# CHAPTER 3

# Materials and Methods

## 3.1 Materials

1) soy sauce odor liquid (food grade, Fatisco Co., Ltd., Thailand)
2) soy sauce odor powder (food grade, A2B Food, Thailand)
3) sodium chloride (food grade, Union Science Co. Ltd., Thailand)
4) sodium chloride (food grade, Thai Refined Salt Co. Ltd., Thailand)
5) peanut (Tawan produce Co. Ltd., Thailand)
6) roasted peanut (Tong Garden Co. Ltd., Thailand)
7) methocel (food grade, Vicchi Enterprise Co., Ltd., Thailand)
8) distilled Water (Chiang Mai Polestar (1992) Co., Ltd., Thailand)
9) sugar (Mitr Phol Sugar Co. Ltd., Thailand)
10) citric acid (food grade, Union Science Co. Ltd., Thailand)
11) caffeine (Sigma-aldrich, Inc., Germany)
12) artificial flavors (Greathill Co Ltd., Thailand) including banana, orange, grape, peach, fish sauce and soy sauce
13) reduced sodium products (Ampol Food Processing Ltd., Thailand) including salt, soy sauce and tomato sauce
14) absolute ethanol (AR grade, MERCK, Germany)
15) nitric acid 65% (AR grade, RCI Labscan Limited, Thailand)
16) hydrochloric acid 37% (AR grade, RCI Labscan Limited, Thailand)
17) perchloric acid 70% (Rankem, India)

### 3.1.1 Instruments

1) hot air oven (FD115, Serial No. 08-36764, Binder, Germany)
2) hot plate (IKA®C- MAGHS7, USA)
3) spray dryer )JMC Group Engineering Concept., Ltd., Thailand(
4) atomic absorption spectrometer, ASS (Avanta M1, USA)
5) micropipet 10 – 100 and 100 – 1,000 microlitre (Nichiryo, Japan)
6) particle size analyzer laser, Mastersizer ® S (Malvern Instrument Ltd., U.K.)
7) hood (Toplab design and technology, Sartorius, A120S, Germany)
8) weighing scale (A&D, Model HR-2000i/ FX-2000i, Japan)
9) water activity analyzer, aquaLab LITE (DECAGON Devices Inc., USA)
10) color analyzer (Konica Minolta: CR-400 series, Japan)
11) X-ray diffractometer (Model Xpert MPD, Philips, Netherlands)

### 3.1.2 Apparatus

1) Kitchenware
    1.1) blender (HR2001, Phillips, Thailand)
    1.2) deep fryer (FR 1265, Fritel, Belgium)
2) Glassware
    2.1) beaker (Pyrex, England)
    2.2) erlenmeyer flask (Pyrex, England)
    2.3) volumetric flask (Pyrex, England)
    2.4) measure cylinder (Pyrex, England)
    2.5) brown glass bottle
3) Miscellaneous
    3.1) moisture can
    3.2) spatulas
    3.3) forceps
    3.4) foil package (Chiang mai Plastic, Thailand)
    3.5) zip-lock package (Siam pack, Thailand)
    3.6) thermometer

3.7) 3 oz plastic cup with lid

3.8) cotton ball (Evergreen, Thailand)

3.9) sticker

3.10) nose clip (Spirometics, US)

### 3.1.3 Software use for data analysis

1) Minitab version 16 (Minitab Inc., USA)
2) SPSS version 11.0 (SPSS Inc., USA)

## 3.2 Methods

### 3.2.1 Phase 1: Identification of enhanced saltiness perception odors

To identify odors that enhanced saltiness perception, two steps were conducted as follows:

**1) Gathering information of food and food ingredients related to salty perception**

Food and food ingredients related to salty perception used in this study were come from literature reviews (e.g., anchovy, bacon, carrot, soy sauce and tuna) and focus group interviewing.

Six focus groups with a total of 48 participants were used to identify odor/food items associated with saltiness and to further develop concepts of reduced sodium products. Each focus group consisted of 8 participants who were between 18-65 years of age. Of these 6 groups, three groups were those who did not prefer salty taste/foods, while the other three groups were those who preferred salty taste/foods. In each focus group session, participants seated comfortably in a conference-style meeting room, while observer seated behind the participants, taking note during the session. Each participant received necessary stationary items.

For each session, the experienced moderator started with self-introduction and brief orientation, and stated the ground rule of a focus group session, i.e., participants should respect others' opinion and only one person should speak at a time. The three main topics of discussion included

attitude toward reduced sodium salt, development of reduced sodium salts using OISE ingredients, and some critical factors affecting such development. To stimulate conversation, participants were given samples of reduced sodium salt available in the market, a menu of food items, prices, and locations where these products were available. Each focus group session lasted averagely for ninety minutes. All participants received non-monetary souvenirs for their participation. The protocol of the focus group interview session is shown in Table 3.1.

**Table 3.1** A protocol for a focus group interview session

| Activities/content | Time (mins( |
|---|---|
| 1. Self-introduction and brief orientation | 10 |
| 2. Attitude about reduced sodium salts in food products | |
| ▪ Do you prefer salty food? | 5 |
| ▪ Where does salty taste in food products come from? | |
| ▪ What is sodium? | 5 |
| ▪ What is the importance of salt/sodium reduction in food products? | 5 |
| ▪ How would you reduce salt/sodium in food products/in your diet? | 5 |
| 3. Explanation of sodium reduction in food products. Information was given by visual aids. | 5 |
| 4. Development of reduced sodium salt using OISE. Information was given by visual aids. Examples of reduced sodium products were given. | |
| ▪ Introduction of reduced sodium salt | 5 |
| ▪ What is your opinion about reduced sodium salt? | 5 |
| ▪ List the type of odors, ingredients and food items that are associated with salty taste and flavor? Some explanations were given without introducing bias. | 20 |
| 5. Factors affecting development of reduced sodium salts by OISE | |
| ▪ List desirable attributes and package of reduced sodium salt with OISE | 10 |
| ▪ How much are you willing to pay for this product? | 5 |
| ▪ Where would you purchase this salt and what type of promotion of this salt would you like to see? | 5 |
| 6. Thanks and closing remarks | 2 |

**Data collection and interpretation**

All six focus group sessions were recorded by an audio tape recorder and a video recorder. After all six sessions had been completed, all recorders were transcribed and analyzed for each topic. Data collected by the observers were combined with those from recorders. All written comments of participants were also incorporated.

**2) Selection of odor on saltiness enhancement by salty intensity rating**

Four-hundred ten consumers were participated in this study. These subjects were recruited on the basis of interesting and availability. They were not be informed any information about the aim of the study. The sample was divided in three subgroups by age: respondent from 18-30 years old, 31-45 years old and those from 46-65 years old.

The research data were collected through self–administrated questionnaire (Appendix C). The questionnaires were divided into two parts: demographic part and salty intensity rating part. Demographical data were collected including gender, age and occupation. In salty intensity rating section, food and food ingredient names were used in this experiment based on the results of previous experiment 3.2.1(1) (e.g., anchovy, bacon, carrot, soy sauce and tuna). Only, food items available in the market were selected in this study. For fifty seven food odor names, panelists were asked to estimate salty intensity from 0-9. The scales were anchored as "0=unknow, 1=no salty, 9=extremely salty". The food name or food ingredients that received the top ten highest rating scores were selected for the further experiment.



The survey was performed during January-May, 2012 and the research area was the provincial center of Chiang Mai, Thailand such as Chiang Mai University, Faculty of Agro-industry and Public Park. The participants were selected through non probability sampling (purposive sampling). Questionnaires were self-administered at these places. The participants took 15–20 min to answer all questions in questionnaire.

**Statistical data analysis**

All statistical manipulations were performed using SPSS program. The salty intensity differences of each food items in the questionnaire study were analyzed using ANOVAs. All effects that have a *P*-value of 0.05 or lower were reported as 'significant'. Post hoc contrasts were used to compare food items using the Tukey HSD. The chi-squared test was used to determine if there were an association between the demographic characteristics and the salty intensity of the odor items.

### 3.2.2 Phase 2: Investigation of odor induced saltiness enhancement in solution model

**1) Odor tasteless testing**

Soy sauce odor was selected among top five food names from the previous experiment that it was used for verification of tasteless odors. Triangle test was performed in this experiment to check whether odor solution was tasteless.

All panelists comprised of students in Faculty of Agro-Industry, Chiang Mai University, all ranging in age between 20 and 40 years. They were recruited following Appendix C. All panelists were experienced with sensory test (especially discriminant test). Before testing, there was a short talk presenting the procedure to the panelists. No information was given about real objective of this study. The panelists were also introduced to the sensory ballot of triangle method. General questions about the experiment, how to use clip nose and how to assign the answer of each method were described to all panelists.

**Samples preparation**

The food-grade soy sauce odor was purchased from Fatisco Co., Ltd., Thailand. This soy sauce odor was prepared in solution (at 40,000 ppb without salt in distilled water). Thirty milliliters of the samples were

presented in 1-oz white plastic cups coded with 3-digit numbers at room temperature (25 ± 1 °C).

**Procedure for triangle test**

This triangle tests was carried out in the Sensory Laboratory at the faculty of Agro-Industry Chiang Mai University. This test was held under red light to eliminate effect of the odor color on the panelist evaluation. The three solution samples were served to the panelists, two samples were similar and the other was different. Thirty panelists were instructed to evaluate each odor solution (A) and water (W) by using nose-clip in the order they were given and to examine the odd sample. The order in which the samples were presented was randomized (AAW, AWA, WAA, WWA, WAW, or AWW). Between each sample, the panelists were to cleanse their palate with water. Two triangle test sessions were held on separate days and each of the two triangle test sessions was replicated.

**Data Analysis**

The numbers of correct responses were found out, and considered significantly different if they differed at a $P < 0.05$ level of significance (Meilgaard et al., 2007). The odor that no significant difference from water was used in this studied.



**2) Determination of odor concentration threshold and odor induced saltiness enhancement in solution model**

In this experiment, investigation of odor induced saltiness enhancement in solution model by trained panelists and consumers was performed. ASTM 679-04 (2011) was used to determine of odor concentration threshold and odor induced saltiness enhancement in solution model.

A pool of students and staff from the Product Development Technology Division, Faculty of Agro-Industry, Chiang Mai University, Thailand, who had previous experience in the sensory testing were screened

for this study. Screening criteria were (1) panelists were able to perceive smell or taste, (2) panelists were able to correctly identify the four basic tastes (sweet, salty, sour and bitter), (3) panelists were able to correctly identify or describe some common odors (banana, orange, grape, peach, fish sauce and soy sauce), (4) panelists were able to correctly rank the increasing order of taste (sweet, salty, sour, bitterness) solutions at different concentration levels (one reverse pair for the adjacent samples was allowed), and (5) panelists were available to participate. Ten panelists were selected (seven females and three males; 20-30 years of age). The meeting session was taken place at sensory evaluation room and taken about two hours per session (ten sessions). In each session the panelists was calibrated with reference salt solution. The details for recruit, screening and training methodologies were described in Appendix C.

**Samples preparation**

Soy sauce odor (Fatisco Co., Ltd., Thailand) was mixed with distilled water and/or 0.02 M salt solutions (NaCl, Union Science Co., Ltd., Thailand) to obtain seven sets of solutions with different concentration levels, ranging from 0.4-400,000 ppb for distilled water and 32-500,000 ppb for 0.02M NaCl solutions (Figure 3.1). These concentration ranges were based on preliminary studies. All solutions were freshly prepared, and 20 ml of each sample was poured into a two-ounce plastic cup coded with 3-digit random numbers, and kept at 25 °C prior to threshold analysis.



**Procedure for sensory threshold analysis**

The absolute and recognition thresholds of soy sauce odor in water solutions were determined by using the ascending forced choice method of limits (ASTM E679-04). The three alternative forced choice (3-AFC) test was used. Water solutions with different concentration levels of soy sauce odor were presented one set at a time in the order of increasing concentration (from 0.4 to 400,000 ppb in 10-fold increments). The panelists evaluated one set of three sample solutions at a time. For each set, two samples were control (distilled water) and one was distilled water with

one concentration level of the soy sauce odor (Figure 3.1). The panelists first selected the odd sample (absolute threshold) and further identified specific tastes of the odd sample that exhibited recognisable difference (recognition threshold). The choices of recognisable tastes included four basic tastes: sweet, salty, sour, bitter, and "not sure" in case the panelists were unsure of their selection.

In the study of the salty taste perception level of 0.02 M salt (NaCl) solutions containing soy sauce odor, the ascending forced choice method of limits (ASTM E679-04) was used to measure the difference threshold. The three alternative forced choice (3-AFC) test was used. Salt solutions (0.02 M of NaCl) with different concentration levels of soy sauce odor were presented one set at a time in the order of increasing concentration (from 32 to 500,000 ppb in 5-fold increments). The panelists evaluated one set of three sample solutions at a time. For each set, two were control (0.02 M salt solution) and one was the 0.02 M salt solution with one concentration level of the soy sauce odor (Figure 3.1). The panelists must choose the sample which was saltier than the other two samples.

All threshold determinations were independently carried out in triplicate in partitioned booths in the sensory analysis laboratory with controlled temperature (23-25°C) and adequate lighting. The nose clips were not used to allow the influence of soy sauce odor on salty taste perception. Between each sample, panelists cleansed their mouth and palate with distilled water and unsalted crackers. Each panelist evaluated 3-4 sets per day and completed their evaluation in 4 days. Panelists were advised not to drink nor eat one hour prior to the test.

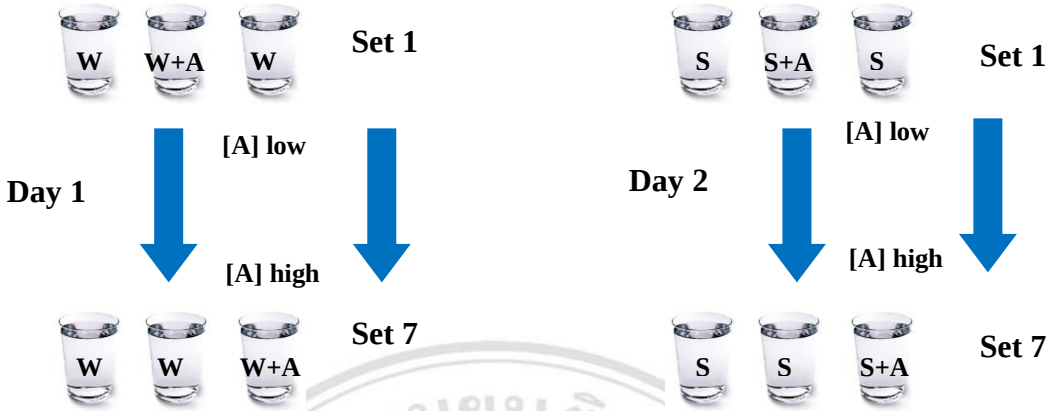


**Figure 3.1** Determination of odor concentration threshold

**Data Analysis**

Absolute threshold was determined when panelists were able to correctly identify the different sample (i.e., the water sample with the soy sauce odor), while saltiness recognition threshold was determined when panelists were able to recognise salty taste of the odd sample that exhibited recognisable difference. A series of each panelist judgments was tabulated with a sequence containing "0" for an incorrect choice or "+" for a correct choice, which was arranged in the order of judgments of ascending concentrations of soy sauce odor. As the distribution is typically skewed, a geometric mean rather than an arithmetic mean was used to measure the center location of the distribution (ASTM E679-04). Therefore, the best-estimate threshold (BET) concentration of soy sauce odor for the absolute threshold was the geometric mean of the last missed (0) concentration and the next (adjacent) higher concentration (+). The BET concentration for the saltiness recognition threshold was the geometric mean of the two lowest concentrations at which correct responses occurred and a recognisable taste was identified as "salty." (See Table 4.7 for sample calculation). The group best-estimate threshold (GBET) was obtained by the arithmetic average of summation of the logarithm with base 10 (log10) of the individual BET

values. The standard deviation log10 provided a measure of the group variation. The arithmetic average of GBETs of three independent replicates was reported for the absolute and saltiness recognition thresholds in ppb of soy sauce odor concentration. The percentage of panelists recognising specific tastes of the odd sample that exhibited recognisable difference was calculated for each soy sauce odor concentration.

The saltiness difference threshold was determined when panelists were able to choose the sample which was saltier than the other two samples. The BET concentration for the saltiness difference threshold was the geometric mean of the two lowest concentrations at which correct responses occurred. The GBETs of three independent replicates were calculated as above. For all threshold values, the relative standard deviation (%RSD = [mean/standard deviation] x 100) was also reported.

### 3.2.3 Phase 3: Study of using odor-induced saltiness enhancement with modified salt particle and its utilization in roasted peanut

This study was set to investigate OISE from experiment 3.2.2 comparing with using a modified salt particle by various processing, 3 steps were conducted as follows:

**1) Suitable of odor concentration for modified salt particle**

The three concentrations of soy sauce odor powder from A2B Food, Thailand (35, 50 and 70 %) were used in this experiment with sodium chloride (Union Science Co. Ltd., Thailand). Completely Randomized Design (CRD) with 3 replications was applied in this experiment. All treatments were prepared at the concentration of 0.90 %w/w of roasted peanut (without salt). Two-hundred consumers were taken to evaluate in overall liking, and saltiness attributes with 9-point hedonic scale where 1 = dislike very much, 5 = neither like nor dislike and 9 = like extremely (Peryam and Pilgrim, 1957) and the trained panelists was be involved in this experiment to evaluate the salty intensity (Lawless and Heymann, 2010).

The details for recruit, screening and training methodologies were described in Appendix C. Moreover, sodium content was determined each sample by Atomic absorption spectrometry (Appendix B).

**2) Modification salt particle using different processes with odor-induced saltiness enhancement**

The effects of using modified salt particle on salty perception were investigated. Completely Randomized Design (CRD) was conducted in this experiment. One commercial salt (Union Science Co. Ltd., Thailand), two different salt particles from spray drying and foam-mat drying were involved in this study. For spray drying, 30% commercial salt (Union Science Co. Ltd., Thailand) in water solution was dried using a spray dryer (speed rate 5 L/h, nozzle diameter 0.5 mm, inlet air temperature 180ºC and outlet air temperature 90ºC). For foam-mat drying, 30% commercial salt (Union Science Co. Ltd., Thailand) with 4% methocel in water solution was dried at a temperature of 60˚C using a hot air oven (Seakow et al., 2012). All samples were taken to evaluate in physical, chemical and sensory properties.

**Physical and chemical properties of modified salt particle**

All salt samples were taken to evaluate the physical properties as follows (see Appendix C):

- Color CIE )L* a* and b*) and water activity )$a_w$) (Vongsawasdi et al., 2002)
- Bulk density (Vongsawasdi *et al.*, 2002) and yield (%) (Vongsawasdi et al., 2002)
- Crystallite determination was studied using an X-Ray Diffraction.
- Average particle size and morphology was studied using Scanning Electron Microscopy (SEM) (Sa-Uram, 2004(.
- Moisture content (Thai Industrial Standard institute, 2001)

**Sensory evaluation**

Two sensory evaluation methods were conducted. One was rating method by trained panelist and the other was consumer acceptance testing. Roasted peanut (no salt) was used in this experiment. Panelists (n=10) were trained and two practice sessions were conducted. The details for recruit, screening and training methodologies were described in Appendix C. Evaluate salty taste by rating intensity method. Two-hundred consumers were taken to evaluate in overall liking, and saltiness attributes with 9-point hedonic scale where 1 = dislike very much, 5 = neither like nor dislike and 9 = like extremely ) Peryam and Pilgrim, 1957( . Sodium content was determined each sample by Atomic absorption spectrometry

**3) Comparison the effect of using spray dried salt and odor-induced saltiness enhancement**

The effects of using spray dried salt and OISE was conducted. The treatments were arranged in a completely randomized 2x2 factorial design. All treatments in this study show in Table 3.2. All samples were taken to evaluate sensory properties. Roasted peanut (no salt) was used in this experiment. Panelists (n=10) were trained and two practice sessions were conducted. The details for recruit, screening and training methodologies were described in Appendix C. Evaluate salty taste by rating intensity method. Two-hundred consumers were taken to evaluate in overall liking, and saltiness attributes with 9-point hedonic scale where 1 = dislike very much, 5 = neither like nor dislike and 9 = like extremely ) Peryam and Pilgrim, 1957(.

**Table 3.2** Experiment condition in a completely randomized 2x2 factorial design for study the effect of using spray dried salt and OISE

| Treatments | Salt types | Soy sauce powder content (%)* |
|---|---|---|
| 1 | commercial salt | 0 |
| 2 | commercial salt | 50 |
| 3 | spray dried salt | 0 |
| 4 | spray dried salt | 50 |

* The percentage of soy sauce powder was the replacement of 0, 50 % salt in formula.

The best modified salt particle from this experiment was selected for using with odor powder from experiment 3.2.3 (1) and this sample was evaluated in moisture content, $a_w$, color, density and sensory properties. Roasted peanut (no salt) was used in this experiment. Two hundred consumers were taken to evaluate in overall liking, saltiness and overall taste attributes with Label Affective Magnitude where a line scale includes two additional anchors: 'greatest imaginable like = 100' and 'greatest imaginable dislike = 0' (Cardello and Schutz, 2004).