Chapter 3

Reserch designs, scope and methods

3.1 Equipment

- A colorimeter (Chrometer, Minolta CR-300, Japan).
- A viscometer (Brookfield Programmable DV *– II + VISCOMETER, England).
- A separatory funnel (Pyrex, Germany).
- A muffle furnace (Gallenkamp, Canada).
- Burettes (Diffico, Germany).
- A Kjeldahl Digester DK 6 Model (Velp Scientifica, Italy).
- A Kjeldahl Distillation (Foss Tecator, U.S.A).
- A hot air oven (National, Taiwan).
- A pH meter (Precisa, Switzerland).
- An analytical balance (Precisa 610C Model, Switzerland).
- An anaiytical balance (Sartorius A120S Model, Germany).
- An autoclave (Gallenkamp, Canada).
- An incubator (Gallenkamp, Canada).
- Measurement pipettes (1, 5 and 10 ml) (HBG, Germany).
- Filter papers no. 2 and 4 (Whatman, England).
- Test tubes (10ml) (Pyrex, U.S.A).
- A water bath (Gallenkamp, Canada).
- A spectrophotometer (ThermoSpectronic, Biomate 5, U.S.A).
- A hand refractometer (ATAGO, Model N1, Japan).
- A hot plate and magnetic stirrer (Whatman model HPMS, England).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright © by Chiang Mai University
All rights reserved

- A GC-MS (GC 6890 Agilent Technologies and Hewlett Packard Model 5973 (EI) mass spectrometry).

3.2 Solvents and Chemicals

- Phenolpthalein; $C_{20}H_{14}O_4$ (Fluka, Germany).
- Sodium hydroxide; NaOH (Merck, Germany).
- Potassium hydrogen phthalate; $KHC_8H_4O_4$ (Univar, New Zealand).
- Ammonium hydroxide; NH_4OH (Merck, Germany).
- Hydrochloric acid; HCL (BDH, England).
- Ethyl alcohol; C_2H_5OH (Merck, Germany).
- Ammonium sulphate; $(NH_4)_2SO_4$ (Univar, New Zealand).
- Chloroform; $CHCl_3$ (Lab-Scan Ltd., Ireland).
- Diethyl ether; $(C_2H_2)O_2$ (Lab-Scan Ltd., Ireland).
- Petroleum ether; $(C_2H_5)O_{20}$ (J.T. Baker, U.S.A).
- Standard β – carotene (Sigma-Aldrich, U.S.A).
- Hexane (Fisher Scientific, U.S.A).
- Acetone (J.T. Baker, U.S.A).
- Sulfuric acid; H_2SO_4 (Merck, Germany).
- Sodium sulfate; Na_2SO_2 (Fisher Scientific, U.S.A).
- Copper sulfate; $CuSO_4.5H_2O$ (Carlo Erba Regenti, Italy).
- Zinc acetate dehydrate (J.T. Baker, U.S.A).
- Acetic acid glacial (Merck, Germany).
- Potassium ferrocyanide (Asia Pacific Specialty, Australia).
- Potassium sodium tartrate (J.T. Baker, U.S.A).
- Citrus pectin (Sigma-Aldrich, U.S.A).
- Methyl red; $(CH_3)_2NC_6H_4N$ (Unilab, Australia).
- Boric acid; H_3BO_3 (Univar, New Zealand).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

- Sodium sulfide; Na_2SO_3 (May and Baker Ltd., England).
- Methylene blue (Merck, Germany).
- Tartaric acid (Carlo Erba Reagent, Germany).
- Orange Serum Agar (Himedia M 933, India).
- Potato Dextrose Agar (Lab-Scan Asia Co. Ltd, Ireland).
- MRS Agar; Lactobacillus MRS Agar M 640 (Himedia, India).
- Carrez I was composed of zinc acetate dehydrate 21.9 g and adjust to 100 ml with distilled water.
- Carrez II was composed of potassium ferrocyanide 10.6 g and adjust to 100 ml with distilled water.
- Fehling solutiom no. 1 was made from copper sulfate 69.278 g and adjust to 1 l with distilled water.
- Fehling solution no. 2 was made from sodium hydroxide 100 g, sodium potassiumtartrate 346 g and adjust to 1 l with distilled water.

3.3 Material

- Orange varieties used in this study were Keaw Wan Prae and Sai Namphung. Both of orange fruits were bought at a local market in Chiang Mai.

- Orange juice sac from commercial orange juice.

3.4 Analysis

3.4.1 Physical analysis

1. Color was measured by a colorimeter.
2. Cloudiness of samples was determined by a spectrophotometer.
3. Viscosity analysis was carried out using a viscometer.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

3.4.2 Chemical analysis

1. pH value of samples was measured directly using a pH meter.

2. Total soluble solids content was determined by a hand refractometer with a measurement range between 0-32%.

3. Total titrable acidity was assessed by a titration method with sodium hydroxide (AOAC, 2000) and expressed as % citric acid.

4. Moisture content and total solid were determined by AOAC methods (AOAC, 2000) specified for an oven-dry method.

5. Reducing and total sugar contents were determined by a Lane and Eynon method (AOAC, 2000).

6. Protein content was determined by a Kjeldahl method (AOAC, 2000).

7. Fat content was assessed by a Rose-Gottlieb method (AOAC, 2000).

8. Ash content was determined by an AOAC method (AOAC, 2000).

9. Fiber content was determined by digestion of acid and alkaline methods (AOAC, 2000).

10. Carbohydrate content was determined by an AOAC method (AOAC, 2000).

11. Pectin esterase (PE) enzyme content was determined by a titration method (Bull *et al.*, 2004).

12. Flavor was determined by a GC-MS.

3.4.3 Microbial analysis

1. Total plate counts were enumerated using pour plate method on Orange Serum Agar (Jia *et al.*, 1999).

2. Total yeast and moulds were enumerated on Potato Dextrose Agar (Jia *et al.*, 1999).

3. Lactic acid bacteria were enumerated on MRS Agar (de Man *et al.*, 1960).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

3.4.4 Nutritional analysis

1. Vitamin C content was determined as ascorbic acid by a titrimetric method using 2, 6 – dichloroindophenol (AOAC, 2000).

2. Carotenoid content was measured using a spectrophotometer.

3.4.5 Statistical analysis

A factorial in Completely Randomized Design (CRD) was used to evaluate the effect of different treatments on the dependent variables (for experiments in the sections 3.5.2 and 3.5.3) using SPSS 11.0 for Windows (SPSS Inc., U.S.A.). Statistical significance was determined at $P < 0.05$. Duncan comparisons were used to analyze the differences between means. For the experiment of different orange varieties (section 3.5.1) and the effect of storage periods (section 3.5.2 and 3.5.3), a statistical design of CRD was applied.

3.5 Methods

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

In this section, two orange varieties of Sai Namphung and Keaw Wan Prae were used and their orange juices were compared based on physical, chemical, nutritional and microbiological characteristics.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Preparation of juice samples

Samples of two orange varieties were purchased from Warorot market, Chiang Mai. Oranges were processed within 2 days after purchased. After appropriate cleaning and washing in warm water (at 80^0C) for 2 min, the orange juice was extracted by a juice extractor (Suklampoo, 2003). The extracted orange juice was passed a clean cloth

to remove any solid residues, filled into 500 ml polyethylene terephthalate (PET) bottles and kept immediately in a refrigerator at chilled temperature.

Physical analysis: the samples of orange juice were analyzed for color, cloudiness and viscosity characteristics. Yield of oranges juices were also calculated. Each test was performed in triplicate.

Chemical analysis: the samples of orange juice were measured for their pH values, total soluble solids contents, total titrable acidities, moisture contents, total solids, protein contents, fat contents, ash contents, carbohydrate contents, reducing sugars, total sugars, PE enzyme activities, fiber contents and flavors. Each test, except for flavor analysis, was performed in triplicate.

Microbiology analysis: the samples of orange juice were determined for their microbial qualities based on Total Plate Count, yeast and mold count and lactic acid bacteria. Each test was performed in duplicate and results were expressed as colony-forming units (CFU) per milliliter.

Nutritional analysis: the samples of orange juice were determined for their vitamin C and carotenoid contents. Each test was performed in triplicate.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

3.5.2 Study the effects of sugar and salt additions on the quality of fresh orange juice during storage at chilled temperature

One orange variety from the section 3.5.1 was used in this section. Orange fruit was purchased from Warorot market, Chiang Mai. The fruit was processed within 2 days after purchasing according to the procedure in the section 3.5.1. After removing

any solid residues, the juice was divided into 9 batches. Into each batch of orange juice, salt and sugar were added following the salt and sugar concentrations in Table 3.1. After the salt and sugar additions, the orange juice samples were kept in a refrigerator at chilled temperature for 15 days. During storage time, orange juice samples were analysed for their physical, chemical, nutritional and microbial changes.

Physical analysis: orange juice samples were analyzed for color, cloudiness and viscosity properties. The color and cloudiness testing were carried out every 3 days during the storage of orange juice, while the viscosity property was determined on 0, 7 and 15 days of storage. Each test was performed in triplicate.

Table 3.1 Different salt and sugar concentrations for orange juice treatments

Salt concentration (%) ($^{w}/_{v}$)	**Sugar concentration (%) ($^{w}/_{v}$)**		
	0	**2.5**	**5**
0	1 (control)	4	7
0.05	2	5	8
0.1	3	6	9

Chemical analysis: samples of orange juice were determined for pH values, total soluble solids contents, total titrable acidities, moisture contents, total solids, reducing sugars and PE enzyme activities. Each test was performed in triplicate and was conducted every 3 days during 15 days storage of orange juice.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Microbiological analysis: samples of orange juice were measured for Total Plate Count, yeast and mold and lactic acid bacteria counts. Each test was performed in duplicate and results were expressed as colony-forming units (CFU) per milliliter (ml).

The analyses of Total Plate Count and lactic acid bacteria were conducted every 3 days for 15 days storage of orange juice, while the enumeration of yeast and mold was carried out only on 0, 7 and 15 days of storage.

Nutritional analysis: samples of orange juice were measured for their vitamin C and carotenoid contents. The vitamin C content was done every 3 days for 15 days storage of orange juice, while the carotenoid content was determined on 0, 7 and 15 days of storage. Each test was performed in triplicate.

3.5.3 Study the effects of orange juice sacs and pH values on the quality of fresh orange juice during storage at chilled temperature

One orange juice variety from the section 3.5.1 and one combination concentration of salt and sugar from the section 3.5.2 were applied in this section. Orange fruit was purchased from Warorot market, Chiang Mai. The fruit was processed within 2 days after purchasing according to the procedure in the section 3.5.1. After a filtration step to remove any solid residues, the juice was divided into 6 batches. Each batch of orange juice received different treatments of orange juice sac addition and/or pH alteration (Table 3.2). There were 3 pH values of 3, 3.5 and 4 that were studied in this section (the pH value of the control orange juice was 3 at 0 day storage). The pH alteration was done by adding 0.5 N NaOH. For the orange juice sac factor, 2 levels of no orange juice sac and 3% ($^{w}/_{v}$) orange juice sac were studied. After adjusting different treatments for each batch of orange juice, all the orange juice samples were stored in a refrigerator at chilled temperature for 15 days. During the storage period, orange juice samples were analyzed for their physical, chemical, nutritional and microbial analyses.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Physical analysis: orange juice samples were analyzed for color, cloudiness and viscosity properties. The color and cloudiness testing were carried out every 3 days during the storage of orange juice, while the viscosity property was determined on 0, 7 and 15 days of storage. Each test was performed in triplicate.

Table 3.2 Different pH values and orange juice sac addition for fresh orange juice treatments

Orange juice sac (%) ($^{w}/_{v}$)	**pH value**		
	3.0	**3.5**	**4.0**
0	1 (control)	2	3
3	4	5	6

Chemical analysis: samples of orange juice were determined for pH values, total soluble solids contents, total titrable acidities, moisture contents, total solids, reducing sugars, PE enzyme activities and fiber contents. All the analysis, except for the fiber content, were done every 3 days during 15 days storage of orange juice. The fiber content was analyzed on 0, 7 and 15 days of storage. Each test was performed in triplicate.

Microbiological analysis: samples of orange juice were measured for Total Plate Count, yeast and mold and lactic acid bacteria counts. Each test was performed in duplicate and results were expressed as colony-forming units (CFU) per milliliter (ml). The analyses of Total Plate Count and lactic acid bacteria were conducted every 3 days for 15 days storage of orange juice, while the enumeration of yeast and mold was carried out only on 0, 7 and 15 days of storage.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Nutritional analysis: samples of orange juice were measured for their vitamin C and carotenoid contents. The vitamin C content was done every 3 days for 15 days storage of orange juice, while the carotenoid content was determined on 0, 7 and 15 days of storage. Each test was performed in triplicate.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved