

## CHAPTER 3

### MATERIALS AND METHODS

The oranges used in this studied was *Citrus reticulata* Blanco. cv. Sai Nam Pung and cv. Khieo Waan harvested on the 8<sup>th</sup>-10<sup>th</sup> month after the fruit set. Orange juice prepared from fresh oranges cv. Sai Nam Pung. Samples were obtained from a private farm at Fang district, Chiang Mai province, Thailand during the 2004-2005 harvest seasons. Random samples of a uniform size and colour. Fresh oranges were hand-peeled and squeezed with stainless press for the juice. The orange juice was filtered to remove its pulp. Samples of filtered juices was placed in sterilized bottle and kept for the experiments in this study. The main topics for the experiments were four sections.

1. Physical, chemical, microbiological and sensory analysis of fresh orange juice.
2. Monitoring the shelf life of orange juice at different storage temperatures.
3. The effect of storage condition on carotenoids content in orange juice.
4. The combination effect of nisin and pH on the quality of orange juice.

#### **3.1 Physical, chemical, microbiological and sensory analysis of fresh orange juice**

Orange juice prepared from healthy fresh oranges cv. Sai Nam Pung and cv. Khieo Waan of a uniform size and colour. Fresh oranges were hand-peeled and squeezed with stainless press for the juice. The orange juice were filtered to remove its pulp. The juices prepared for experiment 1, 2 and 3. Samples of filtered juices was placed in sterilized bottle and kept for analyzed its Physical, chemical, microbiological and sensory subsequently.

## Physical analysis

### Colour determination

The orange juice colour was evaluated by colourimeter (Minolta CR-300, Japan) for the values of L\*, a\* and b\*.

L\* (L\* whiteness/darkness, ranged from 0 to 100)

a\* (a\* redness for positive value and greenness for the negative one)

b\* (b\* yellowness for positive and blueness for negative value)

All measurements were done in triplicate.

### Chemical analysis

**Total soluble solids** (AOAC, 1995) The total soluble solids of orange juice was read from a hand refractometer (Atago, Japan).

**Titrateable acidity** (AOAC, 1995) The titrateable acidity of orange juice was measured by diluting 10 ml of orange juice with distilled water and titrated against 0.1 N NaOH to a pH 8.1 as the end-point by using a pH meter. The titrateable acidity was expressed in g acid per 100 ml based on citric acids. All measurements were done in triplicate.

**pH value** (AOAC, 1995) The measurement of pH values of orange juice was done using a pH meter (Consort C830 CE, Belgium).

**Reducing sugar** (Pearson *et al*, 1976) Reducing sugar by a Lane and Eynon method.

An amount of 5 ml orange juice was transferred to a volumetric flask adjust to the 100 ml with pure water, add 5 ml of carrez solution no.1, which composed of zinc acetate dihydrate 21.9 g in acetic acid (glacial) 3 ml adjust to 100 ml with water and 5 ml of carrez solution no.2, that made of potassium-ferrocyanide 10.6 g adjust to 100 ml with water. Add the volumetric flask with carrez solution no.1 and 2 to make a mixed solution of 250 ml. Keep the mixed solution at room temperature for 20 min. After that, filter the mixed solution with a whatman filter paper no.4 and the supernatant of the solution was transferred into a burette. Prepare a fehling's solution no.1 which was made from dissolve 69.3 g copper sulphate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) in water and make up to 1 litre and Fehling's solution no.2 that was composed of dissolve 100

g sodium hydroxide and 345 g sodium potassium tartrate ( $\text{KNaC}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ ) in water and make up to 1 litre. Into a flask 250 ml, transfer 5 ml of Fehling's solution no.1 and 2 and add 8-10 glass beads. Add 3-4 drop of methylene blue indicator that was composed of dissolve 1g methylene blue in water and make up to 1 litre. Add methylene blue indicator 5 seconds before the completion of boiling period, then titrate until the final orange-red colour. The titration should be in the range 15-50 ml.

**Ascorbic acid** (AOAC, 1995) Ascorbic acid reduces oxidation-reduction indicator dye 2,6 dichloroindophenol, to colorless solution. At end point, excess unreduced dye was rose pink in acid solution. Vitamin was extracted and titration performed in presence of  $\text{HPO}_3$  - $\text{CH}_3\text{COOH}$  solution. Reagents that used were metaphosphoric acid-acetic acid solution, ascorbic acid standard solution and indophenol standard solution

-Metaphosphoric acid-acetic acid solution. Dissolve, with shaking, 15 g  $\text{HPO}_3$  pellets or freshly pulverized stick  $\text{HPO}_3$  in 40 ml  $\text{CH}_3\text{COOH}$  and 200 ml  $\text{H}_2\text{O}$ ; dilute to ca 500 ml, and filter rapidly through fluted paper into glass-stoppered bottle.

-Ascorbic acid standard solution 1 mg/ml. Accurately weight 50 mg ascorbic acid reference standard that has been stored in desiccator away from direct sunlight. Transfer to 50 ml volumetric flask. Dilute to volume immediately before use with  $\text{HPO}_3$ - $\text{CH}_3\text{COOH}$  solution.

-Indophenol standard solution. Dissolve 50 mg 2,6 dichloroindophenol Na salt, that has been stored in desiccator over soda lime, in 50 ml  $\text{H}_2\text{O}$  to which has been added 42 mg  $\text{NaHCO}_3$ ; shake vigorously, and when dye dissolves, dilute to 200 ml with  $\text{H}_2\text{O}$ . Filter through fluted paper into amber glass-stoppered bottle.

Transfer three 2.0 ml ascorbic acid standard solution to each of three 50 ml Erlenmeyers containing 5.0 ml  $\text{HPO}_3$ - $\text{CH}_3\text{COOH}$  solution. Titrate rapidly with indophenol solution from 50 ml burette until light but distinct rose pink persists  $\geq 5$  s. (Each titration should require 15 ml indophenol solution, and titration should check within 0.1 ml). Similarly titrated 3 blanks composed of 7.0 ml  $\text{HPO}_3$ - $\text{CH}_3\text{COOH}$  solution, plus volume  $\text{H}_2\text{O}$  equal to volume indophenol solution used in direct titration. After subtracting average blanks (usually ca 0.1 ml) from standardization titrations, calculate and express concentration of indophenol solution as mg ascorbic

acid equivalent to 1.0 ml reagent. Standardization indophenol solution daily with freshly prepared ascorbic acid standard solution.

Orange juice prepared by mix and filter. Pipette orange juice sample to each of three 50 ml Erlenmeyers containing 5.0 ml  $\text{HPO}_3\text{-CH}_3\text{COOH}$  solution. Titrate rapidly with indophenol solution from burette until light but distinct rose pink persists  $\geq 5$  s. Calculate ascorbic acid in mg/ml

### **Sensory evaluation**

Sensory evaluation was carried out by 9-points Hedonic scale: 1=extremely dislike, 2=dislike most, 3=dislike very much, 4=dislike, 5=neither dislike nor like, 6=like, 7= like very much, 8= like most, 9= extremely like. Panelists (undergraduate students) of the department of Food Science and Technology, Chiang Mai Rajabhat university participated in this study. The 40 panelists were served with 25 ml orange juice sample that are coded with 3-digit random numbers. The panelists described the sensory characteristics of the orange juice including colour, flavour and taste.

### **Microbial analysis**

Orange juice samples were tested for total plate count, yeast and mold, lactic acid bacteria and spore count. Total plate count was used orange serum agar (Linchong, 2003). The analysis of total plate count was done by preparing the medium as directed. Add 1 ml of an orange juice sample into a sterile petri dish. Add 20 ml of the medium at 50°C to the plate and mix the sample and the medium properly. After that the plate were incubated at 30°C and examine for 2 days. Report as a number of colony- forming units per ml of orange juice (Harrigan, 1998).

Yeast and mold were enumerated using potato dextrose agar (Harrigan, 1998).

Lactic acid bacteria were measured by Man Rogosa Sharpe (MRS agar) Spore count was measured using an orange serum agar (Harrigan, 1998).The number of spore in orange juice samples was measured by heating the samples at 80°C for 10 min. The samples were then cooled and pour plated. The plates were incubated in duplicate sets at 30°C and 55°C, for the mesophilic and the thermophilic spore counts, respectively.

### **Statistical analysis**

The data results were analyzed by analysis of variance using a Completed Randomized Design (CRD). If the F values from the analysis of variance was significant, then the Duncan's New Multiple Range Test (DMRT) used to determine differences between treatment means (Montgomery, 2001).

### **3.2 Monitoring the shelf life of orange juice at different storage temperatures.**

Fresh orange juice prepared according to the condition in the experiment number 1 and stored at 3 different storage temperatures 4°C, -18°C and room temperature (30°C). The orange juice were analyzed on the 0, 3, 6, 9, 12, 15 days for the juices that stored at 4°C and -18°C and on the 0, 1, 2, 3, 4, 5, 6, 7 days for the juices that stored at room temperature.

Physical, chemical, microbiological and sensory evaluation were conducted with the same methods as in section 1 experiment.

### **3.3 The effect of storage condition on carotenoids content in orange juice.**

Orange juice prepared from fresh oranges cv. Sai Nam Pung and cv. Khieo Waan. Fresh orange juice are prepared according to the condition in the experiment number 1 and stored at 3 different storage temperatures 4, -18°C and at room temperature (30°C). After that, the orange juice was analyzed for total carotenoid contents according to the method described by Pearson *et al.* (1976). The total carotenoid content in orange juice were determined by Spectrophotometer. Samples of orange juice ( $10 \pm 0.1\text{g}$ ) were extracted with diethyl ether in a separating funnel. The anhydrous sodium sulphate was added, the diethyl ether extract were evaporated to a volumn of 25 ml then transferred to a small beaker. The petroleum ether was

added, the sample were kept and then filtered. The solution was determined the optical density at 450 nm. Total carotenoid content ( $\mu\text{g/g}$ ) was calculated as

$$\text{total carotenoid content} = A \times 4.17 / W$$

Where, A= value of absorbance at 450 nm

W= g sample/ml of solution.

The  $\beta$ -carotene in orange juice was determined by HPLC (Method of food analysis, 2002) with an HPLC Series 1100 Agilent. Samples of orange juice ( $10 \pm 0.05\text{g}$ ) were extracted with petroleum ether 15 ml, vortex for 2 min. The solution was well mixed and further centrifuge at 2,000 rpm/min for 15 min. The clarified juice was transferred in round bottom flask and extract for 3 times. The petroleum ether was evaporated to dryness in a vacuum rotary evaporator at  $40^\circ\text{C}$ . The extract in round bottom flask was collected. The isopropanol (HPLC grade) was added and the volume was made up to 25 ml. Afterwards, the extract was passed through the syringe filter 13 mm, 0.45 micron, nylon membrane attached to its outlet. An aliquot of filtrate was injected into the HPLC equipment. The mobile phase was acetonitrile: methanol at a ratio of 70:30. The column was a C18 Hypersil ODS (4x125 mm, 5  $\mu\text{m}$ ), flow rate of 1.6 ml/min, temperature of  $40^\circ\text{C}$ , Diode Array detector at wavelength of 450 nm.

### 3.4 The combination effect of nisin and pH on the quality of orange juice

Effect of nisin and pH on effectiveness to extend shelf life of orange juice Sai Nam Pung and cv. Khieo Waan. The value 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  $4^\circ\text{C}$  during 0 to 42 days.

Orange juice prepared from fresh oranges cv. Sai Nam Pung and cv. Khieo Waan. Fresh orange juice was adjusted pH 3 levels: 3.6, 4.2 and 4.8 with citric acid and then added nisin 3 levels: 0, 50 and 100 IU/ml after that stored at  $4^\circ\text{C}$ . The orange juice was evaluated the spoilage parameters every 3 other days for 42 days or until spoilage. Physical, chemical, microbiological and sensory evaluation conducted with the same methods as in section 1 experiment.