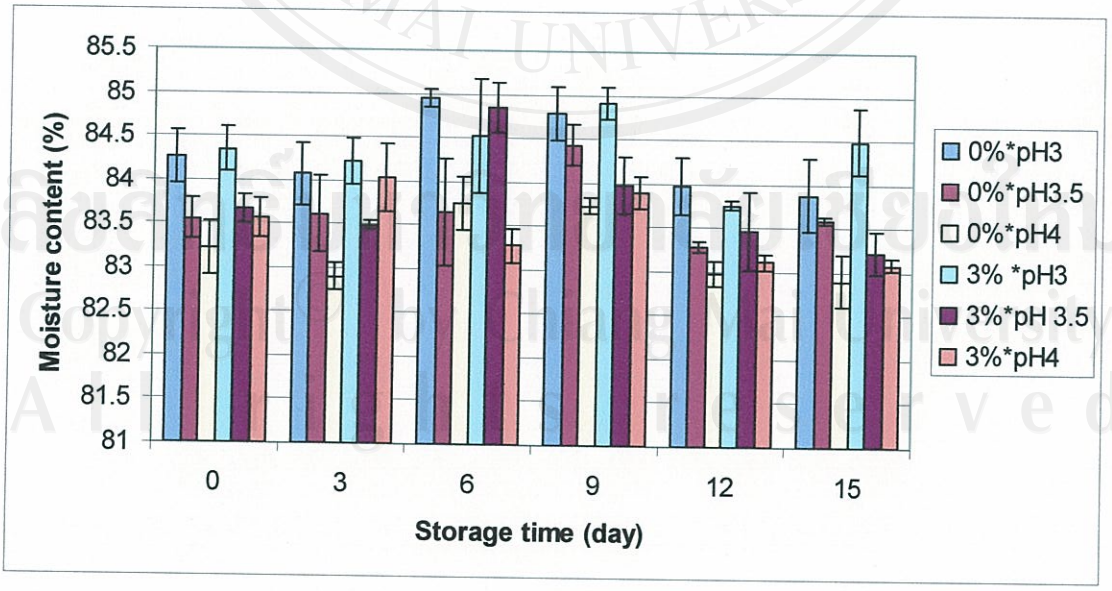


Fig. 4.17 The result of moisture content of orange juice during chilled storage as affected by orange juice sac and pH levels

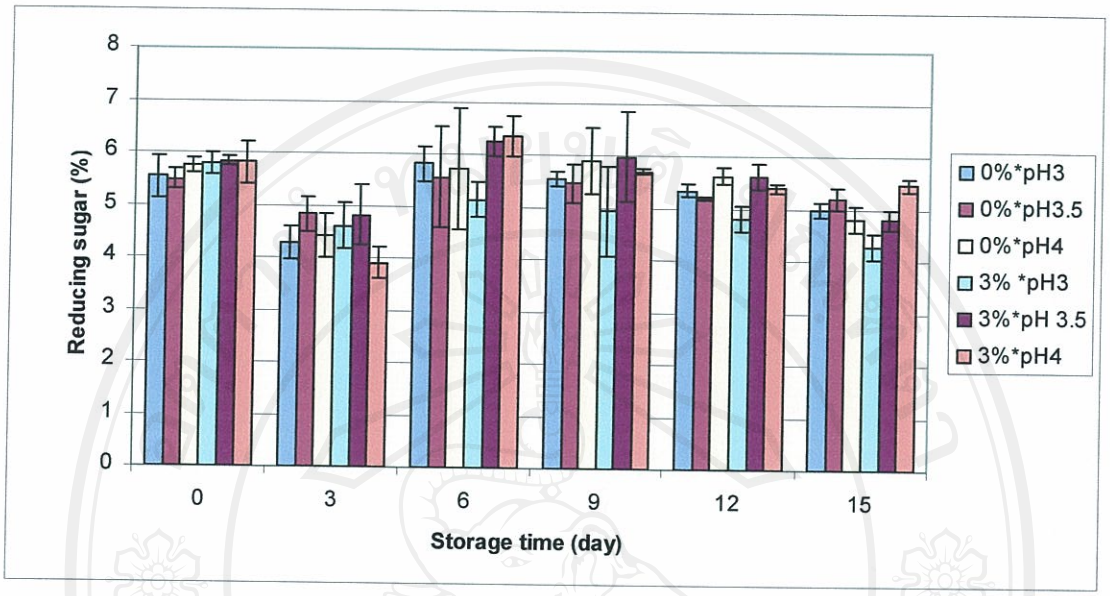


Fig. 4.18 The result of reducing sugar of orange juice during chilled storage as affected by orange juice sac and pH levels

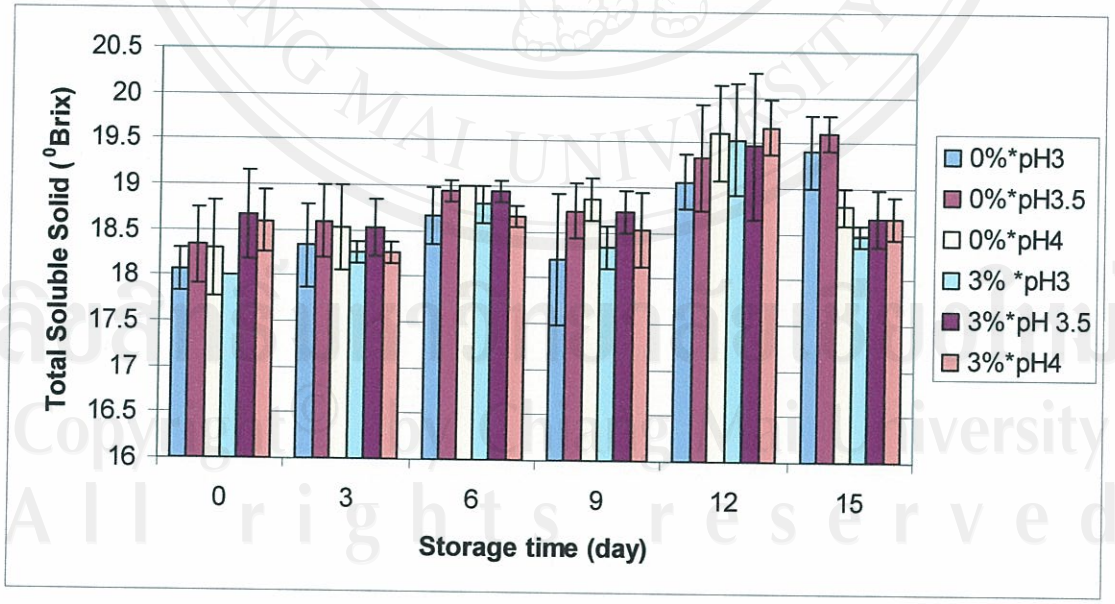


Fig. 4.19 The result of TSS of orange juice during chilled storage as affected by orange juice sac and pH levels

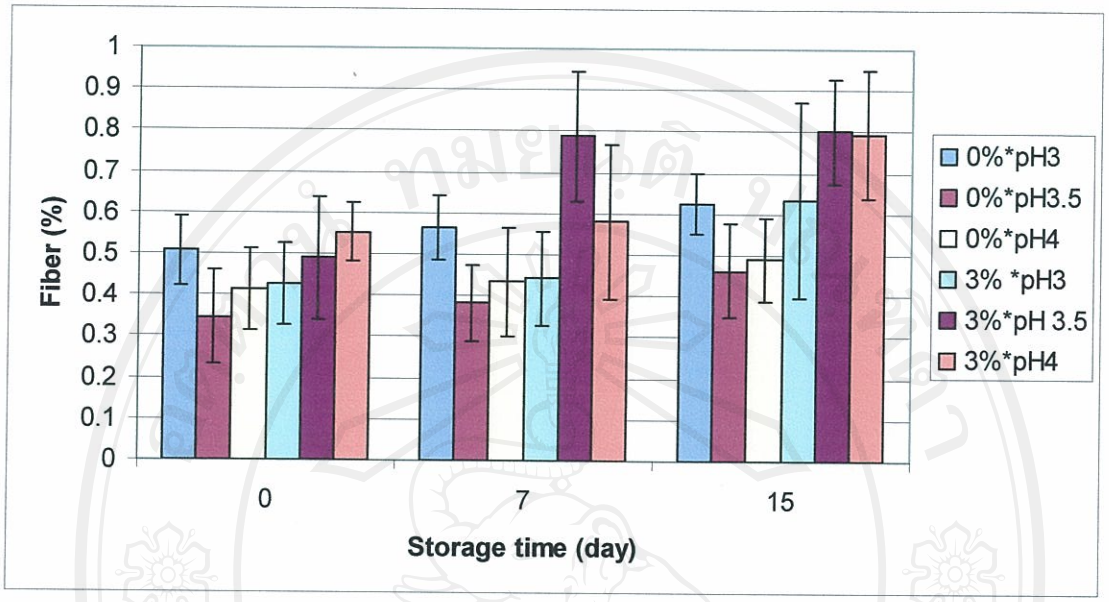


Fig. 4.20 The result of fiber content of orange juice during chilled storage as affected by orange juice sac and pH levels

Table 4.27 The result of moisture content (%) of orange juice during chilled storage as affected by orange juice sac

Storage time (day)	No added orange juice sac	Added 3% ($^{w}/_{v}$) orange juice sac
0	83.68±0.52$^{a*)}$	83.86±0.42^{a}
3	83.53±0.59^{a}	83.92± 0.41^{b}
6	84.12± 0.71^{a}	84.22± 0.81^{a}
9	84.32± 0.50^{a}	83.89± 0.19^{a}
12	83.41± 0.48^{a}	83.44± 0.36^{a}
15	83.45± 0.51^{a}	83.60± 0.71^{a}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

Table 4.28 The result of fiber content (%) of orange juice during chilled storage as affected by orange juice sac

Storage time (day)	No added orange juice sac	Added 3% ($^{w}/_{v}$) orange juice sac
0	$0.42 \pm 0.11^{a*)}$	0.49 ± 0.11^{b}
7	0.46 ± 0.12^{a}	0.60 ± 0.10^{b}
15	0.53 ± 0.11^{a}	0.74 ± 0.18^{b}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

Table 4.29 The result of total titrable acidity (%) of orange juice during chilled storage as affected by different pH values

Storage time (day)	pH 3	pH 3.5	pH 4
0	$0.33 \pm 0.01^{a*)}$	0.28 ± 0.01^{a}	0.23 ± 0.01^{b}
3	0.33 ± 0.02^{a}	0.28 ± 0.01^{b}	0.24 ± 0.01^{c}
6	0.32 ± 0.02^{a}	0.30 ± 0.02^{b}	0.24 ± 0.01^{c}
9	0.32 ± 0.01^{a}	0.28 ± 0.02^{b}	0.22 ± 0.01^{c}
12	0.32 ± 0.01^{a}	0.29 ± 0.02^{b}	0.23 ± 0.01^{c}
15	0.33 ± 0.01^{a}	0.29 ± 0.01^{b}	0.24 ± 0.02^{c}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

Table 4.30 The result of moisture content (%) of orange juice during chilled storage as affected by different pH values

Storage time (day)	pH 3	pH 3.5	pH 4
0	$84.31 \pm 0.26^{a*)}$	83.61 ± 0.19^{b}	83.39 ± 0.31^{b}
3	84.15 ± 0.30^{a}	83.55 ± 0.29^{b}	83.47 ± 0.68^{b}
6	84.74 ± 0.47^{a}	84.25 ± 0.78^{a}	83.51 ± 0.35^{b}
9	84.85 ± 0.23^{a}	84.20 ± 0.36^{b}	83.81 ± 0.16^{c}
12	83.87 ± 0.24^{a}	83.37 ± 0.30^{b}	83.04 ± 0.13^{c}
15	84.19 ± 0.49^{a}	83.40 ± 0.26^{b}	82.99 ± 0.22^{c}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

Table 4.31 The result of total soluble solids (TSS) (^{0}Brix) of orange juice during chilled storage as affected by different pH values

Storage time (day)	pH 3	pH 3.5	pH 4
0	$15.69 \pm 0.26^{a*)}$	16.39 ± 0.19^{b}	16.61 ± 0.31^{b}
3	15.85 ± 0.30^{a}	16.45 ± 0.29^{b}	16.53 ± 0.68^{b}
6	15.26 ± 0.47^{a}	15.75 ± 0.78^{a}	16.49 ± 0.35^{b}
9	15.16 ± 0.23^{a}	15.80 ± 0.36^{b}	16.19 ± 0.16^{c}
12	16.14 ± 0.24^{a}	16.63 ± 0.30^{b}	16.96 ± 0.13^{c}
15	15.81 ± 0.49^{a}	16.60 ± 0.26^{b}	17.01 ± 0.22^{c}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

Table 4.32 The results of reducing sugar (%) of orange juice during chilled storage as affected by different pH values

Storage time (day)	pH 3	pH 3.5	pH 4
0	$5.66 \pm 0.30^{a*)}$	5.66 ± 0.29^{a}	5.79 ± 0.22^{a}
3	4.44 ± 0.32^{a}	4.83 ± 0.21^{a}	4.17 ± 0.69^{a}
6	5.48 ± 0.56^{a}	5.89 ± 0.47^{a}	6.03 ± 0.83^{a}
9	5.24 ± 0.65^{a}	5.72 ± 0.43^{a}	5.79 ± 0.28^{a}
12	5.08 ± 0.63^{a}	5.40 ± 0.46^{a}	5.50 ± 0.24^{a}
15	4.63 ± 0.47^{a}	4.99 ± 0.30^{b}	5.14 ± 0.44^{b}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

4.3.3 Microbiological characteristics of Keaw Wan Prae orange juice during chilled storage as affected by orange juice sacs and pH values

4.3.3.1 Total Plate Count

The first analysis to monitor the microbial content of orange juice samples was carried out based on Total Plate Count. This analysis was used to monitor the total microorganisms that were present in orange juice, including bacteria, yeast and mold. The analysis result for the count was shown in Fig. 4.21 and Tables 4.33 – 4.34. Fig. 4.21 and Appendix E, Table 2.16 showed clearly that the number of total micro-organisms in the orange juice samples was increased significantly throughout the storage period. This result was similar to the previous result in the section 4.2.3, confirming that microorganisms would have a significant contribution in the spoilage of fresh orange juice during refrigerated storage.

Analyzing more details about the effect of pH values on the microbial quality of orange juice, the data showed that the orange juice samples at a pH value of 3 had lower microbial counts than those of the other orange juices in the middle of the storage time (3rd to 9th days of storage) (Table 4.33). Since bacteria are grow faster at pH values of 6.0–8.0 (Adams and Moss, 2000), this data confirmed that at lower pH values, microorganisms, particularly bacteria, would need a longer time to adapt themselves with acid environments. However, once this adaptation period was over, the microorganisms could grow quicker. Therefore at the end of the storage time, different pH values of orange juice samples did not affect significantly the result of Total Plate Count. In addition, Worobo and Padilla-Zakour (1998) reported that pH is a critical factor for microbial growth. If the minimum pH for specific microorganism is known, it is possible to design or adjust food products to control pathogens and extend the product's shelf-life.

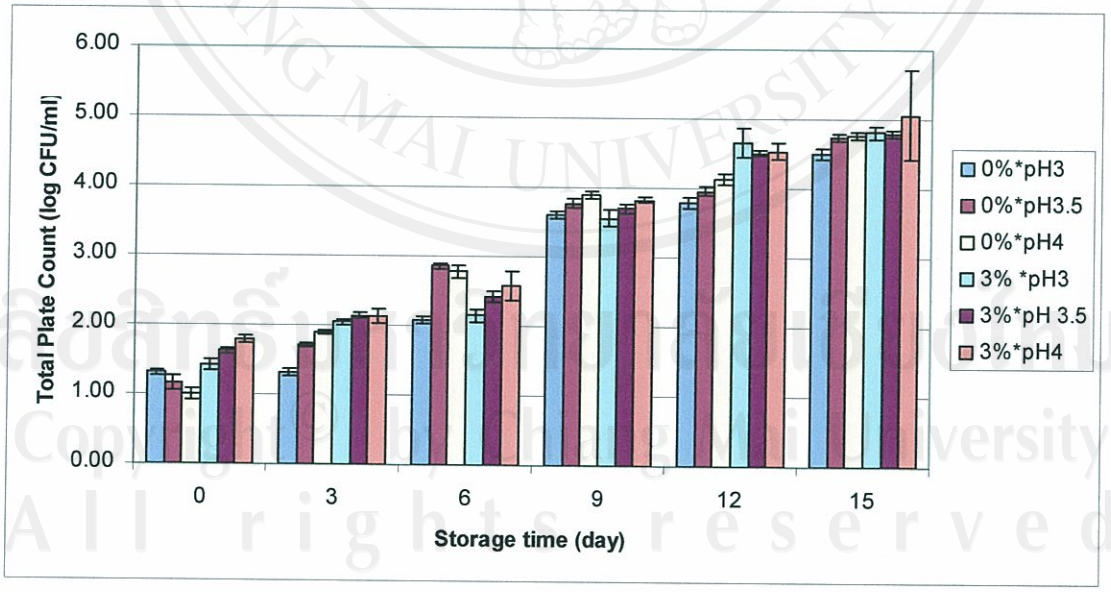


Fig. 4.21 The result of Total Plate Count of orange juice during chilled storage as affected by orange juice sac and pH levels

Table 4.33 The result of Total Plate Count (log CFU/ml) of orange juice during chilled storage as affected by different pH values

Storage time (day)	pH 3	pH 3.5	pH 4
0	$1.37 \pm 0.09^{a*)}$	1.39 ± 0.26^{a}	1.40 ± 0.45^{a}
3	1.68 ± 0.40^{a}	1.93 ± 0.24^{b}	2.01 ± 0.15^{c}
6	2.12 ± 0.08^{a}	2.65 ± 0.25^{b}	2.69 ± 0.18^{b}
9	3.58 ± 0.08^{a}	3.74 ± 0.07^{b}	3.86 ± 0.05^{c}
12	4.22 ± 0.50^{a}	4.23 ± 0.31^{a}	4.34 ± 0.23^{a}
15	4.65 ± 0.6^{a}	4.76 ± 0.05^{a}	4.92 ± 0.44^{a}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

Table 4.34 The result of Total Plate Count (log CFU/ml) of orange juice during chilled storage as affected by orange juice sacs

Storage time (day)	No added orange juice sac	Added 3% ($^{w}/_{v}$) orange juice sac
0	$1.15 \pm 0.15^{a*)}$	1.62 ± 0.20^{b}
3	1.64 ± 0.25^{a}	2.11 ± 0.07^{b}
6	2.58 ± 0.38^{a}	2.39 ± 0.23^{b}
9	3.89 ± 0.05^{a}	3.69 ± 0.14^{a}
12	3.96 ± 0.16^{a}	4.57 ± 0.13^{b}
15	4.67 ± 0.13^{a}	4.88 ± 0.35^{a}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

Beside pH values, orange juice samples were also significantly affected by the presence of orange juice sacs. In Table 4.34, it could be seen that orange juice samples that contained 3% ($^{w}/_{v}$) orange juice sac significantly had higher numbers of micro-organism than those of the orange juice samples without orange juice sac at the

beginning of the storage time. However, during the storage time the microorganisms in the orange juice samples without orange juice sacs could grow faster, making both treatments to be not significantly different at the end of the storage time. A higher microbial load in the orange juice samples with orange juice sacs could be due to a contamination during the process of the orange juice sac addition and/or letting the orange juice samples longer at room temperature, which gave an opportunity for the microorganisms to grow. For the interaction between pH values and orange juice sac, the Total Plate Count result showed that the orange juice samples with 3% ($^{w}/_{v}$) orange juice sac and had a pH value of 4 had higher microbial loads compared to that of the orange juice samples at a pH value of 3 and without the presence of orange juice sac throughout the storage time (Appendix E, Table 4.16).

4.3.3.2 Lactic acid bacteria count

The result of lactic acid bacteria measurement in orange juice samples was shown a similarity to the result of Total Plate Count. During storage at chilled temperature, the number of lactic acid bacteria in the orange juice samples was shown to be significantly increased (Fig. 4.22 and Appendix E, Table 2.17). In general, the increase of lactic acid bacteria count in this section was higher than the previous section of 4.2.3. In the section 4.2.3, the data showed that the number of lactic acid bacteria was increased from 2.11 log CFU/ml to 3.22 log CFU/ml, whereas this section found an increase from 1.24 log CFU/ml to 3.89 log CFU/ml. This result might indicate that the growth of lactic acid bacteria was affected more by the presence of orange juice sacs and pH values compared to the presence of salt and sugar.

The effect of orange juice sacs and pH values on the lactic acid bacteria of orange juices was shown to have similar patterns as these factors did to the Total Plate Count. Orange juice samples at a pH value of 4 were shown to be significantly different in the number of lactic acid bacteria compared to those in the other orange

juice samples only at the beginning of the storage time which was on the 3rd and 6th days of storage (Table 4.35). Adams and Moss (2000) reported that lactobacilli and acetic acid bacteria grow faster at a pH range of 5.0-6.0. Since the optimum growth pH of these bacteria was lower than general bacteria, the bacteria could do a quicker adaptation time in orange juices, which was 3 days earlier than the result of the Total Plate Count. On the other hand, the presence of 3% ($^{w}/_{v}$) orange juice sacs in orange juices caused the orange juice samples to have significantly higher number of lactic acid bacteria compared to those in the orange juice samples without orange juice sacs throughout the storage period (Table 4.36). A similar explanation as for the Total Plate Count result could be seen in the section 4.3.3.1. For the interaction between orange juice sac and pH value, the lactic acid bacteria measurement showed that the bacteria was not significantly be affected by this interaction throughout the storage period (Appendix E, Table 4.17).

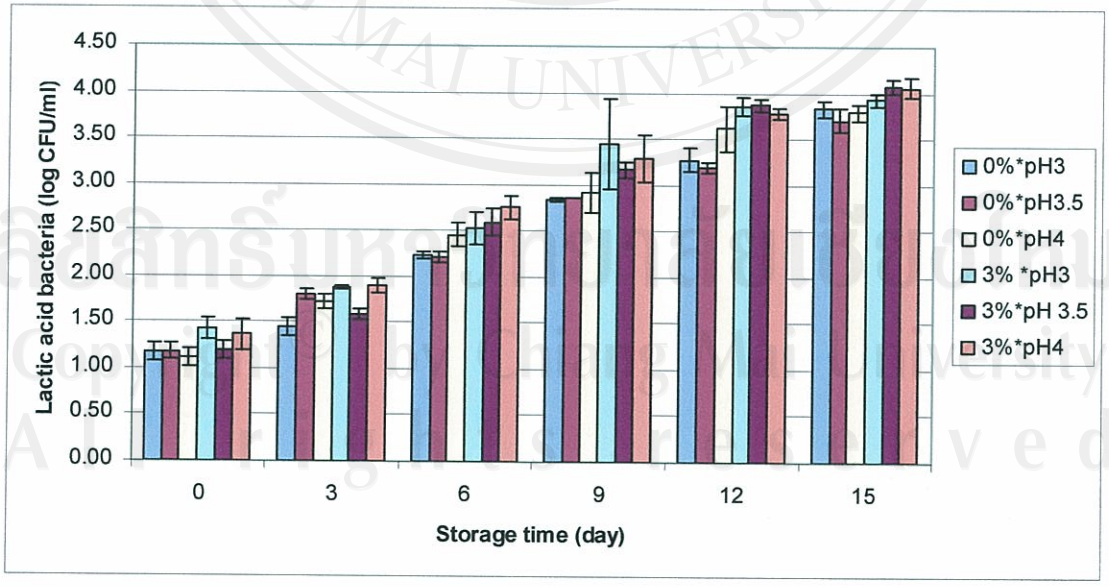


Fig. 4.22 The result of lactic acid bacteria of orange juice during chilled storage as affected by orange juice sac and pH levels

Table 4.35 The result of lactic acid bacteria (log CFU/ml) of orange juice during chilled storage as affected by different pH values

Storage time (day)	pH 3	pH 3.5	pH 4
0	$1.30 \pm 0.17^{a*)}$	1.19 ± 0.08^{a}	1.24 ± 0.19^{a}
3	1.67 ± 0.25^{a}	1.69 ± 0.13^{a}	1.81 ± 0.12^{b}
6	2.37 ± 0.19^{a}	2.40 ± 0.23^{a}	2.61 ± 0.20^{b}
9	3.13 ± 0.46^{a}	3.01 ± 0.18^{a}	3.10 ± 0.29^{a}
12	3.57 ± 0.34^{a}	3.53 ± 0.37^{a}	3.69 ± 0.18^{a}
15	3.88 ± 0.09^{a}	3.88 ± 0.22^{a}	3.92 ± 0.17^{a}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

Table 4.36 The result of lactic acid bacteria (log CFU/ml) of orange juice during chilled storage as affected by orange juice sacs

Storage time (day)	No added orange juice sac	Added 3% ($^{w}/_{v}$) orange juice sac
0	$1.15 \pm 0.09^{a*)}$	1.33 ± 0.15^{b}
3	1.66 ± 0.17^{a}	1.80 ± 0.17^{b}
6	2.30 ± 0.14^{a}	2.62 ± 0.17^{b}
9	2.87 ± 0.11^{a}	3.30 ± 0.30^{b}
12	3.36 ± 0.24^{a}	3.83 ± 0.08^{b}
15	3.77 ± 0.11^{a}	4.02 ± 0.10^{b}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

4.3.3.3 Yeast and mold count

The measurement of yeast and mold was carried out at the beginning and at the end of the storage period. The yeast and mold enumeration showed that these micro-organisms were significantly increased during storage at chilled temperature (Fig. 4.23,

Appendix E, Table 2.17). At higher pH values of orange juice samples, the number of the yeast and mold was significantly higher than those of the orange juice samples at a pH value of 3.0 (Table 4.37). This result confirmed the report that wrote yeast has an optimum growth pH of 4.5 to 6.0 and fungi has an optimum growth pH of 3.5 – 4.0 (Adams and Moss, 2000). At the same time, the presence of 3% ($^{w}/_{v}$) orange juice sac in orange juices caused the orange juice samples to have significantly higher number of yeast and mold compared to those of the orange juice samples without orange juice sacs at the end of the storage period (Table 4.38). For the interaction between orange juice sacs and pH values, Table 4.18 in Appendix E showed that the orange juice samples without any addition of orange juice sac and a pH value of 3.0 significantly contained the lowest number of yeast and mold compared to those of the other orange juice treatments at the beginning of the storage period. However, at the end of the storage period, orange juice samples at a pH value of 3.0 either with or without 3% ($^{w}/_{v}$) orange juice sac significantly had lower numbers of yeast and mold compared to those of the orange juices with pH values of 3.5 and 4.0. This finding indicated that the pH value may have a more significant contribution to the growth of yeast and mold in orange juices compared to the presence of orange juice sac. In addition, it could be noted that the increase in the number of yeast and mold in this section (Fig. 4.23) was lower than that of the section 4.2.3 (Fig. 4.12). This result might reflect that the growth of yeast and mold in orange juices could be affected more by the presence of salt and sugar compared to the presence of orange juice sacs and different initial pH values.

As an overall, it could be concluded that microorganisms could grow in fresh orange juice at a pH value range of 3.0 to 4.0 during refrigerated storage. At higher pH values of orange juices, the microorganisms could grow faster compared to the presence of these microorganisms in the orange juices that had a low pH value. The presence of orange juice sacs in the fresh orange juice would increase the initial

microbial load of the juice and give a positive impact in supporting the growth of some microorganisms.

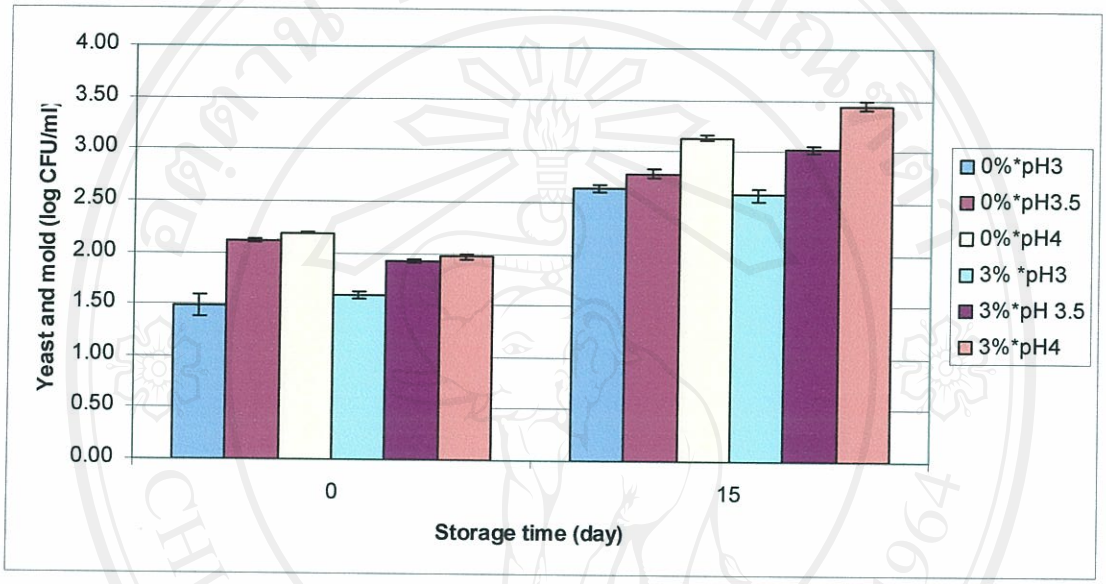


Fig. 4.23 The result of yeast and mold of orange juice during chilled storage as affected by orange juice sac and pH levels

Table 4.37 The result of yeast and mold (log CFU/ml) of orange juice during chilled storage as affected by different pH values

Storage time (day)	pH 3	pH 3.5	pH 4
0	$1.54 \pm 0.09^{a*)}$	2.02 ± 0.11^{b}	2.08 ± 0.12^{b}
15	2.61 ± 0.05^{a}	2.91 ± 0.14^{b}	3.29 ± 0.18^{c}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

Table 4.38 The result of yeast and mold (log CFU/ml) of orange juice during chilled storage as affected by orange juice sacs

Storage time (day)	No added orange juice sac	Added 3% ($^{w}/_{v}$) orange juice sac
0	$1.93 \pm 0.34^{a*)}$	1.83 ± 0.18^{b}
15	2.85 ± 0.22^{a}	3.02 ± 0.37^{b}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

4.3.4 Nutritional values of Keaw Wan Prae orange juice during chilled storage as affected by orange juice sacs and pH values

4.3.4.1 Vitamin C content

The result of vitamin C of orange juices was displayed in Fig. 4.24 and Tables 4.39 and 4.40. From the Figure and Appendix E, Table 2.19, it showed that the vitamin C content in the orange juices was significantly reduced during refrigerated storage for 15 days. Factors of pH values and orange juice sacs were found to significantly affect the retention of the vitamin. As the pH values of orange juice were higher the retention of the vitamin C in the juice was found to be significantly lower (Table 4.39).

At the end of the storage time, the best vitamin C retention was shown to be present in the orange juice at a pH value of 3.0. Bull *et al.* (2004) reported that degradation of ascorbic acid in orange juice could be due to the pH of the juice and the amount of oxygen dissolved in the juice. In addition, a better vitamin C retention could be achieved in the presence of higher concentrations of citric acid (Nagy, 1980). The vitamin C retention would also be significantly higher in the orange juices without orange juice sacs (Table 4.40).

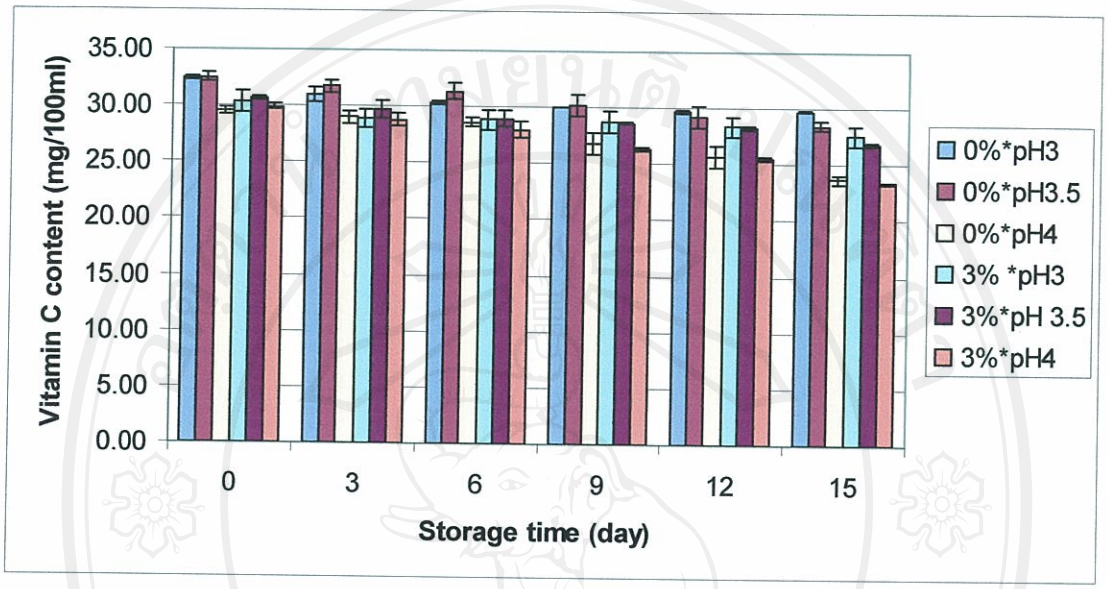


Fig. 4.24 The result of vitamin C of orange juice during chilled storage as affected by orange juice sac and pH levels

Table 4.39 The result of vitamin C (mg/100ml) of orange juice during chilled storage as affected by different pH values

Day	pH 3	pH 3.5	pH 4
0	$31.43 \pm 1.31^{a*)}$	31.59 ± 1.01^{a}	29.73 ± 0.32^{b}
3	29.94 ± 1.28^{a}	30.72 ± 1.26^{a}	28.90 ± 0.49^{b}
6	29.57 ± 0.99^{a}	30.17 ± 1.50^{a}	28.30 ± 0.64^{b}
9	29.38 ± 0.97^{a}	29.42 ± 1.08^{a}	26.43 ± 0.65^{b}
12	29.03 ± 0.92^{a}	28.74 ± 0.82^{a}	25.60 ± 0.59^{b}
15	28.67 ± 1.33^{a}	27.64 ± 0.94^{b}	23.56 ± 0.32^{c}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

Table 4.40 The result of vitamin C (mg/100ml) of orange juice during chilled storage as affected by orange juice sacs

Storage time (day)	No added orange juice sac	Added 3% ($^{w}/_{v}$) orange juice sac
0	$31.51 \pm 1.49^{a*)}$	30.33 ± 0.60^{b}
3	30.56 ± 1.32^{a}	29.15 ± 0.75^{b}
6	30.10 ± 1.29^{a}	28.59 ± 0.83^{b}
9	29.03 ± 1.78^{a}	27.89 ± 1.28^{b}
12	28.22 ± 2.01^{a}	27.37 ± 1.47^{b}
15	27.32 ± 0.77^{a}	25.93 ± 1.98^{b}

*) Values within a row followed by different letters were significantly different ($P \leq 0.05$)

4.3.4.2. Carotenoid content

Carotenoids are quality indicators for orange juice as they contribute both to the color and the nutritive value of the juice (Bull *et al.*, 2004). For the color measurement, the section 4.3.1.1 had shown that the L, a* and b* values of the orange juices were not significantly affected by storage at chilled temperature. A similar result was also displayed in this section. Fig. 4.25 and Appendix E, Table 2.20 showed that the carotenoid content of orange juices was not significantly affected during the storage period, eventhough the carotenoid values were reduced. This result confirmed the previous result by Bull *et al.* (2004), who reported that the β-carotene content in fresh, high pressure processed and heat treated orange juices did not significantly decrease during storage at 4 and 10^{0}C. Furthermore, the carotenoid content in the orange juice was not significantly affected by orange juice sacs and pH values (Appendix E, Table 4.20).

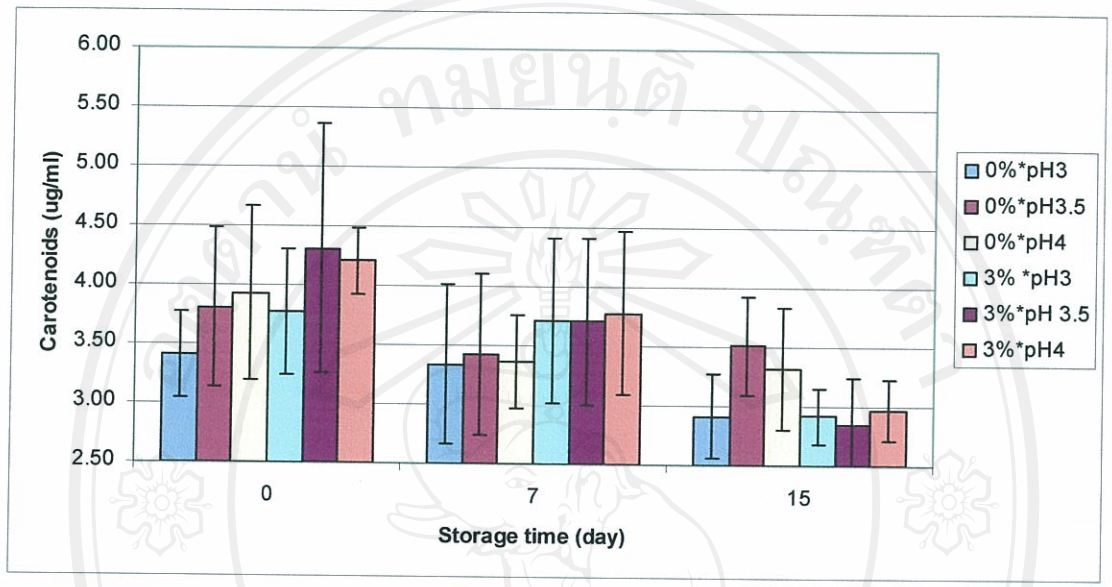


Fig. 4.25 The result of carotenoid of orange juice during chilled storage as affected by orange juice sac and pH levels

Chapter 5

Conclusion

From the first experiment, it could be concluded that Keaw Wan Prae orange juices had a better microbial and nutritional content than those of Sai Namphung orange juices. The total microorganism of the Keaw Wan Prae orange juices was 2.83 ± 0.18 log CFU/ml and the vitamin C and carotenoid contents of the juices were 30.71 ± 8.13 mg/100ml and 6.61±0.61 µg/ml, respectively.

A study about the effects of sugar and salt addition on the quality of Keaw Wan Prae fresh orange juices during storage at chilled temperature showed that an addition of 5% ($^{w}/_{v}$) sugar produced the best quality of orange juices based on the microbiological and nutritional assessment. The amounts of total microorganisms and lactic acid in the orange juices were 4.38 ± 0.34 log CFU/ml and 2.83 ± 1.10 log CFU/ml, respectively, at the end of storage time. In addition, the orange juice had a vitamin C content of 22.74 ± 3.82 mg/100 ml after 15 days at refrigerated storage. The presence of salt in the orange juices contributed to higher microbial counts and lower vitamin C contents.

In the last experiment section, the result data showed that Keaw Wan Prae fresh orange juices had a better quality at a pH value of 3.0 and without any addition of orange juice sacs. Applying this condition, the fresh orange juice had a total microbial load of 4.51 ± 0.08 log CFU/ml and a vitamin C content of 29.77 ± 0.10 mg/100 ml at the end of 15 day storage at chilled temperature.

Result from this study concluded that an addition of 5% ($^{w}/_{v}$) sugar could improve the microbial quality of fresh orange juice compared to that in the no-added sugar orange juices. For orange juices that had pH values more than 3.5, reduction in

the orange juice pH value to be 3.0 by adding food grade acids could also improve the qualities of the juice during refrigerated storage.

References

Anonymous. 1996. The International Carotenoid Society. Carotenoid. [Online]. Available on: *http://www.carotenoidsociety.org/carotenoids/fcarotenoids.html.* [2006, August 15].

Anonymous. 2000. Citrus: It Only Begins With C. [Online]. Available on: *http://www.berkeleywellness.com/html/wl/2000/wlFeatured1000.html.* [2005, April 27].

Anonymous. 2003. ACT Government Health Information. Preservatives, sugars and metals in fruit juices. [Online]. Available on: *http://www.health.act.gov.au/c/health?a=da&did=10018228&pid=1053860055.* [2005, April 15].

Anonymous. 2005a. Mandarins (Tangerines). [Online]. Available on: *http://www.innvista.com/health/foods/fruits/mander.html.* [2005, April 25].

Anonymous. 2005b. Thanathon Orchard (Products). [Online]. Available on: *http://www.tntorchard.Com/product.html.* [2005, April 26].

Anonymous. 2005c. Nutrition of orange juice. [Online]. Available on: *http://www.drinksmixer.com/desc69.html* [2005, April 26].

Anonymous. 2006a. Carotenoid. [Online]. Available on: *http://info.productfinder.net/hsi/MultiCarotene--30-Mg--60-Sgels.html.* [2006, August 15].

Anonymous. 2006b. On-line microbiology text book. Bacterial structure. [Online]. Available on: *http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=toc&book_id=2.* [2006, November 23].

Adams, M. R. and Moss, M. O. 2000. *Food Microbiology.* 2nd ed. The Royal Society of Chemistry. UK.

Alklint, C. 2003. Carrot juice processing-effects on various quality aspects. [Online]. Available on: *www.lub.lu.se/cgi-bin/show_diss.pl/tec_668.html.* [2006, August 15].

AOAC. 2000. *Official Methods of Analysis of the Association of Official Analytical Chemists*. AOAC International. Washington, DC.

Bayindirli, A., Alpas, H., Bozoglu, F. and Hizal, M. 2006. Efficiency of high pressure treatment on inactivation of pathogenic microorganisms and enzymes in apple, orange, apricot and sour cherry juices. *Food Control*. 17. p 52-58.

Bull, M. K., Zerdin, K., Howe, E., Goicoechea, D., Paramanandhan, P., Stockman, R., Sellahewa, J., Szabo, E. A., Johnson, R. L. and Stewart, C. M. 2004. The effect of high pressure processing on the microbial, physical and chemical properties of Valencia and Navel orange juice. *Innovative Food Science and Emerging Technology*. 5. p 135-149.

Chiesa, A., Valera, M. S. and Fraschina, A. 1996. Acidity and pigment changes in tomato (*Lycopersicon Esculentum* Mill.) fruit ripening. [Online]. Available on: *http://www.actahort.org/books/464/464_81.htm*. [2006, December 23].

Choi, M. H., Kim, G. H. and Lee H. S. 2002. Effect of ascorbic acid retention on juice color and pigment stability in blood orange (*Citrus sinensis*) juice during refrigerated storage. *Food Research International.* 35 (8). p 753-759.

Codex. 2005. Orange juice preserved exclusively by physical means 1. [Online].Available on: *http://www.codexalimentarius.net/web/standard_list.jsp*. [2005, April 26].

Cortés, C., Torregrosa, F., Esteve, M. J. and Friágola, A. 2006. Carotenoid profile modification during refrigerated storage in untreated and pasteurized orange juice and orange juice treated with high-intensity pulsed electric fields. *Journal Agricultural and Food Chemistry*. 54. p 6247-6254.

Collet, L.S.F.C.A., Shigeoka, D.S., Badolato, G.G. and Tadini, C.C. 2005. A kinetic study on pectinesterase inactivation during continuous pasteurization of orange juice. *Journal of Food Engineering*. 69. p 125-129.

de Man, J. D., Rogosa, M. and Sharpe, M. E. 1960. A Medium for the cultivation of *Lactobacilli. Journal Applied of Bacteria*. 23. p 130-135.

Denby, D. 1996. Chemistry Review. [Online]. Available on: *http://en.wikipedi a.org/wik i/DCPIP*. [2006, July 16].

de Souza, M. C.C., Benassi, M. de T., Meneghel, R. F. de A. and de Silva, R. S. dos S. F. 2004. Stability of unpasteurized and refrigerated orange juice. *Brazilian Archives of Biology and Technology*. 47 (3). p 391-397.

di Mascio, P., Kaiser, S. and Sies, H. 1989. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Archives of Biochemistry Biophysics*. 274. p 532-538.

Eren, F. G. S. 1998. Effect of temperature and pH on nonenzymic browning in minced dried pepper during storage. *Turkish Jounal of Engineering and Environmental Sciences*. 22. p 33-38.

Farnworth, E. R., Lagace, M., Couture, R., Yaylayan, V. and Stewart, B. 2001. Thermal processing, storage conditions and the composition and physical properties of orange juice. *Food Research International*. 34 (1). p 25-30.

Gardner, P. T., White, T. A. C., McPhail, D. B. and Duthie, G. G. 2000. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chemistry*. 68. p 471-474.

Gregory III, J. F. 1996. Vitamins. In: *Food chemistry*. 3rd edition. Fennema, O. R. (ed.). Marcel Dekker, Inc., New York, p 531-616.

Holden, J. M., Eldridge, A. L., Beecher, G. R., Buzzard, I. M., Bhagwat, A. S., Davis, C. S., Douglass, L. W., Gebhardt, E. S., Haytowitz, D. and Schakel, S. 1999.

Carotenoid Content of U.S. Foods: An Update of the Database. *Journal of food composition and analysis*. 12. p 169-196.

Ingallinera, B., Barbagallo, R. N., Spagna, G., Palmeri, R. and Tonaro, A. 2005. Effects of thermal treatments on pectinesterase activity determined in blood oranges juices. *Enzyme and Microbial Technology*. 36. p 258-263.

Jia, M., Zhang, Q. H. and Min, D. B. 1999. Pulsed electric field processing effects on flavour compounds and microorganisms of orange juice. *Food Chemistry*. 65. p 445-451.

Jordan, M.J., Goodner, K.L. and Laencina, J. 2003. Deaeration and pasteurization effects on the orange juice aromatic fraction. *Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology*. 36. p 391-396.

Koca, N., Burdurlu, H. S. and Karadeniz, F. 2003. Kinetics of nonenzymatic browning reaction in citrus juice concentrates during storage. *Turkish Journal of Agricultural and Forestry*. 27. p 353-360.

Lee, H. S. 1992. Antioxidative activity of browning reaction products isolated from storage–aged orange juice. *Journal Agricultural Food Chemistry*. 40. p 550-552.

Lee, H. S. and Coates, G.A. 1999. Vitamin C in frozen, fresh squeezed, unpasteurized, polyethylene-bottled orange juice: a storage study. *Food Chemistry*. 65 (2). p 165-168.

Leffingwell, J. C. 1999. Aldehydes. [Online]. Available on: *http://www.leffingwell.com /ald1.htm*. [2006, October 15].

Leslaw, J. and Teresa, F. 2003. Viscosity of concentrated strawberry juice effect of temperature and soluble solids content. [Online]. *Electronic Journal of Polish Agricultural Universities*. 6 (2). Available on: *http://www.ejpau.media.p1/volume6/issue2/food/art-11.html*.

Murata, M., Shinoda, Y. and Homma, S. 2002. Browning of model orange juice solution and changes in the components. *International Congress Series.* 1245. p 459-460.

Namutebi, A. 1998. Effects of preservation method and storage conditions on the flavour and colour of passion fruit juice. *African Crop Science Journal.* 6 (4). p 397-405

Nagy, S. 1980. Vitamin C contents of citrus fruit and their products: a review. *Journal of Agricultural and Food Chemistry.* 28 (1). p 8-18.

Nagy, S., Chen, C. S. and Shaw, P. E. 1993. *Fruit Juice Processing Technology.* AgScience, Inc., Auburndale, Florida.

Nienaber, U. and Shellhammer T. H. 2006. Shelf life study of high pressure processed orange juice. [Online]. Available on: *http://grad.fst.ohio-state.edu/hpp/83A-16.pdf.* [2006, October 15].

Pandell, A. J. 1999. The acidity of wine. [Online]. Available on: *http://wineperspective.com/the-acidity_of_wine.htm.* [2006, November 22].

Parish, M. 2006. How do salt and sugar prevent microbial spoilage. Available on: *http://www.sciam.com/askexpert_question.cfm?articleID=000DCCA9-088E-13F6-881A83414B7F4945&catID=3&topicID=3.* [2006, December 27].

Parish M. E. 1998. Orange juice quality after treatment by thermal pasteurization or isostatic high pressure. *Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology.* 31. p 439-442.

Ren, C. and Kermode, A. R. 2000. Pectin methyl esterase and its potential role in dormancy breakage of yellow cedar (*Chamaecyparis nootkatensis*) seeds. American Society of Plant Biologists. [Online]. Available on: http://abstracts.aspb.org/pb2000/public/P24/0815.html. [2006, October 15].

Rieger, M. 2005. Citrus. [Online]. Available on: *http://www.uga.edu/fruit/citrus.htm* [2005, April 26].

Riu-Aumatell, M., Castellari, M., Lopez-Tamames, E., Galassi, S. and Buxaderas, S. 2004. Characterisation of volatile compounds of fruit juices and nectars by HS/SPME and GC/MS. *Food Chemistry*. 87. p 627-637.

Roig, M. G., Bello, J. F., Rivera, Z. S. and Kennedy, J. F. 1999. Studies on the occurrence of non-enzymatic browning during storage of citrus juice. *Food Research International*. 32 (9). p 609-619.

Rujanakaikan, L. and Rattanapanon, N. 1997. *Analysis of Food*. 5th ed. Department of Food Science and Technology, Faculty of Agro-Industry, Chiang Mai University. Chiang Mai.

Sánchez-Moreno, C., Plaza, L., De Ancos, B. and Cano M. P. 2003. Vitamin C, provitamin A carotenoids and other carotenoids in high-pressurized orange juice during refrigerated storage. *Journal of Agricultural and Food Chemistry*. 5. p 647-653.

Sánchez, F., Korine, C., Pinshow, B. And Dudley, R. 2004. The possible roles of ethanol in the relationship between plants and frugivores: first experiments with egyptian fruit bats. *Integrative and Comparative Biology*. 44. p 290–294.

Schreibman, T. 2005. The 8 Healthiest Drinks. [Online]. Available on: *http://www.fitnessmagazine.com/food_and_nutrition/031804_feature6_print.jsp*. [2005, April 27].

Selli, S., Cabaroglu, T. and Canbas, A. 2004. Volatile flavour components of orange juice obtained from the cv. Kozan of Turkey. *Journal of Food Composition and Analysis*. 17. p 789-796.

Spagna, G., Barbagallo, R. N. and Ingallinera, B. 2003. A specific method for determination of pectin esterase in blood oranges. *Enzyme and Microbial Technology*. 32. p. 174-177.

Suklampoo, L. 2003. Preservation of Mild Heat and Ginger Extract. *Food*. 33(3). p 211- 221.

Sun, D., Dure, L., Grant, T. and Wicker, L. 2006. Secondary structure and stability of marsh grapefruit pectinesterase - a circular dichroism study. [Online]. Available on: *http://ift.confex.com/ift/98annual/techprogram/accepted/983.htm.* [2006, November 23].

Suntornsuk, L., Gritsanapun, W., Nilkamhank, S. and Paochom, A. 2002. Quantitation of vitamin C content in herbal juice using direct titration. *Journal of Pharmaceutical and Biomedical Analysis*. 28. p 849-855.

Todar, K. 2004. Nutrition and growth of bacteria. [Online]. Available on: *http://textbo-okofbacteriology.net/nutgro.html*. [2006, November 22].

Torregrosa, F., Esteve, M.J., Frigola, A. and Cortes, C. 2006. Ascorbic acid stability during refrigerated storage of orange-carrot juice treated by high pulsed electric field and comparison with pasteurized juice. *Journal of Food Engineering*. 73(4). p 339-345.

Tran, M. T. T. and Farid, M. 2004. Ultraviolet treatment of orange juice. *Innovative Food Science and Emerging Technology*. 5. p 495-502.

Tressler, D. K. and Joslyn, M. A. 1971. *Fruit and Vegetable Juice Processing Technology*. 2. The AVI Publishing Co., Inc. California.

USDA. 1972. United States Standards for Grades of Grapefruit Juice and Orange Juice. [Online]. Available on: *http://www.ams.usda.gov/standards/cngrapoj.pdf.* [2006, October 10].

Worobo, R. and Padilla-Zakour, O. 1998. Food safety and you. *The Newsletter of the New York State Food Venture Center*. Winter 99. 1 (4). [Online]. Available on: *http://www.nysaes.cornell.edu/necfe/pubs/pdf/Venture/venture4_safety.html.* [2006, October 10].

Zanoni, B., Pagliarini, E., Galli, A. and Laureati, M. 2004. Shelf–life prediction of fresh blood orange juice. *Journal of Food Engineering*. 70. p 512-517.

Zierler, B., Siegmund, B. and Pfannhauser, W. 2004. Determination of off-flavour compounds in apple juice caused by microorganisms using headspace solid phase microextraction-gas chromatography-mass spectrometry. *Analytica Chemica Acta.* 520. p 3-11.

Appendices

Appendix A

Picture of orange and orange juice

Fig. A1 Fruit and juice of Keaw Wan Prae orange

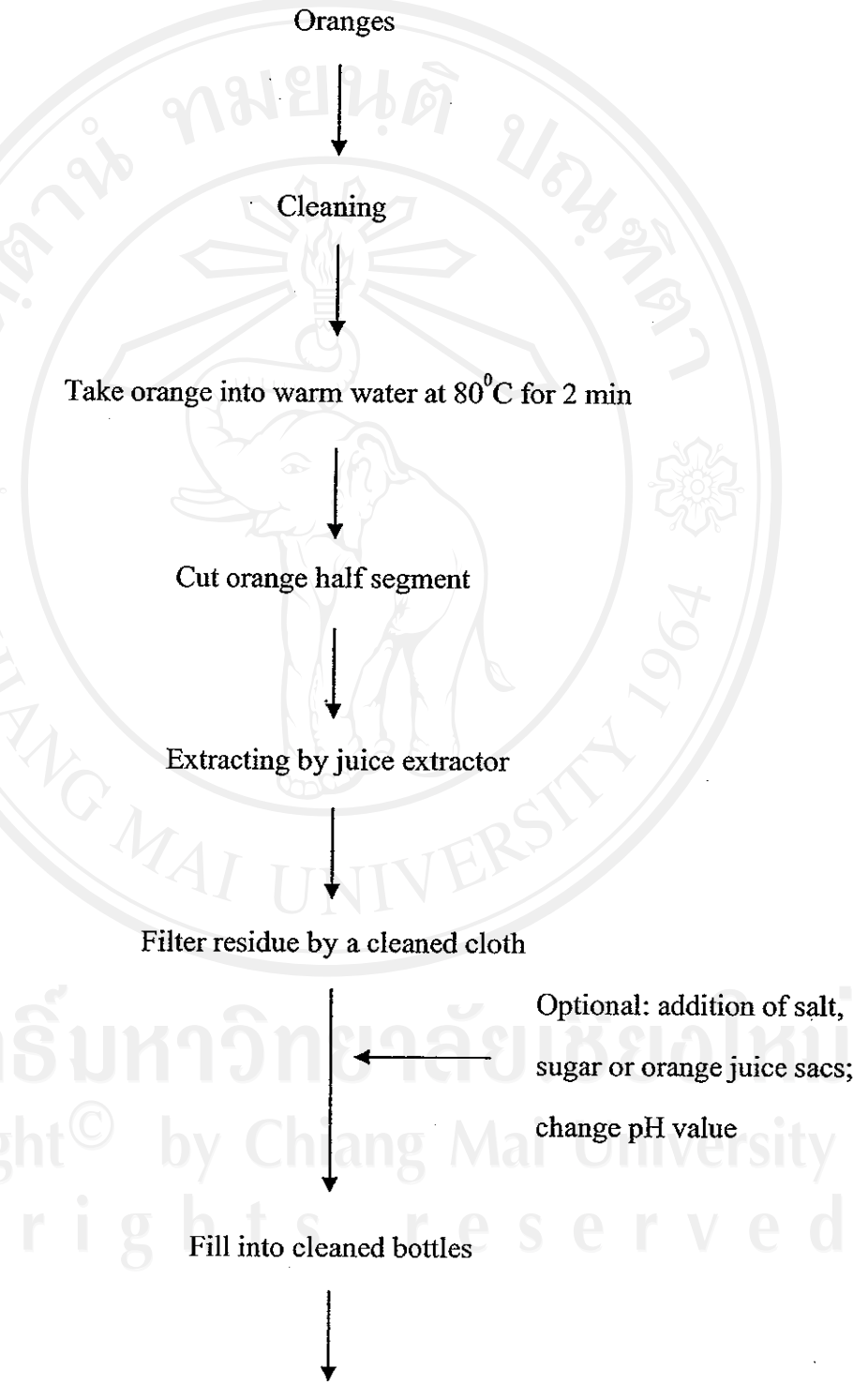
Fig. A2 Fruit and juice of Sai Namphung orange

Fig. A3 A color comparison between Keaw Wan Prae orange juice (left) and Sai Namphung orange juice (right)

Appendix B

Orange juice processing

Steps in the orange juice process



Appendix C

Physical, chemical, microbiological and nutritional analyses

1. Physical method

1.1 Color analysis

CIE L, a* and b* values were measured with a colorimeter (Chrometer, Minolta CR-300, Japan). Samples of juice were prepared by pouring 30 ml of orange juice into a white plastic cup.

L value = a lightness factor with a value range of 0-100 %.

a* and b* value = chromaticity coordinates, with a value range from (–) 60 to (+) 60.

Total color difference (Δ E*) was calculated using a formula of : $(\Delta L^2 + \Delta a^{*2} + \Delta b^{*2})^{1/2}$ (Lee and Coates, 1999).

Browning index was calculated from $[(C_{rt} - C_{ro})^2 + [(C_{yt} - C_{yo})^2]^{1/2}$ (Suklampoo, 2003).

C_{rt} = a* value of samples.

C_{ro} = a* value of control.

C_{yt} = b* value of samples.

C_{yo} = b* value of control.

1.2 Cloud loss analysis (Farnworth *et al.*, 2001)

Cloudiness of orange juice samples was determined by measuring % transmittance (%T) in a spectrophotometer at a wavelength of 640 nm.

1.3 Viscosity analysis

Viscosity of orange juice was measured using a viscometer. An amount of 8 ml orange juice was poured into a 100 cylinder and placed in the correct position under the viscometer equipment. The orange juice sample was measured using a viscometer speed at 200 rpm. Measurements were conducted at room temperature.

1.4 Yield analysis

The yield of orange juice was measured based on a formula:

$$\% \text{ yield} = \frac{\text{weight of orange juice}}{\text{weight of orange fruit}} \times 100$$

2. Chemical method

2.1 Total Titratable Acidity analysis (AOAC, 2000)

Pipette 10 ml orange juice sample into a flask and dilute the sample with distilled water. Then pipette 10 ml diluted solution sample into another flask. Drop phenopthalein 2-3 drops and titrate the sample with 0.1 M NaOH until sample reach the end point (sample solution became pink that was persisted for 30 s).

$$\% \text{ Citric acid} = \frac{\text{ml of 0.1 M NaOH} \times 0.1 \times 0.007 \times 100}{\text{weight of sample}}$$

2.2 Moisture and Total solid analysis (AOAC, 2000)

Heated an empty moisture dish in a hot air oven about 20-30 min. Cool in a desiccator and weigh the dish. Into the cooled and weighed dish (provided with cover), previously heated to 100 ± 3°C, accurately weigh 2 g of orange juice sample. Uncover the dish and dry the dish with its cover and contents for 3 h in an oven provided with opening for ventilation and maintained at 100 ± 3°C. Cover the dish while it is still in the oven, transfer to a desiccator, and weigh the dish soon after it reached a room temperature. Dry the sample again for several times until the sample has a constant weight.

$$\% \text{ moisture content} = \frac{\text{Loss in the sample weight during drying x 100}}{\text{Initial weight of the sample}}$$

% total solid = 100 - % moisture content

2.3 Sugar analysis (AOAC, 2000)

2.3.1 Reducing sugar

Transfer 15 ml of orange juice sample into a 100 volumetric flask and adjust to 100 ml with deionized water. Add 5 ml of Carrez I and II sulotions. Shake and adjust to 200 ml with distilled water. Put aside the mixed solution for precipitation about 20 min and filter the solution with a Whatman filter paper no. 4. Pour the solution filtrate into a 50 ml burette and pipette 5 ml of Fehling solution no. 1 and 2 into a flask.
Heat the Fehling solution on a hot plate and add one drop of methylene blue indicator. Titrate the Fehling solution with the filtrate solution in the burette until the Fehling solution has a color of orange–red.

2.3.2 Inversion sugar

Pipette 50 ml of filtrate solution from the previous determination into a volumetric flask. Add 10 ml of 6.34 N hydrochloric acid. Place the volumetric flask in a water bath at 70°C for 10 min and cool immediately. Make the mixture solution neutral with 5 N NaOH and adjust to 100 ml with water. Do titration following the procedure of reducing sugar.

% sucrose (S) = % different between D_1 and D_2 x 0.95

% total sugar = D_1 + S

D_1 = % Reducing sugar

D_2 = % Inversion sugar

2.4 Protein analysis (AOAC, 2000)

Place weighed sample (15 ml) in a digestion flask. Add 8 g catalyst mixture and 20 ml H_2SO_4. Place the flask in an inclined position in a digestion machine and heat the machine gently until frothing ceases. Continue boil briskly until the solution clears (~ 2 h).

Cool, add distilled water to dilute the mixture solution and pour into a distilling flask. Add 400 ml H_2O (ammonia-free water) and a few Zn granules to prevent bumping. Immediately immerse a condenser tip into a receiver that contains 50 ml of 2% boric acid solution in a 500 ml flask and 5-7 drops indicator. Add 75 ml of 50% sodium hydroxide using a funnel into the distilling equipment. Rotate the distilling flask to mix the contents thoroughly; then heat until all NH_3 has been distilled (≥ 150 ml distillate). Remove the receiver, wash the tip of the condenser and titrate excess standard acid in distillate with 0.05 M H_2SO_4. Do blank determination to correct any nitrogen content in reagents.

$$\%N = \frac{(V_a - V_b) \times N.H_2SO_4 \times 1.4007}{W}$$

V_a = ml of standard acid for sample titration

V_b = ml of standard acid for blank titration

$N.H_2SO_4$ = normality acid

W = weight of sample (g)

%Protein = %N x factor (factor value = 6.25)

2.5 Fat analysis (AOAC, 2000)

Weigh sample (0.5-1.0 g) and place into a separated funnel. Add 10 ml water and shake. Add 1.25 ml ammonia solution, 10 ml ethyl alcohol and 25 ml diethyl ether, close with a stopper and shake vigorously for 1 min. Carefully release the pressure of the funnel. Add 25 ml petroleum ether, close the stopper and shake vigorously for 1 min. Carefully release the pressure. Let stand until an upper liquid is practically clear (~ 30 min). Pour the upper clear solution into a previously weighed beaker. Take the beaker to stand in a hood until diethyl ether and petroleum are evaporated and place the beaker in a hot air oven ($T = 102 \pm 2^{0}C$) for 2 h. Cool in a desiccator and weigh the sample.

$$\%\text{Fat content} = \frac{(W_2 - W_3) \times 100}{W_1}$$

W_1 = Weight of sample

W_2 = Weight of beaker and fat

W_3 = Weight of beaker

2.6 Ash analysis (AOAC, 2000)

Weigh 3-5 g sample into an ashing dish that has been heated, cooled in a desicator, and weighed soon after reaching room temperature. Before ashing the sample, heat the sample on a bunchen lamp until no more black smoke appeared. Then ash the sample in a muffle furnace at 550°C until light gray ash results or until it reaches a constant weight. Cool in a desicator and weigh soon after reaching room temperature.

$$\%\ \text{Ash} = \frac{\text{Weight of ash} \times 100}{\text{Weight of sample}}$$

2.7 Fiber analysis (AOAC, 2000)

Weigh 5 g sample into a 500 ml beaker. Transfer 1.25 M sulfuric acid (200 ml) into the beaker. Boil the sample solution on a hot plate for 30 min. Filter the sample solution using a Whatman paper no. 4 until it dries by applying a vacuum pump and wash the residue with boiling water until the sample does not have acid (do a test using a litmus paper). Place 200 ml of 1.25% NaOH into a beaker and boil the beaker on a hot plate. Wash the residue on the filter paper with distilled water. Boil the sample again on the hot plate for 30 min. Filter the sample using a Whatman paper no. 4 and wash the residue with boiling water. Transfer the filter paper with the sample residue into a crucible and dry at 102 ± 2^{0}C for 3 h. Cool in a desicator and weigh. Then ash the residue for 2 h at 550 ± 10^{0}C, cool in the desicator, and weigh.

$$\% \text{ Crude fiber} = \frac{(W_4 - W_3 + W_2) + (W_5 - W_3)\,(100 - \%H_2O - \%\text{fat})}{W_1}$$

W_1 = Weight of sample

W_2 = Weight of filter paper

W_3 = Weight of crucible

W_4 = Weight of crucible + filter paper + sample after drying

W_5 = Weight of crucible + ash

$\%H_2O$ = Moisture content of sample

%fat = Fat of sample

2.8 Carbohydrate content (AOAC, 2000)

Carbohydrate content was determined by measuring the difference of the original sample minus the moisture, protein, crude fat and mineral contents calculated at the same moisture level.

2.9 Pectin esterase (PE) activity

Quantitative measurement of PE was based on a method reported by Bull *et al.* (2004). The orange juice sample was mixed properly by inverting the bottle several times and 5 ml of the juice was transferred into 50 ml of a 1% pectin substrate solution in 0.2 M sodium chloride. The sample was titrated to pH 7.5 with 0.2 N NaOH. Then 0.05 N NaOH was titrated into the sample to maintain the sample pH at 7.5 for 30 min. The volume of 0.05 N NaOH consumed during this time was recorded. The PE activity expressed as PE units (PEU) per gram soluble solids was calculated by using the formula:

$$\text{PEU/g.s.s.} = \frac{\text{ml NaOH x normality of NaOH}}{\text{Weight of sample x 30 min x } {}^{0}\text{Brix}/100}$$

2.10 Flavor analysis

The flavor components of orange juice samples Gas Chromatography – Mass Spectrophotometer (GC-MS) were identified. For this portion of the work, a GC 6890 Agilent Technologies equipped with a 30 m x 0.25 mm ID column and 0.25 μm film thickness was used. The GC was linked to a Hewlett Packard Model 5973 (EI) mass spectrometry detector. The initial oven temperature was maintained at 50^{0}C for 1 min. It was then raised at 10^{0}C/min to 70^{0}C. After that, it increased to 140^{0}C at a rate of 5^{0}C/min and maintained at 140^{0}C for 11 min. Finally it increased to 220^{0}C at a rate of 10^{0}C/min without a final hold time. The injection port and ionizing source were kept at 230^{0}C, and the transfer line was kept at 230^{0}C. Helium was used as a carrier gas and the flow through the column was maintained at 1.0 ml/min. Identification of the components was done by comparison of mass spectra and retention time data with those of authentic compounds supplement with standard mass spectra.

3. Microbiological analysis

3.1 Total Plate Counts. The number of total microorganisms were enumerated using a pour plate method on Orange Serum Agar. Incubation was performed at 35^0C for 48 h (Jia *et al.*, 1999).

3.2 Total yeast and moulds were enumerated on Potato Dextrose Agar acidified to pH 3.5 with 10% tartaric acid by a pour plating technique. The incubation for total yeast and mold counts was done at 22^0C for 5 days (Jia *et al.*, 1999).

3.3 Lactic acid bacteria were enumerated on de Man Rogosa Sharpe (MRS) agar. The incubation for lactic acid bacteria counts was done anaerobically at 35^0C for 3 days (de Man *et al.*, 1960).

4. Nutritional analysis

4.1 Vitamin C content (AOAC, 2000)

Pipette 50 ml sample into a 100 ml volumetric flask. Add 25 ml of 0.4% oxalic acid and dilute with distilled water. Then pipette 10 ml of the diluted sample into a 125 ml flask. Titrate this sample with an indophenol standard solution. At the end point, an excess unreduced dye will produce a rose pink color in solution.

A similar procedure as above is done for 0.05 g of vitamin C standard.

1 ml of vitamin C standard 1 ml had a vitamin C content of 0.2 mg

10 ml of vitamin C standard 10 ml had a vitamin C content 0.2 x 10 = 2 mg

If 2 mg of vitamin C used an indophenol standard solution of a ml, then the sample that used a b ml indophenol standard solution would have a vitamin C content of: $= \frac{2 \times b}{a}$ mg

Since the initial sample volume was 50 ml then for 100 ml of orange juice sample, the sample would contained vitamin C of: $\frac{2 \times b \times 100}{a \times 50}$ mg/100 ml

4.2 Carotenoid content (AOAC, 2000)

4.2.1 Make a standard curve of carotenoid

Place a 0.0005 g of standard beta-carotene (β–carotene) into a beaker. Dilute the standard β–carotene with 2.5 ml chloroform. Pour the β–carotene solution into a 50 ml volumetric flask and adjust the volume with hexane. Pipette 5 ml of the standard solution into a 50 ml volumetric flask and adjust the volume with hexane. Then pipette 1, 2, 3, 4, 5, 6, 7, 8 and 9 ml of the standard solution into 10 ml volumetric flasks and adjust with a 10% acetone in hexane solution. Take the solution that has the highest concentration of β–carotene solution to find the highest absorbance using a wavelength range between 400-500 nm in a spectrophotometer. Use 10% acetone in hexane as a blank. Choose the wavelength that shows the highest absorbance and measure the absorbance of the other solutions (1, 2, 3, 4, 5, 6, 7 and 8 ml). Make a standard curve between the concentrations of the standard β–carotene solution and the absorbance values.

4.2.2 Determination of carotenoid in sample

Weigh 5 g sample and place the sample into a flask. Add 100 ml of 10% acetone in hexane, stir for 10 min and filter using a funnel and Whatman paper no. 4. Pour the clear solution into a separate funnel and pour 100 ml distilled water into the separate funnel to separate acetone from the solution. Shake the funnel and pour the acetone in water into a beaker. Filter the carotenoid in hexane solution and place the carotenoid in hexane solution in a hood to evaporate the hexane from carotenoid. Then dilute the dry carotenoid with 10% acetone in hexane solution in a 50 ml volumetric

flask. Measure the absorbance of the sample solution with a spectrophotometer at 450 nm (Use 10% acetone in hexane as a blank). Calculate the carotenoid content in orange juice using the β–carotene standard curve (Fig. C1).

From standard curve

$y = 1.2808x - 0.0072$

y = absorbance value of carotenoid.

x = carotenoid content (ppm).

Take the x value from the standard curve equation to find the carotenoid content in the orange juice sample.

Calculation to find carotenoid content

1000 ml of a dilute solution has carotenoid = x mg.

50 ml of a dilute solution has carotenoid = (x/1000) x 50 mg.

Carotenoid content from 5 g sample

5 g orange juice has carotenoid = z mg.

1g orange juice has carotenoid = z/5 mg.

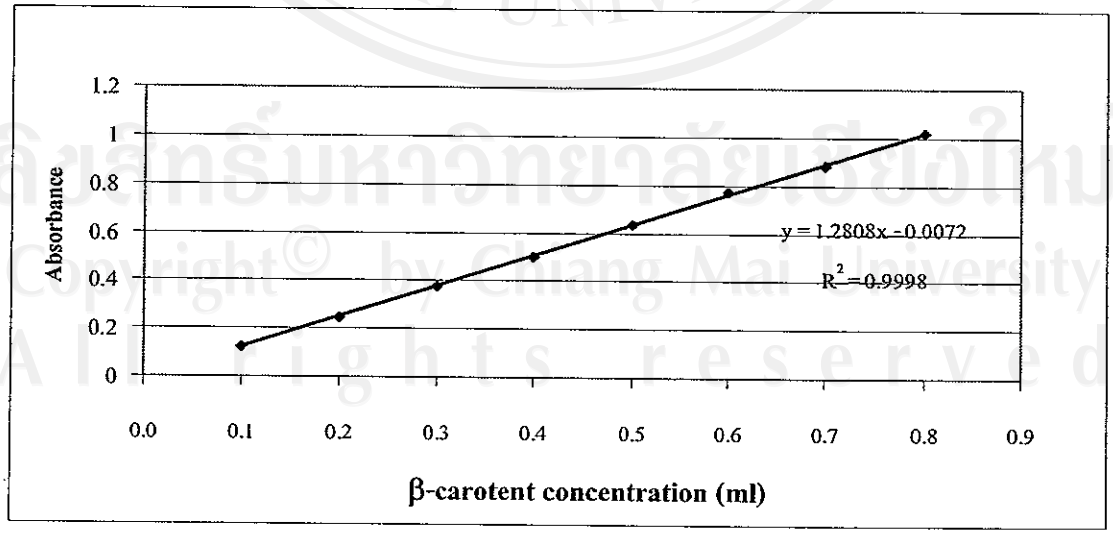


Fig. C1 The standard curve of β – carotene in 10% acetone in hexane solution

Appendix D

Gas Chromatography-Mass Spectrophotometer analysis

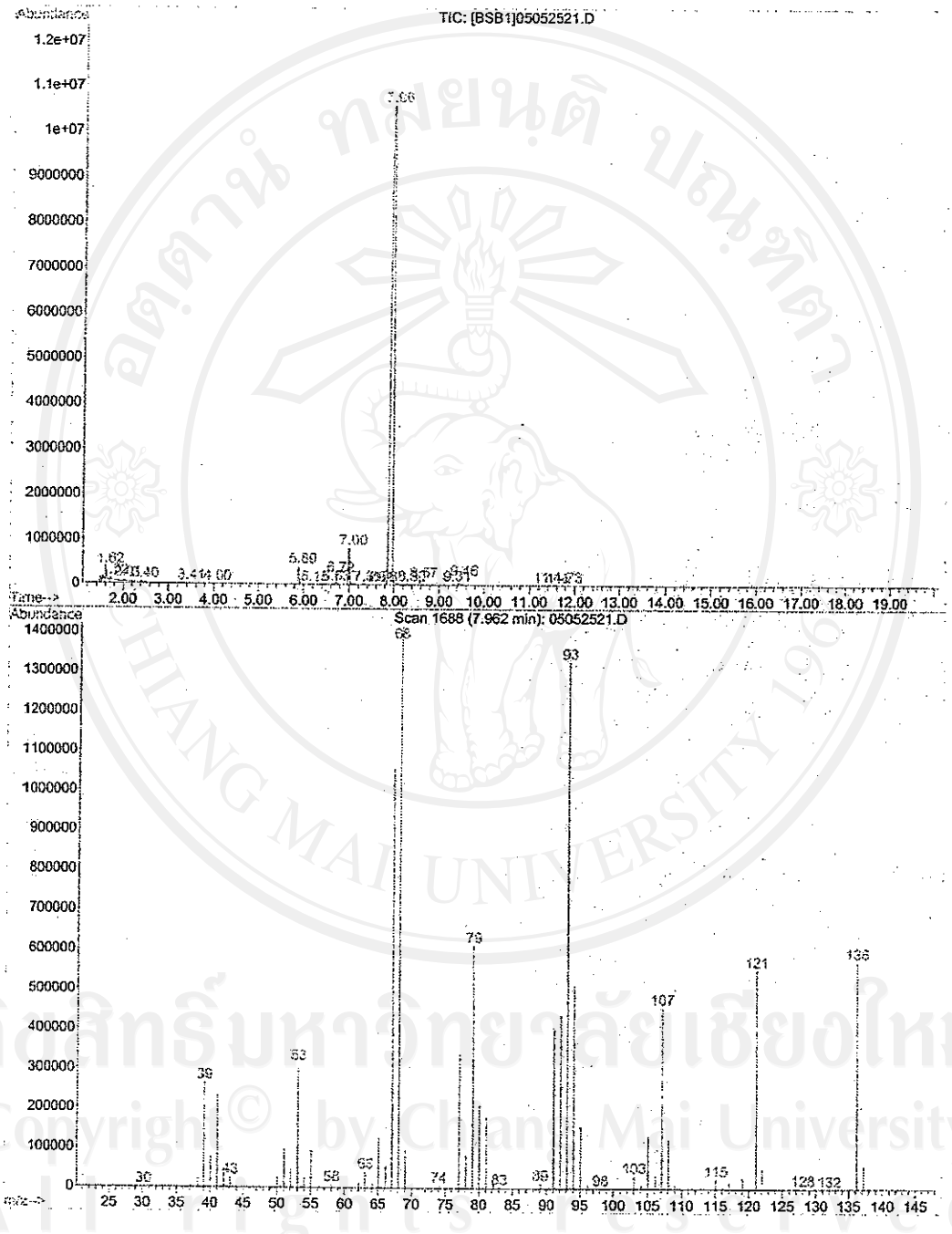


Fig. D1 The chromatogram of flavour components in Keaw Wan Prae fresh orange juice

Table D1 The retention time and percentage of peak area of flavour components in Keaw Wan Prae fresh orange juice

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	1.615	121	129	151	BV	277330	3593799	0.75%	0.687%
2	1.720	151	155	171	PV 2	49569	502632	0.10%	0.096%
3	2.010	222	226	265	PV	31554	367508	0.08%	0.070%
4	2.396	307	321	366	BV 2	35279	1054750	0.22%	0.202%
5	3.414	540	571	596	BB 4	16429	237729	0.05%	0.045%
6	4.005	677	716	727	BB 2	8477	105807	0.02%	0.020%
7	5.890	1167	1179	1213	BB	399216	6910297	1.44%	1.322%
8	6.155	1233	1244	1259	BB 3	7965	152184	0.03%	0.029%
9	6.627	1350	1360	1370	BV 2	18974	348737	0.07%	0.067%
10	6.725	1370	1384	1411	VB	213607	3966350	0.82%	0.759%
11	6.997	1429	1451	1510	BB	819494	15811572	3.29%	3.024%
12	7.315	1514	1529	1552	BB 2	17316	432512	0.09%	0.083%
13	7.608	1577	1601	1609	BV 5	15212	355802	0.07%	0.068%
14	7.677	1609	1618	1643	VB 2	15373	415154	0.09%	0.079%
15	7.962	1653	1688	1764	BV	10574484	481065447	100.00%	91.997%
16	8.304	1764	1772	1822	VB 3	23142	744378	0.15%	0.142%
17	8.569	1823	1837	1870	BV 2	103014	2099966	0.44%	0.402%
18	9.310	2007	2019	2040	PV 4	31260	669758	0.14%	0.128%
19	9.465	2040	2057	2104	PB 2	152839	3658152	0.76%	0.700%
20	11.436	2532	2541	2556	BB 6	6687	135896	0.03%	0.026%
21	11.725	2596	2612	2636	BB 6	10558	283599	0.06%	0.054%