CHAPTER 4
RESULTS AND DISCUSSION

4.1 Physical, chemical, microbiological and sensory analysis of orange juice cv. Sai Nam Pung and cv. Khieo Waan

Physical and chemical properties of the fresh orange juice cv. Sai Nam Pung and cv. Khieo Waan showed in Table 4.1.The fresh orange juice cv. Sai Nam Pung and Khieo Waan showed the values of L*: 27.45 and 37.18; a*: 1.20 and 2.58; b*: 13.88 and 23.80 respectively, there were significant difference ($p<0.05$). The Khieo Waan was brighter (L*) and darker yellow (b*) than the Sai Nam Pung. The total soluble solid, titratable acidity, reducing sugar and ascorbic acid of fresh orange juice cv. Sai Nam Pung and Khieo Waan were non significant difference ($p<0.05$). They contained the values of reducing sugar (mg/100g): 3.46 and 2.83; ascorbic acid (mg/100g): 28.23 and 25.33 respectively. Sai Nam Pung was higher β-carotene (μg/100g) than Khieo Waan significant difference ($p<0.05$), They contained the values 42.73 and 19.85; respectively. In general the fresh orange juice cv. Sai Nam Pung showed the value of reducing sugar, ascorbic acid and β-carotene more than Khieo Waan. Similar results were observed for the data in the literature of Linchong (2003). According to Thailand Institute of Scientific and Technological research (2004), there were few studies on an effective quality control for natural unpasteurized orange juice. The internal retail market for orange juice in Thailand was base mainly on the trade of the natural product, refrigerated and packed in pastic bottles.

Table 4.1 Physical and chemical properties of orange juice cv. Sai Nam Pung and cv. Khieo Waan

Assay	Sai Nam Pung	Khieo waan	Significant level
Colour L*	27.45 ± 4.14 a	37.18 ± 4.04 b	*
Colour a*	1.20 ± 0.81 a	2.58 ± 0.03 b	*
Colour b*	13.88 ± 0.08 a	23.80 ± 0.11 b	*
Total soluble solids (%)	11.93 ± 0.06	11.46 ± 0.12	NS
Total titratable acidity (%)	0.65 ± 0.15	0.52 ± 0.17	NS
Reducing sugar (mg/100g)	3.46 ± 0.17	2.83 ± 0.11	NS
Ascorbic acid (mg /100g)	28.23 ± 0.25	25.33 ± 0.31	NS
β-carotene (μg/100g)	42.73± 0.15 b	19.85± 0.09 a	*

Values are means ± standard deviations.
Values followed by different letters are significantly different ($p < 0.05$).
NS = not significant

4.2 Monitoring the shelf life of the orange juice cv. Sai Nam Pung and cv. Khieo Waan at different storage temperatures.

Table 4.2 Changes in Total Plate Count in orange juice during storage at room temperature (30°C), 4 and -18°C.

		Total Plate Count (CFU/ml)	
Temp.	Days	Sai Nam Pung	Khieo Waan
30°C	0	3.83×10^{2} a	4.10×10^{2} a
	1	4.91×10^{3} a	4.37×10^{3} a
	3	1.91×10^{6} a	2.37×10^{6} b
4°C	0	3.86×10^{2} a	4.10×10^{2} a
	1	7.93×10^{2} a	8.13×10^{2} a
	3	1.06×10^{3} a	1.09×10^{3} a
	6	1.06×10^{4} a	1.09×10^{4} a
	9	1.77×10^{6} a	1.94×10^{6} a
-18°C	0	3.80×10^{2} a	4.16×10^{2} a
	1	5.53×10^{2} a	5.50×10^{2} a
	3	6.5×10^{2} a	6.4×10^{2} a
	6	7.1×10^{2} a	7.4×10^{2} a
	9	7.5×10^{2} a	7.8×10^{2} a
	12	8.1×10^{2} a	8.3×10^{2} a
	15	9.4×10^{2} b	8.3×10^{2} a
	18	9.8×10^{2} b	8.6×10^{2} a
	21	1.10×10^{3} b	1.02×10^{3} a

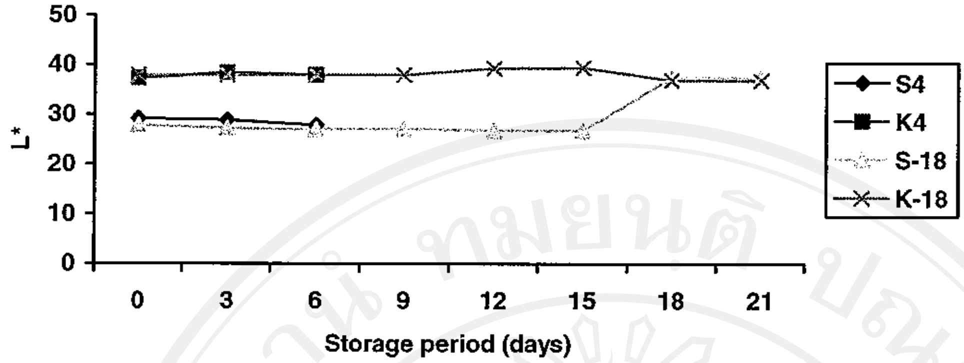
Values followed by different letters are significantly different ($p < 0.05$).

Table 4.3 Shelf life of orange juice cv. Sai Nam Pung and cv. Khieo Waan at different storage temperatures (based on microbiological)

Storage temperature (°C)	Shelf life(days)	
	Sai Nam Pung	Khieo Waan
30	1	1
4	6	6
-18	> 21	> 21

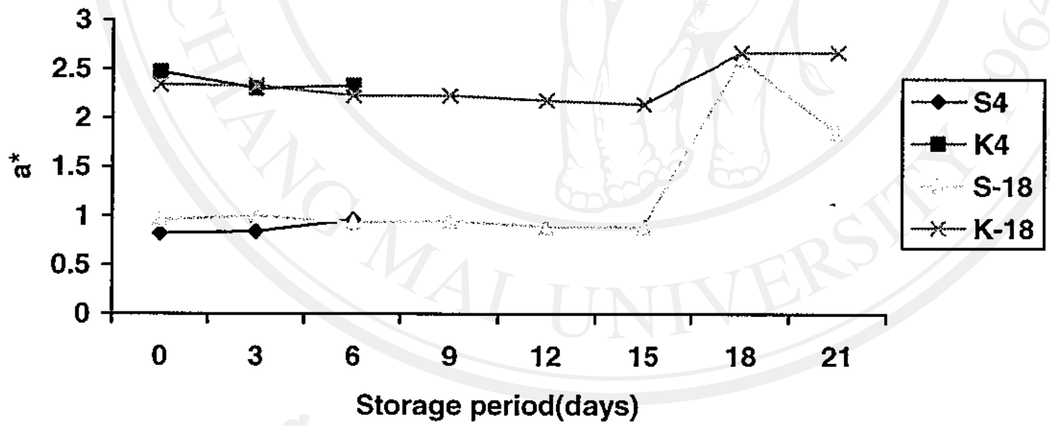
Consider the limited for shelf life estimation of orange juice (Table 4.2-4.3). The criteria of orange juice cv. Sai Nam Pung and Khieo Waan used for total plate count (TPC) was limit of $5x10^4$ CFU/ml (Directive 92/46/EEC, 2006). Fresh orange juice had only 1 day shelf life. Furthermore, the orange juice qualities at 4, -18°C, and room temperature were evaluated. It was observed that 4°C and -18°C, the orange juice still have a good quality throughout the storage time, however ascorbic acid contents were reduced. So, it can be concluded from this trial that the end of storage time at each temperature condition should be in 1 day at room temperature, 6 days at 4°C and more than 21 days at -18°C. The number of microorganisms in orange fruits can affect the microbial quality of fresh orange juice. A specific research related to this issue was done to quantify the transfer rates of microorganisms during an extraction process. It was found that about 1.7–2.6% of total aerobic organisms and 2.3–2.6% of aciduric organisms from the washed oranges were introduced into the fresh juice during the extraction process. The quality of fresh juice is essentially depending on careful fruit handling and strict processing sanitation (Steven and Davis, 2001). The low pH values of orange juices cv. Sai Nam Pung and cv. Khieo Waan greatly limit the number and types of bacteria that can survive or grow. Orange juice have a pH value between 3.4 to 4.4 and in these conditions *Lactobacillus* and *Leuconostoc* can survive and grow. These bacteria can produce undesirable flavours and odours. The growth of lactic acid bacteria in some fruit beverages, like wines, a fermented product from grapes, or cider, a fermented product from apple, can be useful, but in orange juice the lactic acid bacteria considered to be spoilage organisms. *Lactobacilli* plays an important role in the spoilage of soft drinks, fruit juices and related products. *Lactobacillus plantarum* and *Lactobacillus brevis* can multiply in orange juice causing the formation of slime, gas, off-flavour, turbidity and changes in acidity (Murdock and Hatcher, 1975).

Colour determination (Figure 4.1 -4.3) The L* (L* whiteness/darkness, ranged from 0 to 100) showed in Figure 4.1 a* (a* redness for positive value and greenness for the negative one) showed in Figure. 4.2 and b* (b* yellowness for positive and blueness for negative value) showed in Figure. 4.3. The fresh Khieo Waan had a significant higher L*, a*, b* ($p<0.05$) than the fresh Sai Nam Pung (Table 4.1). The orange juice graphs (Fig. I) showed that there is a steady trend in the L* values of the Khieo Waan storage at 4°C (K4) and Sai Nam Pung storage at 4°C (S4) for 6 days and the Khieo Waan storage at -18°C (K-18) for 21 days, no variation in L* were seen. During storage of orange juice cv. Sai Nam Pung and Khieo Waan at 4°C and -18°C, it was observed that b* parameters did not present significant variations until 15 days of storage (Fig.4.3). After this time values of b* slightly changes its colour from initial. This change may be attributed to non-enzymatic browning (Linchong, 2003). Lee and Chen (1998) evaluated colour changes during storage of concentrated orange juice at temperature between 4°C and 24°C. They observed a slight decrease in value of L* in relation to storage time and temperature and an increase in the value of b*.



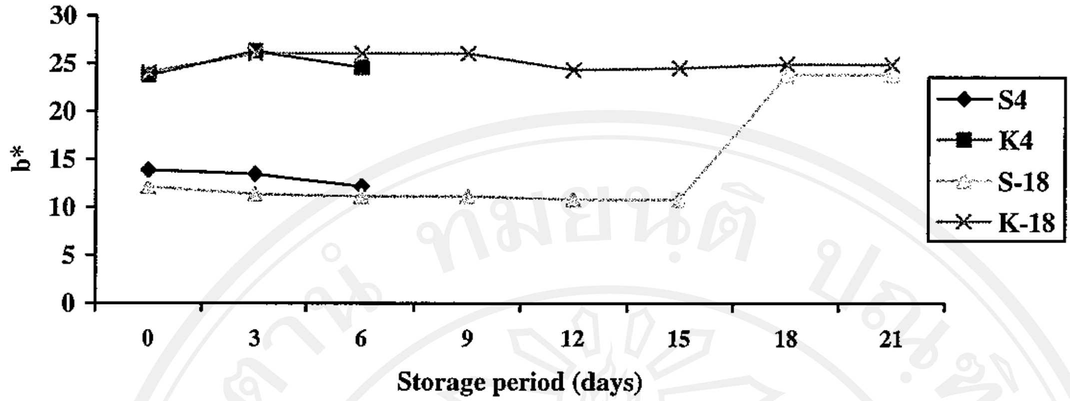
Sai Nam Pung storage at 4°C (S4), Sai Nam Pung storage at -18°C (S-18) Khieo Waan storage at 4°C (K4), Khieo Waan storage at -18 °C (K-18)

Figure 4.1 Changes in colour L* in orange juice cv. Sai Nam Pung and cv. Khieo Waan during storage at 4 °C and -18°C.



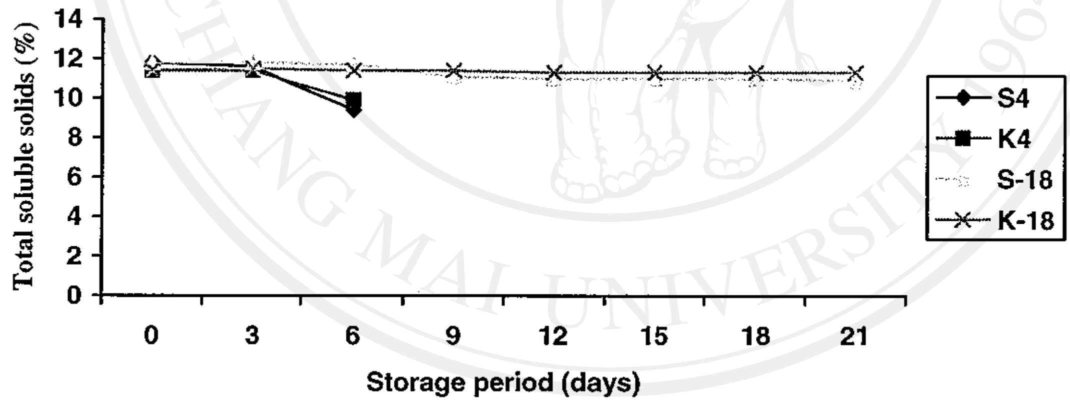
Sai Nam Pung storage at 4°C (S4), Sai Nam Pung storage at -18°C (S-18) Khieo Waan storage at 4°C (K4), Khieo Waan storage at -18 °C (K-18)

Figure 4.2 Changes in colour a* in orange juice cv. Sai Nam Pung and cv. Khieo Waan during storage at 4 °C and -18°C.



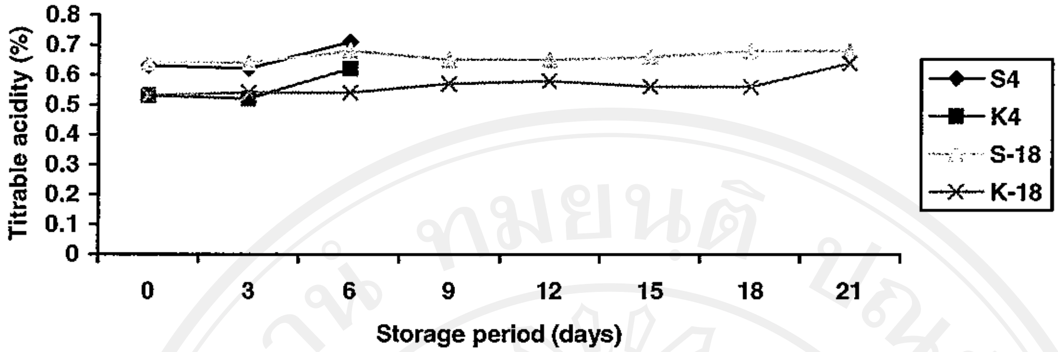
Sai Nam Pung storage at 4°C (S4), Sai Nam Pung storage at -18°C (S-18)
Khieo Waan storage at 4°C (K4), Khieo Waan storage at -18 °C (K-18)

Figure 4.3 Changes in colour b* in orange juice cv. Sai Nam Pung and cv. Khieo Waan during storage at 4 °C and -18°C.



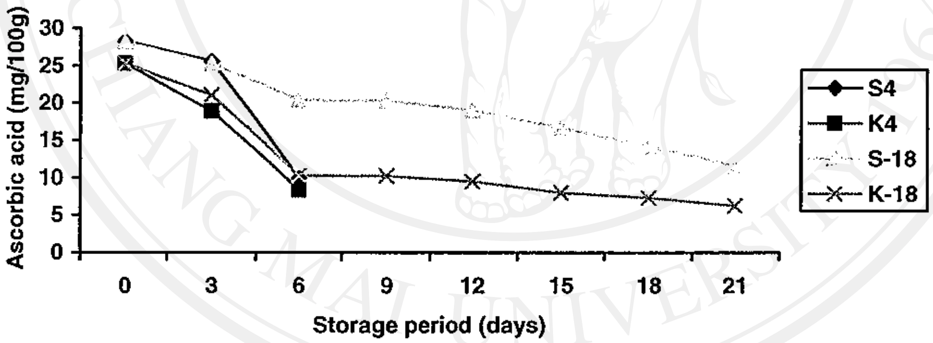
Sai Nam Pung storage at 4°C (S4), Sai Nam Pung storage at -18°C (S-18)
Khieo Waan storage at 4°C (K4), Khieo Waan storage at -18 °C (K-18)

Figure 4.4 Changes in total soluble solids (%) in orange juice cv. Sai Nam Pung and cv. Khieo Waan during storage at 4 °C and -18°C.



Sai Nam Pung storage at 4°C (S4), Sai Nam Pung storage at -18°C (S-18)
Khieo Waan storage at 4°C (K4), Khieo Waan storage at -18 °C (K-18)

Figure 4.5 Changes in titratable acidity (%) in orange juice cv. Sai Nam Pung and cv. Khieo Waan during storage at 4 °C and -18°C.



Sai Nam Pung storage at 4°C (S4), Sai Nam Pung storage at -18°C (S-18)
Khieo Waan storage at 4°C (K4), Khieo Waan storage at -18 °C (K-18)

Figure 4.6 Changes in ascorbic acid (mg/100g) in orange juice cv. Sai Nam Pung and cv. Khieo Waan during storage at 4 °C and -18°C.

Total soluble solid (Figure 4.4) The results indicated that total soluble solid of fresh orange juice cv. Sai Nam Pung and Khieo Waan were not significant difference ($p<0.05$) (Table 4.1). During storage, orange juice both cv. Sai Nam Pung and Khieo Waan at 4°C had a same steady total soluble solid about 11.8 % for 3 days and decrease until 6 days (Figure. 4.4). This decrease may be attributed to consumption of sugar as a result of the onset of fermentation. The K-18 and S-18 have been remaining steadily. It was found that total soluble solid were not significant difference ($p<0.05$). Both of them had total soluble solid closely until storage at 21 days.

Titratable acidity (Figure 4.5) During storage of orange juice cv. Sai Nam Pung and Khieo Waan at -18°C, no variations in titratable acidity .This implies that the same quality of two varieties in term of citric acid. Whereas when they were stored at 4°C the acidity began to increase in 3- 6 days (Figure 4.5). The increase in acidity may indicate fermentation of orange juice (Murdock and Hatcher, 1975).

Ascorbic acid (Figure 4.6) Ascorbic acid of the orange juice both cv. Sai Nam Pung and cv. Khieo Waan (Table IV) showed the decrease trend there were significant difference ($p<0.05$). The results from our experiment agree with previous reported Hoare *et al.* (1993) in orange juice, the ascorbic acid levels significantly decreased during a storage period even in unopened containers. Therefore, the presence of dehydroascorbic acid needs to be measured besides the ascorbic acid to get the overall activity of the vitamin C in orange juice. The content of vitamin C in orange juice was in a range of 26 to 84 mg/100 ml (Ashurst *et al.*, 1999). Unfortunately, the concentration of this vitamin was reduced during storage of the juices (Li *et al.*, 1989; Lee and Coates, 1999; Johnston and Bowling, 2002). For example, vitamin C in fresh squeezed unpasteurized orange juice was declined during a frozen storage in a polyethylene bottle at -23°C (Lee and Coates, 1999). Li *et al.* (1989) also found the concentration of vitamin C in Valencia orange juice was decreased during storage at 5 and 25°C. Beside these researches, Kabasakalis *et al.* (2000) found that if the juices stored in open containers in a refrigerator for 31 days, the ascorbic acid loss was around 60 to 67%.

The effect of keeping temperature on shelf life of orange juice cv. Sai Nam Pung and Khieo Waan can be evaluated by the microbial spoilage, increasing amount of colony forming unit as total plate count not more than $5x10^4$CFU/ml. Yeast and mold, lactic acid bacteria and spore count were observed (Table A 4.10- A 4.12). At the end of the storage time of each keeping temperature the two kinds of orange juice showed the same data of yeast and mould, lactic acid bacteria and spore count, less than 10 CFU/ml. The results of sensory properties of orange juice showed (Table A 4.1). The appearance, colour, odour sweet and sour of storage at -18°C were significantly different from the other method. All treatments were not different in turbidity. Overall acceptance of the storage at room temperature and 4°C were good sensory properties than at -18°C.

4.3 The effect of storage condition on total carotenoid content in orange juice cv. Sai Nam Pung and cv. Khieo Waan

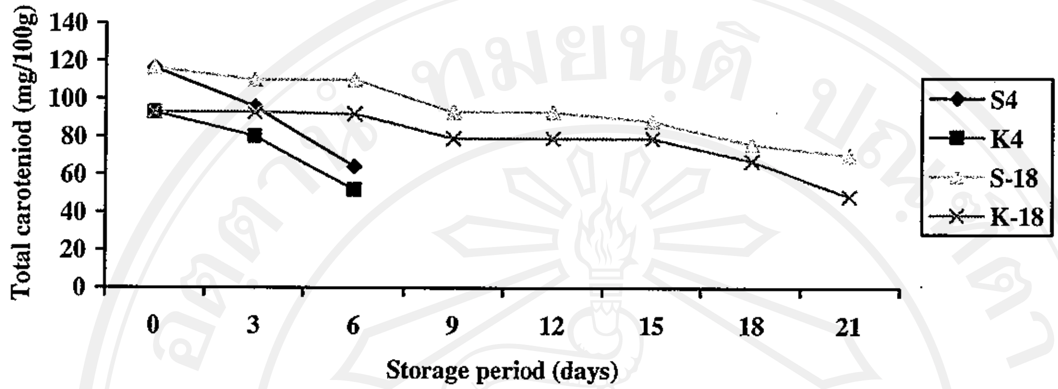


Figure 4.7 Changes in total carotenoid content in orange juice during storage at 4 and-18 °C

Total carotenoid contents in Sai Nam Pung more than in Khieo Waan. The outstanding decrease of carotenoid contents in both kinds of juice begins on the first day at 4°C while this begins on the 6 day at -18 °C in orange juice. The results of the total carotenoid content showed in Figure 4.7. All treatments have the same decrease trend in the total carotenoid content. The storage at room temperature has a sharp decrease in the carotenoid after 1 day (Table A 4.13). In case of storage at 4°C, The values decrease rapidly after 6 day and not detected since then. Clara *et al.* (2006) reported that decreased in the concentrations of total carotenoids during storage in refrigeration was greater in the untreated orange juice and the pasteurized juice than in the juice treated with high-intensity pulsed electric fields (HIPEF).

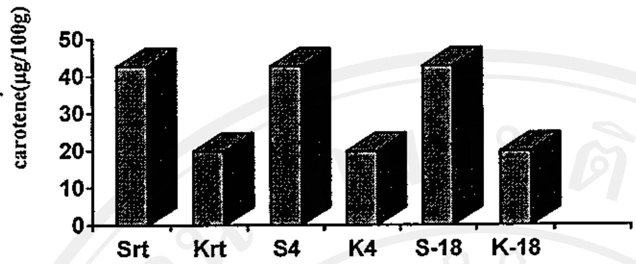


Figure 4.8 β-carotene (µg/100g) in orange juice cv. Sai Nam Pung and Khieo Waan at first day storage

Figure 4.8 showed the results of the β-carotene (µg/100g) in orange juice cv. Sai Nam Pung and Khieo Waan during storage at first day. All treatments have the same trend. The storage at room temperature, 4°C and -18°C, had a sharp decrease until not detected. It may be concluded that β-carotene in orange juice cv. Sai Nam Pung and Khieo waan decreased with increasing temperature. The loss of β-carotene may be caused by chemical alterations and storage condition (Clara *et al.*, 2006).

4.4 The combination effect of nisin and pH on the quality of orange juice.

Several factors played important roles in the combination of nisin and reduced pH including orange juice quality, nisin concentration, types of microorganism, storage temperature, processing conditions. Good quality orange juice should have a low spore count and low microorganism. The effect of nisin and pH on shelf life of two kinds of orange juice cv. Sai Nam Pung and Khieo waan, can be evaluated by the microbial spoilage, increasing amount of colony forming unit as total plate count per millilitre of juice not more than $5x10^4$, Yeast and mold, lactic acid bacteria and changing in spore count (Table A4.26- A4.33). The other parameter to co-indicate decomposition of the juice were a number of physicochemical properties colour in CIE system (L*, a* and b*), total soluble solid, titratable acidity, pH, reducing sugar, ascorbic acid and total carotenoid content (Table A4.14- A4.25).

Table A4.14 showed L* value of orange juice Sai Nam Pung which combination of nisin and adjusted pH value during storage time at 4°C. The orange juice was more brightness as the higher of nisin content, whereas the pH value was not significantly affected on L* value. Nevertheless the concentration of 100 IU of nisin at several pH value, the data showed that the brightness reduced with the reduction of pH value. Moreover, the L* value would be reduced as longer storage time in orange juice which supplemented with a combination of nisin and pH value adjustment and the orange juice which did not added nisin, adjusted at 4.2, 4.8 of pH values. Whereas the L* value were increased as longer storage time in orange juice which were adjusted pH value but no added nisin.

L* value of orange juice cv. Khieo Waan added with different concentration of nisin and adjusted pH value during storage time at 4°C were showed in Table A 4.15. The L* value of orange juice were decreased as the higher concentration of nisin and lower pH value. Moreover, the duration storage time affected to L* value reduction with longer storage time.

The a* value of orange juice cv. Sai Nam Pung and Khieo Waan which supplemented with different concentration of nisin and value adjustment were the same trend (Table A 4.16 and A 4.17). However, the influence of storage time on a* value between those varieties of orange juice were contrasted. The most of a* value of orange juice cv. Sai Nam Pung were increased with longer storage time. On the other hand, the most of a* value of orange juice cv. Khieo Waan were reduced as longer storage time.

The b* value of orange juice which prepared from both of cv. Sai Nam Pung and cv. Khieo Waan varieties, whereas the addition of nisin caused to increased b* value in orange juice cv. Sai Nam Pung but reduced that value in orange juice cv. Khieo Waan (Table A4.18 and A4.19). Storage time influence was different trend in orange juice cv. Sai Nam Pung and Khieo Waan. b* value were reduced as the higher storage time in orange juice cv. Sai Nam Pung, excepted the orange juice which no added nisin and adjusted pH 3.6 was not significant different in b* value during storage time. Whereas the b* value of fresh orange juice cv. Khieo Waan were not significant during storage time. It might be related to the orange juice cv. Sai Nam Pung contain higher ascorbic acid content than the orange juice cv. Khieo Waan and the ascorbic acid degradation caused to brown colour (Nelson *et al.*, 1980), led to the b* value of the orange juice cv. Sai Nam Pung reduced during storage (Mc Guire, 1992).

The influences of nisin concentration and pH value adjustment on the total soluble solid in orange juice cv. Sai Nam Pung and Khieo Waan were as same trend (Table A4.20 and A4.21). The addition of nisin and pH were not affected on the total soluble solid value in the orange juice. Storage time caused to reduce the total soluble solid in both of orange juice varieties. It might be the microorganism grow during storage and utilized the nutrition substances in orange juice such as reducing sugar. It was clearly in Table A4.22 - A4.25. Moreover, the ascorbic acid in orange juice also reduced as higher storage time.

The effect of nisin concentration and pH value adjustment were not significant different in reducing sugar content of both of orange juice varieties during first 6 days storage time (Table A4.22 and A4.23). After that, the remained reducing sugar content would be increased as higher concentration of nisin and lower pH value. In addition, storage time also influenced on reducing sugar content as the same trend in both two orange juice varieties. The reducing sugar content was reduced with longer storage time. Moreover, the nisin concentration and pH value also related to amount of reducing sugar during storage time. The data was shown, the higher concentration of nisin and lower pH value could be retained the reducing sugar content in orange juice for longer storage time. The reduction of reducing sugar content also corresponded with amount of total viable count. The reducing sugar content would be reduced as increasing of amount of total plate count. This refer to the microorganisms might be use the reducing sugar for their growth.

The addition of nisin and pH value adjustment were not influenced on ascorbic acid content in both varieties of fresh orange juice (Table A4.24 and A4.25). The ascorbic acid content also reduced as the longer storage time in both of orange juice varieties. However, the orange juice cv. Sai Nam Pung could be retained ascorbic acid content for longer storage time than the orange juice cv. Khieo Waan. It might be due to the orange juice cv. Sai Nam Pung were contained the original ascorbic acid content much more than the orange juice cv. Khieo Waan. Moreover, the influence of nisin content and pH value also related to the decomposed of ascorbic acid in both of orange juice varieties during storage. The data shown that the higher of nisin content and lower pH value could be retained the ascorbic acid concentration in the both of orange juice varieties for longer time. Therefore, the nisin content and low pH value could be delayed the decomposed of ascorbic acid in orange juice.

Table A4.26 and A4.297 showed the amount of total plate count in orange juice cv. Sai Nam Pung and Khieo Waan increased during storage time. This data related to the amount of total soluble solid (Table A4.20 and A4.21) and reducing sugar content (Table A4.22 and 4.23) were reduced during the storage time. It might be due to the microorganisms used the sugar for their growth. After that the concentration of nisin and pH value adjustment were influenced on amount of total viable count in orange juice cv. Sai Nam Pung as the same trend with cv. Khieo Waan during 3 to 42 days storage. The orange juice which added higher nisin concentration and lower pH value would have smaller amount of total viable count, led to extend shelf life of the orange juice. Because of revealed amount of total viable count was more than 5×10^4 CFU/ml led to spoilage product. It might be due to nisin and low pH value could be inhibited the growth of microorganisms (Li *et al.*, 1989). Moreover, the orange juice which added 50 IU or 100 IU of nisin and adjusted at pH value of 3.6 would have longest storage time at 24 days. That might be concluded 50 IU concentration of nisin was enough for extended shelf life of orange juice at pH value of 3.6.

The addition of nisin and pH value adjustment influenced on amount of yeast and mould in orange juice cv. Sai Nam Pung. The trend of the effect of nisin content and pH value on the amount of yeast and mould in orange juice cv. Sai Nam Pung and Khieo Waan were the same. The addition of nisin and reduction of pH value would be delayed the growth of yeast and mould (Table A4.28 and A4.29). Moreover, the lower pH values was affected to reduce the duration time for the growth of yeast and mould at the same level of nisin concentration. Therefore, both of the nisin addition and reduction of pH value also were inhibited the growth of yeast and mould.

Table A4.30 and 4.31 showed amount of lactic acid bacteria in orange juice cv. Sai Nam Pung and Khieo Waan. The influence of nisin concentration and pH value on the amount of lactic acid bacteria in orange juice cv. Sai Nam Pung and Khieo Waan were the same trend. The data displayed the lactic acid bacteria could not grow in both juice varieties which fortified by nisin and adjusted at several pH value. However, the orange juice which no added nisin but differed pH value were delayed the growth of lactic acid bacteria until 12 day storage. It might be due to the lactic acid bacteria could not competed other microorganisms, which showed higher amount during storage time.

Amount of spore in orange juice cv. Sai Nam Pung and Khieo Waan which supplemented with different concentration of nisin and varied pH value during storage time were shown in Table A4.32 and A4.33. It was found that the both of orange juice varieties were not have spore count during storage. It might be due to the low pH value was not suitable for the growth of spore, that showed clearly in the juice which no added nisin. Moreover, the combination of nisin and pH also inhibited the growth of spore.

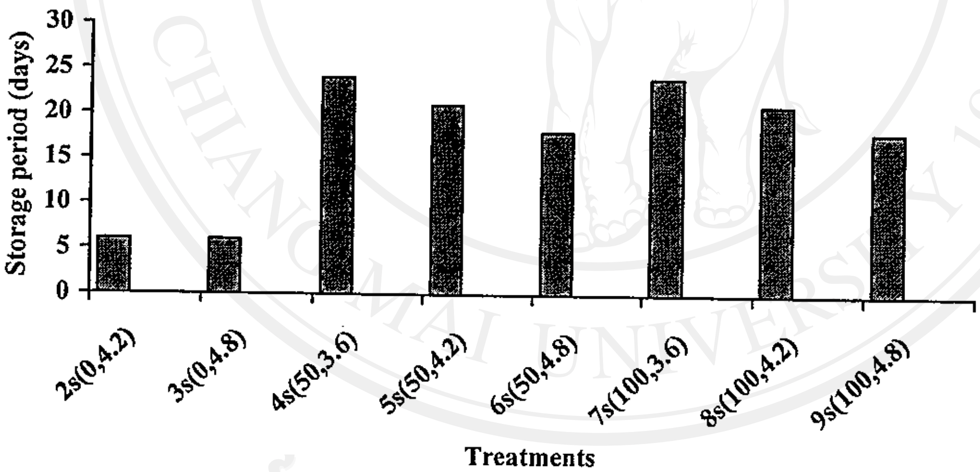


Figure 4.9 Combination of nisin and pH on effectiveness to extend shelf life of orange juice

Effect of nisin and pH on effectiveness to extend shelf life of orange juice cv. Sai Nam Pung and cv. Khieo Waan was studied. The values of pH were studied at 3.6, 4.2 and 4.8 and nisin concentration at 50 and 100 IU/ml compared with the control with out nisin added. All of samples were stored at 4°C during 0 to 42 days. The results showed that these differences in pH levels effected on the effectiveness of nisin. The level of pH at lower (pH 3.6), nisin has more effect than the higher level (pH 4.2 and 4.8) at the same level of nisin (50 and 100 IU/ml). When used the high level of nisin at 100 IU/ml results were similar like 50 IU/ml.

The criteria of orange juice with regards to total plate count regulated limit of $5x10^4$ CFU/ml. For 50 IU of nisin found that orange juice cv. Sai Nam Pung had slow growth of total plate count at pH 3.6, so can extend the shelf life to 24 days with the total plate count of to $2.2x10^3$ CFU/ml from initial at $3.3x10^2$ CFU/ml (Table A4.26). pH was one factor that could affect the action of nisin. Researchers had reported that nisin would be more effective at lower pH values (Delves-Broughton, 1990). The increase in effectiveness of nisin at lower pH values could be explained by intramolecular and intermolecular reactions between nucleophilic groups and the dehydro residues (Breukink and de Kruijff, 1999; Paul Ross *et al.*, 2002). Nisin is a cationic molecule due to a combination of three lysine and one or more histidine residues. It is more soluble at acid pH values and becomes less soluble when the pH value is increased. nisin was effective in a wide range of pH values at 3.5-8.0, although in its application, nisin was more often to be used in acidic foods.

Hodgins (2002) reported that using a hurdle approach of temperature, acidity, antimicrobial compounds (nisin and lysozyme) and number of pulses was also investigated to maximize a microbial killing. An optimal condition tested resulted in over 6 log reductions in the microbial population when applying 20 pulses at an electric field of 80 kV/cm, a pH solution of 3.5, a heating temperature at 44°C and an addition of 100 IU nisin/100 ml solution. The sensory analysis results of the juice after the hurdle treatment also showed favourable characteristics. A 97.5% retention of vitamin C was found in the treated juice. The juice shelf life based on the microbiological quality was improved and determined to be at least 28 days when stored at 4°C (Hodgins, 2002).