CHAPTER III

RESEARCH AND METHODOLOGY

List of Materials and Equipments

Rice cracker raw material

Flour blend

1. Rice flour 3 cultivars were used as following;

    1.1 Chainat1 (CN) (Phitsanulok Rice Research Center, Phitsanulok, Thailand)

    1.2 Phatthumthanee1 (PN) (Phitsanulok Rice Research Center, Phitsanulok, Thailand)

    1.3 Surin1 (SR) (Chaijalearn Co., Ltd., Bangkok, Thailand)

2. Waxy rice flour variety RD6 (Jewhoksang Co., Ltd., Lampang, Thailand)
3. Wheat flour (local market, Thailand)
4. Pregelatinized tapioca starch (Bangkok Starch Co., Ltd., Bangkok, Thailand)

Hydrocolloids (Bronson & Jacobs International Co., Ltd., Bangkok, Thailand)

1. xanthan gum (XN
2. carboxymethylcellulose (CMC)
3. hydroxypropylmethylcellulose (HPMC) [Methocel-K4M]

Ingredients of crackers (purchased from local market)

1. Salt
2. Sugar
3. Milk powder
4. Palm oil
5. Glucose syrup
6. Baking powder
7. Margarine
8. Dry yeast
9. Ammonia powder

10. Lecithin
11. Corn flour
12. Tapioca flour
13. Flavor butter milk and vanilla
14. Sodium bicarbonate
15. Shortening

Equipments

1. Color Reader model CR-10 (Konica Minolta sensing Inc., Osaka, Japan)
2. Scanning Electron Microscope Model 1455VP (Leo Electric Systems, Cambridge, UK)
3. Moisture Meter model Sartorius MA40 (Sartorius, Inc., Goettingen, Germany)
4. The universal testing machine model 441 (Instron, Ltd., Buckinghamshire, England)
5. Water Activity meter model Novasina RS 200 (Novasina, Axair Ltd., Pfaffikon, Switzerland)
6. UV/VIS Spectrophotometer (Perkin Elmer, Lambda 20)
7. Stereo Microscope (Research stereo SZH10, Japan)
8. Vernia Caliper (Thailand)
9. Rheometer (Physica MCR301, Anton Paar, Germany)
10. Mixer (Kitchen-Aid model 5SS, St. Joseph, MI, USA)
11. Digital scales (Mettler Toledo, Model PE 503-S)
12. Incubator (Shel lab, Model 2020, Shaldon Manufacturing Inc., USA.)
13. Autoclave (Model KT-30L, ALP Co., LTD, Tokyo Japan)
14. Hot Sealer (Thailand)
15. A controlled stress-strain rheometer (Physica MCR 301, Physica/Anton Paar, Germany)
17. Blender (Sharp model : EM-11, Japan)
18. Grinder (Tramslab model: Mill3100, England)

Chemical Substances analytical reagent (AR grade)

1. Acetic acid (LAB-Scan Co., LTD, Ireland)
2. 2-thiobarbituric acid (Fluka, Belgium)
3. hydrochloric acid (Merck, Germany)
4. Antifoaming agent (Merck, Germany)
5. Sodium hydroxide (UNIVAR, Australia)
6. Glacial acetic acid (Merck, Germany)
7. Iodine (Merck, Germany)
8. Ethyl alcohol 95% (Merck, Germany)
9. Potato amylose (Fluka, England)
10. Bromothymol blue (Merck, Germany)
11. Potassium hydroxide (UNIVAR, Australia)
12. Pate count agar (Merck, Germany)
13. Bengal rose (Fluka, England)
14. Peptone (Merck, Germany)

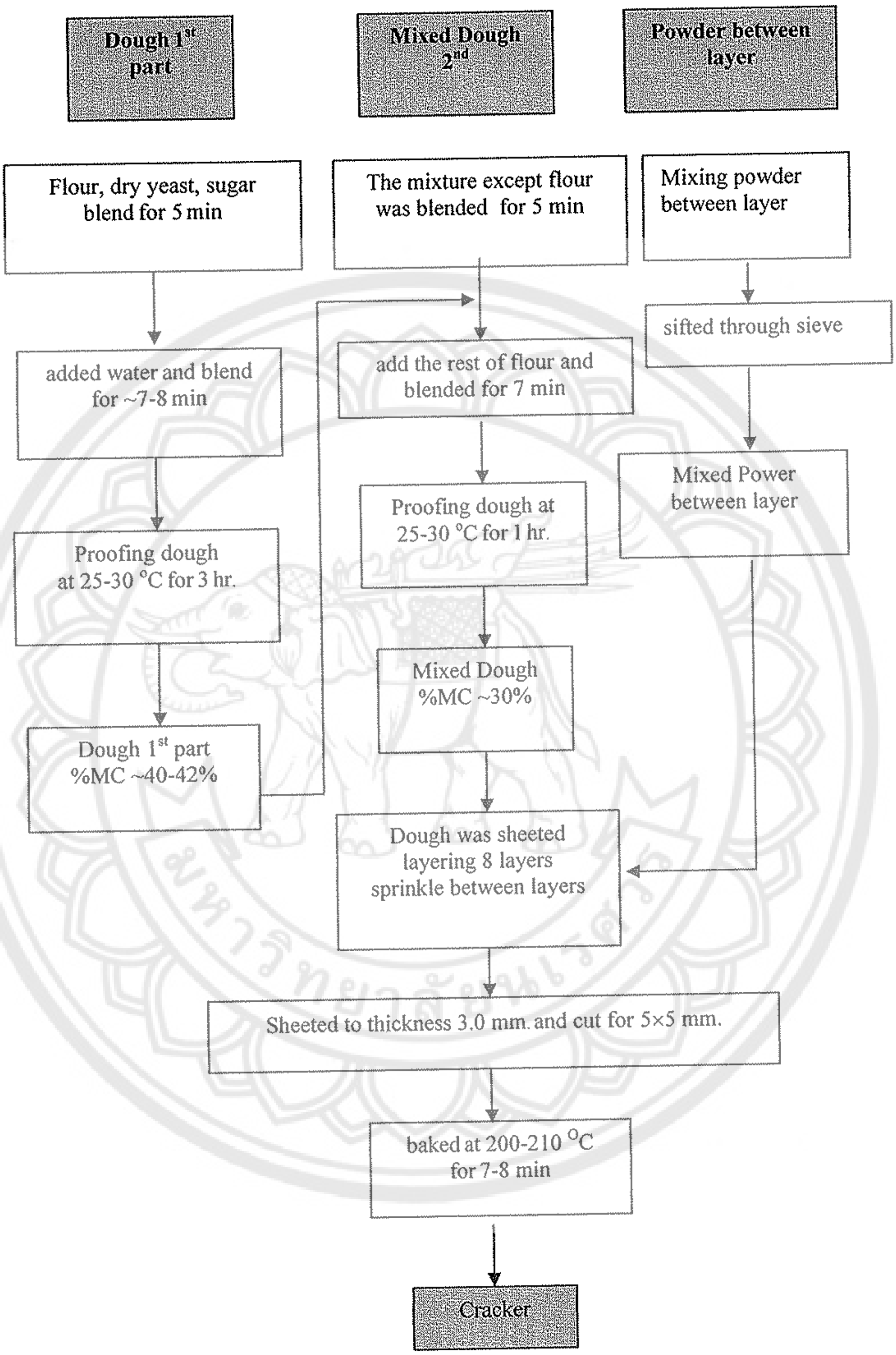

Figure 3 Processing of cracker

Source: Adapted from Sinn Salee Snack & Biscuit Ltd.

Cracker preparation

The cracker process was modified from that of cracker manufacturer (Sinn Salee snack & biscuit Ltd, Thailand). The dough preparation presented in Figure 3, were prepared by mixing 50% of flour, sugar and water in a mixer, adding yeast, blending and proofing dough at 25-30°C for 60 min, resulting in the first-half dough. The other half of mixture consisted of margarine, baking powder, ammonia powder, lecithin, salt, milk powder, palm oil, glucose syrup, flavor butter milk and vanilla were well blended before adding the first dough and the rest of flour. The mixture was blended in a mixer follow by proofing dough at 25-30°C for 60 min. Proofed dough was kneaded, sheeted, layered, cut (a cracker size of 5x5x0.3 cm), baked in the oven at 200-210°C for 7-8 minute and was cooled at 25-30°C for 60 min. Samples were packed in sealed polyproplylene bags and stored at room temperature (30±2°C) until being determined.

Experimental Design

1. **Effects of raw material and primary processing of rice flour on rice crackers.**

1.1 Effects of rice cultivars, amylose content on rice cracker qualities.

The physical and chemical properties of treatment crackers were compared with controls made of 100% commercial rice flour (CRF) and 100% wheat flour (WF). Experimental design in this research was a Completely Randomized Design (CRD). The main effect was rice cultivars. In this case, three types of rice cultivar were used. All treatments were duplicates. The physical and chemical properties of cracker samples were determined as described in 1.5.

1.1.1 The protein content of the rice flour samples were determined by Kjeldahl Method (AOAC, 1990). The result was multiplied by the factor 5.95 to convert to crude protein content.

1.1.2 Amylose content of the rice flour was measured by the iodine colorimetric method (Juliano, 1987).

1.1.3 The gel consistency value of the rice flour samples was determined by the method of Perez and Juliano (1987) of dispersing 100 g. of flour samples. The rice flour samples were wetted with 0.2 ml of 95% ethanol containing

0.025% (w/v) bromthymol blue in tubes and 2.0 ml of 0.2 *N* KOH was added. The tubes were soaked in the water bath for 5 min before removed and cooled for 20 min. The tubes were laid horizontally over a ruled graph paper, and gel length was measured after 1 hr. The gel consistency values may be classified as soft (61-100 mm.), medium (41-60 mm.), or hard (26-40 mm.).

1.1.4 The dough rheological measurement was studied by using the dynamic oscillatory tests. The test was performed by a controlled stress-strain rheometer (Physica MCR 301, Physica/Anton Paar, Germany) using a parallel-plate geometry (PP25/TG 6866) with plate diameter and plate gap of 25 and 2 mm., respectively. The excess of sample was trimmed off by a knife. The amplitude sweep (strain sweep) test was used to identify the linear viscoelastic region by determining the linear viscoelastic range and the critical strain (LVE) of dough by fixing the frequency. The frequency sweep test provided the information of dough rheological changes concerning the structure, molecular structure and viscoelastic behavior (Angioloni and Rosa, 2007). The measurements were conducted at 25°c. After the strain test, dough was rest between the plates for 1 min before testing by the frequency sweep test using a frequency sweep from 0.1 to 10 Hz at 0.1% strain. Treatment samples were compared with controls made of 100% commercial rice flour and 100% wheat flour. The chosen rice cultivar was then used for the next experiment steps.

1.2 Effects of milling type on rice cracker qualities

The influence of milling type on physical and chemical properties of rice cracker samples was determined. Experimental design in this research was a Completely Randomized Design (CRD). The main effect was milling type. In this case, two types of milling were used; dry milling (DM) and wet milling (WM). Rice cracker samples from different milling types were compared with two control; 100% commercial rice flour (CRF) and 100% wheat flour (WF). All treatments were duplicates.

1.2.1 The preparation of dry milling rice flour, modified from Sooksomboon and Naivikul, (2006) started with polishing rice kernels by grinding with blender (Sharp model : EM-11, Japan) followed by a hammer mill grinder (Tramslab model: Mill3100, England) with a 0.5 mm. sieve. Then flour was sieved through a standard sieves of 100 mesh, was packed in polyproplylene bags, and was stored at room temperature (30±2°C) until being used (Sooksomboon and Naivikul, 2006).

1.2.2 The damage starch was determined by following the assay procedure of AACC standard method 76-31, ICC method No.164 using a damage starch assay kit (Megazyme International Ireland Ltd., Ireland).

1.2.3 The physical and chemical properties of cracker samples were determined as described in 1.5. The samples were compared with controls and a selected treatment was used for the next experiments.

1.3 Effects of flour particle size on rice cracker qualities

After the rice cultivar and milling type were chosen, flour particle size was varied into 4 different rice flour particle sizes. The flour sample was ground using hammer mill grinder (Tramslab model: Mill3100, England) with a 0.5 mm. then sieved through a series of standard sieves at 50 mesh (299 μm), 100 mesh (139 μm), 140 mesh (103 μm) and 230 mesh (63 μm). Samples were compared with two controls of 100% commercial rice flour (CRF) and 100% wheat flour (WF). Experimental design in this research was a Completely Randomized Design (CRD). All treatments were duplicates. The physical and chemical properties of cracker samples were determined as described in 1.5. The suitable flour particle size was selected to be used in the next experiments.

1.4 Effects of hydrocolloids on qualities of rice crackers.

From above experiments, the suitable rice cultivar, milling type and flour particle size was chosen for rice cracker. In this part, the influence of hydrocolloids on physical and chemical properties and microstructure of rice cracker was studied. Experimental design in this research was a Completely Randomized Design (CRD). The main effect was hydrocolloid type, and three types of hydrocolloid with three usage levels were used. Hydrocolloids including carboxymethylcellulose (CMC) xanthan gum (XN), and hydroxypropylmethylcellulose (HPMC) [Methocel-K4M] were added at 1.5%, 3.0% and 4.5% (rice flour basis) in treated samples. Two different sets of samples were used to analyze the quality of samples. The dough microstructure, physical and chemical properties of hydrocolloid-treated samples were compared with non-hydrocolloid-added controls which were made of 100% commercial rice flour (CRF) as negative control, wheat flour (WF) as positive control and formulated flour (FF) as formulated control without hydrocolloid addition. All treatments were duplicates.

1.4.1 The physical and chemical properties of cracker samples were determined as described in 1.5.

1.4.2 The dough microstructure was studied using a scanning electron microscope Model 1455VP (Leo Electric Systems, Cambridge, UK). Prior to SEM study, cracker dough samples was cut to size 10×10 mm, freeze dried, and kept in a desicator until further used. Dough sample was mounted on a slide and separately placed on a sample holder using double-sided scotch tape. The internal structure was faced upward and sputter-coated with gold (2 min, 2 mbar) before being transferred to the microscope where it was observed in vacuum at an accelerating voltage of 5 kV.

1.4.3 The dough rheological measurement was studied by using the dynamic oscillatory tests. The test was performed by a controlled stress-strain rheometer (Physica MCR 301, Physica/Anton Paar, Germany), using a parallel-plate geometry (PP25/TG 6866) with plate diameter and plate gap of 25 and 2 mm., respectively. The excess of sample was trimmed off by a knife. The amplitude sweep (strain sweep) test was used to identify the linear viscoelastic region by determining the linear viscoelastic range and the critical strain (LVE) of dough by fixing the frequency. The frequency sweep test provided the information of dough rheological changes concerning the structure, molecular structure and viscoelastic behavior (Angioloni and Rosa, 2007). The measurements were conducted at 25°C. After the strain test, dough was rest between the plates for 1 min before testing by the frequency sweep test using a frequency sweep from 0.1 to 10 Hz at 0.1% strain.

1.4.4 Sensory evaluation of samples was conducted by using a 9-point hedonic scale with attributes including color, surface appearance, odor, taste, hardness and crispness. Fifty untrained panelists were screened based on their interests and consumptions of crackers before participating in the test. In this test, only one suitable usage level of each hydrocolloid was used. Therefore, there were 6 samples including 3 hydrocolloid-added rice cracker samples and 3 controls of 100% CRF, 100% WF and FF. Sensory data was statistically analyzed using the analysis of variance (ANOVA) test and sample difference was analyzed by Duncan' s multiple range test (DMRT) at 95% confidence level.

Then, the hydrocolloid type and its suitable usage level for rice cracker were used for further research in the next steps.

1.5 Determination of physical and chemical properties of rice crackers

1.5.1 The moisture content was measured in three replicates. Samples were ground by a mortar before being subjected to the test using a moisture meter model Sartorius MA40 (Sartorius, Inc., Goettingen, Germany).

1.5.2 Water activity value (a_w) of samples were measured in three replicates. Samples were ground by a mortar before being subjected to the test using a water activity meter (Novasina, Axair Ltd., Pfaffikon, Switzerland).

1.5.3 Hardness of samples was determined using ten replicates by the universal testing machine model 441 (Instron, Ltd., Buckinghamshire, England).

1.5.4 Color value was determined by using five replicates. Color values in CIE system was measured in four areas of each cracker by a color reader model CR-10 (Konica Minolta sensing Inc., Osaka, Japan). The L^*, a^*, b^* values were recorded as well as the hue angle and chroma values were calculated.

1.5.5 Thicknesses of cracker before and after baking were measured by a vernia caliper in five replicates then sample puffiness (%) was calculated from the difference of cracker thicknesses as shown in eq. 1.

$$\%\ \text{puffiness} = \frac{\text{thickness of baked cracker} - \text{thickness of cracker dough}}{\text{thickness of cracker dough}} \times 100 \quad \text{(Eq.1)}$$

2. Sensory Evaluation of Rice Crackers

The acceptance tests were conducted by 150 untrained panelists, selected from coffee shops and supermarkets in Phitsanulok province, Thailand. Selected panelists were screened prior to the test based on their interests and consumptions of bakery products. In addition, demography data of panelists such as education, revenue, career, rice product consumption, numbers of residents in household and their choice of beverages for rice bakery product consumptions were collected.

In this case, rice cracker made of formulated flour with 1.5% HPMC was evaluated and compared with wheat cracker control in an acceptance test. Ten attributes of samples including color, surface appearance, butter odor, odor, sweetnessness, saltiness, taste, crispness and crumbness were assessed. Samples were presented in a serving tray randomly and 3-random-digit number was assigned for each sample to avoid bias.

The 5-point Just-about-right (JAR) scale was used in this study. The JAR scale determined how panelists accept samples based on their ideal product. The scales were divided as following; not nearly enough (nn), slightly not enough (sn), just right (jr), slightly too much (sm) and much too much (mm). JAR data was collected as both frequency of responses and % observed responses. The binomial test was used to analyze JAR data to determine % difference from norm or expected JAR based on the consumption that norm of samples in this study was 60% (Bi, 2006; Prinyawiwatkul, 2010). Stuart Maxwell and McNemar was used to determine JAR distributions between 2 samples (formulated rice cracker and wheat cracker) (ASTM, 2009; Prinyawiwatkul, 2010).

Data analysis of each attribute started with comparing the % observed JAR with the norm (60%), if % observed JAR was higher than norm then there was no further analysis, and it could be concluded that panelists accepted the sample's attribute as just right. However, if the % observed JAR was less than norm then a binomial test, with the expected value of 50%, was used to determine whether significant difference between % non JAR responses on both sides of JAR point existed (Bi, 2006; Prinyawiwatkul, 2010). Further data analyses were conducted to determine whether the sample's attribute was too little or too much in terms of the acceptance of panelists to product attributes. For each attribute, the sum of % observed responses for "below Jar" and "above JAR" categories were calculated. The "below JAR" was the sum of % responses from not-nearly-enough (1) and slightly-not-enough (2)) whereas the sum of "above Jar" was from % responses of slightly-too-much (4) and much-too-much (5) (Prinyawiwatkul, 2010). Sample size for the binomial test was the sum of non JAR responses and the critical value was determined from the Table 2.1 (Bi, 2006) shown in Appendix for minimum number of correct responses for difference and preference tests using forced-choice at (α=0.05, two-tailed) (Prinyawiwatkul, 2010). For each attribute, statistical result was shown as frequency response, % observed response and the binomial analyses for % observation frequency.

3. Shelf-life study of rice cracker during 6-month storage.

The samples rice cracker were packed in sealed polyproplylene bags and stored at room temperature (30±2°C) for six months. Samples were compared with two control of 100% commercial rice flour (CRF) and 100% wheat flour (WF). Physical and chemical properties of samples were determined according to methods described in 1.5.

The TBA assay (2-Thiobarbituric acid test) was determined according to the modified method of AOCS. (1990). It is the most widely use to detect Malondialdehyde (MDA), which is a product of lipid oxidation. Therefore, it can be used as the indicator of lipid oxidation, which results in rancid odor in samples. The TBA assay is a colorimetric assay that is based on the conjugation between malondialdehyde and thiobarbituric acid (Packer, Hanninen and Sen, 2000, p. 466). The absorption is measured at 538 nm. by a UV-VIS spectrophotometer (Lambda 20, Perkin Elmer, USA.). The reported value of Thiobarbituric acid number (TBA no.) is from calculating the Malondialdehyde (mg.MDA/Kg.sample).

Microbiological Evaluations of samples were total plate count and total yeast and mold count. Total plate count was determined by using pour plate method using Plate Count Agar. Samples were incubated at 37°C for period 24-28 hours (AOAC, 1990). Total yeast and mold count was determined by spread plate method using Rose Bengal. Samples were incubated at 25°C for period 3-5 days (AOAC, 1990). The microbial values were calculated as colony-forming unit (CFU)/g. All analyses were conducted in triplicate.

4. Statistical analysis

Data was statistically analyzed using the analysis of variance (ANOVA) test and sample difference was analyzed by Duncan' s multiple range test (DMRT) at 95% confidence level.

For sensory data of Just-about-right scale, the binomial test was used to analyze JAR data to determine % difference from norm or expected JAR based on the consumption that norm of samples in this study was 60% (Bi, 2006; Prinyawiwatkul, 2010). Stuart Maxwell and McNemar test was used to determine JAR distributions (ASTM, 2009; Prinyawiwatkul, 2010). Sample size for the binomial test was the sum of non JAR responses and the critical value was determined from the Table 2.1 shown in Appendix for minimum number of correct responses for difference and preference tests using forced-choice at (α=0.05, two-tailed) (Bi, 2006).