CHAPTER III

MATERIALS AND METHODS

3.1 Raw materials

Four brands of high gluten flour (Diamond, Layang-Layang, Gunung-mas and Hovis which represented the control flour) were purchased from a bakery store in Nilai, Malaysia. Salt, sugar, shortening, milk powder and instant yeast were purchased from Tesco, a supermarket in Malaysia. The following equipment, namely mixer (Kitchen Aid, Michigan USA), Electric oven (Salva, Spain) and other baking equipment were used in the bakery laboratory USIM.

3.2 Determination of percentage gluten in flour

200 grams (g) of each flour sample was weighed into a petri dish of known weight and thoroughly mixed with water to form dough. The dough is kneaded under running water to remove starch, then dried in an oven, and weighed after drying according to method 38-10 (AACC, 2000). The % gluten is calculated as follows:

$$\text{gluten \%} = \frac{\text{Weight of gluten}}{\text{Weight of original flour}} \times 100 \qquad [1]$$

Figure 2: Washing the dough to determine the percentage of gluten

3.3 Bread making

A straight dough method was used for bread preparation. The following ingredients (100 g flour basis) were used: Water (62%), salt (2%), sugar (6%), shortening (3%), milk powder (2%), and instant yeast (3%). The breads were baked in an electric oven for 30 min at 190 C^0. After baking, the loaves were left to cool for 20 min, then were placed in polyethylene bags and stored until analysis.

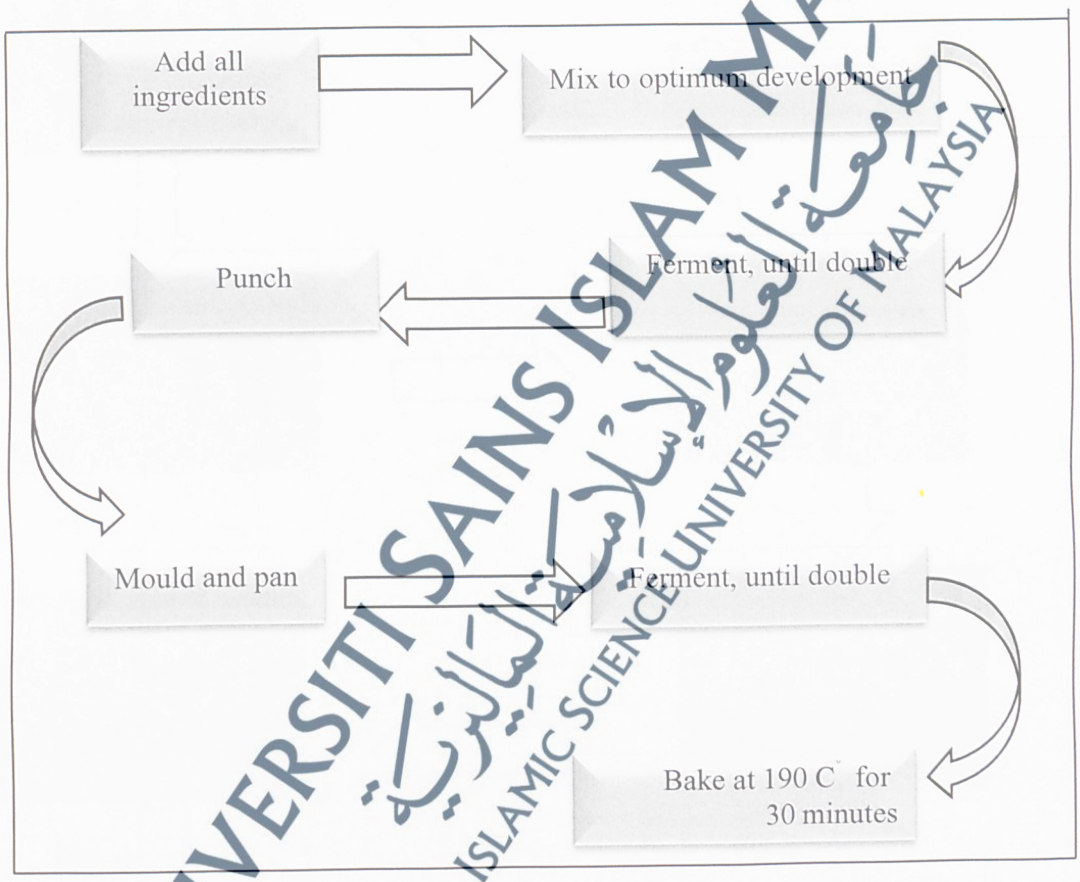

Figure 3: Straight-dough method for making bread

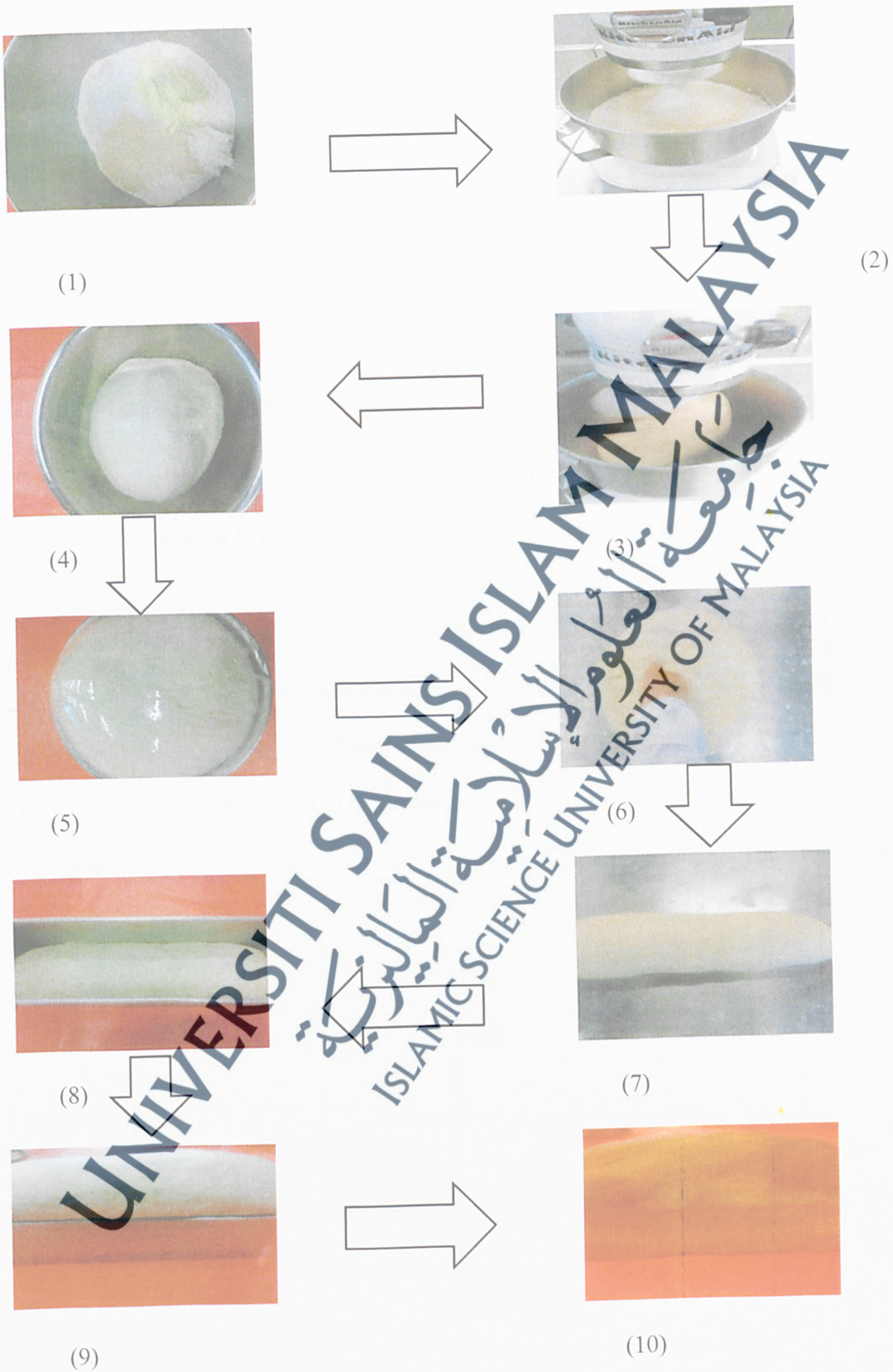

Figure 4: Making of wheat pan bread

Referring to Figure 4, all ingredients were added (1) and mixed together by mixer (2), when dough got optimum development (3); it was put in fermenter (4), after it rose about double (5) it was taken out and punched to release carbon dioxide (CO_2) from dough (6) and was formed as roll (7) then was put in pan (8). The dough was fermented until doubled (9) then the pan was put in oven about 30 minutes at 190°C (10).

3.4 Physical test analysis

The physical characteristics of bread analyzed were the height and the weight, the texture analysis using texture analyzer, and the colour test using hunter labscan XE.

3.4.1 Crust Color test

The color of the bread crust was determined by using hunter labscan XE. By using the International Commission on Illumination (CIE), the color values were recorded as" L" (0, black; 100, white), "a" ($-a$, greenness, $+a$, redness) and" b" (b^-, blueness, b^+, yellowness). The scanner was held in a black box in order to exclude the surrounding light.

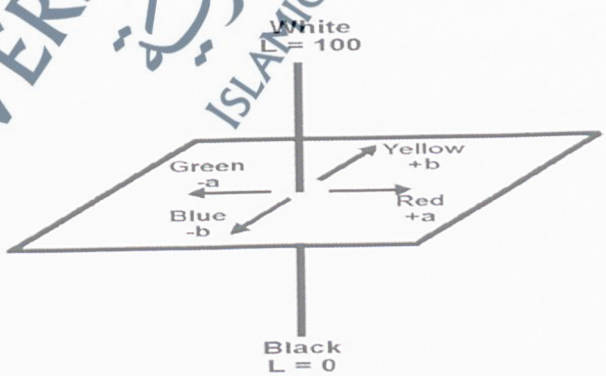

Figure 5: Hunter l, a, b Colour Scale

3.4.2 Texture analysis

The firmness of the bread was measured by using Texture analyzer (Stable Microsystem, UK). Firmness is defined in this method as the force (measured in Newtons) required compressing the product by a pre-set distance, slices have to be equal in thickness (2mm) and was placed centrally under the cylinder probe. 36mm cylinder probe was used to test for the firmness of the bread loaf.

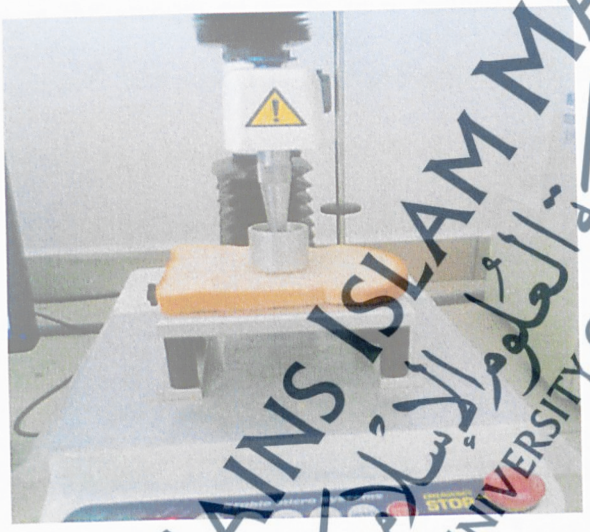

Figure 6: Measurement the firmness of bread samples

3.4.3 Bread height and weight

The height of the bread sample was measured by using standard ruler, while its weight was measured using commercial weighing scale. The height to weight ratio was calculated.

3.5 Proximate analysis

Proximate composition of the bread samples were analyzed for its moisture, ash, protein, crude fiber according to the AOAC (2000) method. The analysis was done in triplicate and the results obtained were recorded.

3.5.1 Moisture content

Moisture content is the amount of water in a material, such as soil (called soil moisture), fruit, or wood. Water content is commonly used in scientific and technical areas, and is expressed as a ratio, which can range from 0 (completely dry) to the value of the materials' porosity at saturation.

3.5.1.1 Procedure

Many of moisture analyzers are available in food industry. Using a digital balance, the sample was placed on aluminum pan (1g at 160 ^{0}C). As the moisture was driven from the sample, the instrument automatically weighed and calculated the percentage of moisture.

3.5.2 Ash content

The ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in foodstuff. There are two types of ashing technique that are used: dry ashing technique that is essential for proximate composition and for some types of specific mineral analysis, and wet ashing technique (oxidation) that is as a preparation for analysis of certain minerals (Nielsen, 2003).

3.5.2.1 Procedure

Three grams (3g) of each sample was weighed into a tared crucible. The sample was charred on an electric hot plate until it ceased smoking. The crucibles (containing charred samples) were then placed in a muffle furnace and were ignited for 12-18 hours at 550 ^{0}C, and then the muffle furnace was turned off.

After the temperature has become at least 250 ^{0}C, the muffle oven door was opened and the crucibles were transferred to a desiccator by using safety tongs, and then was allowed to cool prior to weighing. The ash content was calculated as follows:

$$\% \text{ ash (dry basis)} = \frac{\text{Weight after ashing} - \text{tared weight of crucible}}{\text{Original sample weight}} \quad [2]$$

3.5.3 Protein content

Nitrogen is essential element in protein, and also it includes hydrogen, carbon, nitrogen, oxygen and sulfur. Proteins exist in all cells and they are important for biological function and cell structure. Protein is also present in food and they are very complex (Nielson, 2003).

3.5.3.1 Procedure

One gram (1g) of samples were weighed in a weighing boat and then transferred into a 250 ml Kjedahltherm-digestion tube. 10 ml of H_2SO_4 and 1 Kjeltabs (Gerhardt, Germany) were added. Sulphuric acid was used to wash down the residue, which remain at the glass wall. Digestion with Kjedahltherm uses the following parameters.

Table 1: Parameters in kjeldahltherm

Time in min	Temperature (^{0}C)	Comments
40	400	Digestion tube is put into the preheated block and time it takes for the sample to become translucent.
30	400	Dehydrate the sample.

Distillation and Titration processes were done using the following parameter as listed in Table 2.

Table 2: Steam distillation and Titration programe parameter of Vapodest (Gerhardt, Germany)

Program parameter	Vap 50
Water addition in solution (s)	90
NaOH addition in solution (s)	8
Reaction time in solution (s)	0
Distillation time in solution (s)	240
Steam output in solution (s)	100
Suction sample in solution (s)	25
Boric acid addition in solution (s)	6
Suction receive in solution (s)	25
Titration	Auto
Calculation	Auto

Titration was done automatically using the Vap 50. For the determination of the blank value, the analysis (digestion and distillation) was conducted using the given chemical (Table 2). The consumption of these chemicals has to be taken into account when calculation the protein content, as in the following equation.

$$\%\ N = \frac{1.4007 \times C \times (V\text{-}Vb)}{\text{Sample weight}} \quad [3]$$

% raw protein = %N × 6.25

3.5.4 Crude fiber analysis

Gerhardt fibrebag method is the modern fibretherm that display the fully automated processing of the tedious boiling and filtration steps for the crud fiber. The required boiling times and filtration steps are done for all samples together. This analysis is an instance of gravimetric methods, an official method of the Association of Official Analytical Chemists International (AOAC). Crude fiber includes cellulose and lignin. Hemicelluloses, pectins and hydrocolloids are solubilized in this method and are not exposed as crude fiber (Nielsen, 2003).

3.5.4.1 Procedure

Fibre bags (Gerhardt, Germany) were dried for one (1) hour at 105 ^{0}C, and were transferred to desiccators for 30 minutes, and weighed (A). Fibre bags with glass spacers were inserted into the carousel and crushed bread (1g, B) were undergone defatting by washing three times with petroleum ether.

There were two boiling phases which take place by fibertherm (Gerhardt, Germany); firstly, the samples was boiled in 350 ml sulphuric acid for 30 minutes and the removal of acid by washing three times with hot water. Secondly, it was further boiled

in 350 ml potassium hydroxide solution for 30 minutes and the removal of alkali by washing three times with hot water. Fibre bags were removed from the carousel and were dried at 105 ^{0}C, cooled and weighed (C), followed by incineration at 600 ^{0}C overnight, cooled and weighed (D). The percentage of crude fibre was calculated as the following equation:

$$\% \text{ Crude fiber} = \frac{[(C-A)-(D-E)] \times 100}{B} \quad [4]$$

Blank value E = D-F

3.6 Sensory evaluation

Sixty (60) panelists from students of the faculty of Science and Technology, USIM participated in the evaluation. The attributes for evaluation were colour, aroma, hardness, taste, dryness, appearance and overall acceptability. A 10 point Quantitative Descriptive Analysis method (with scale of 0 = lowest intensity and 10 = highest intensity) and a 7 point Hedonic scale rating (1 = dislike extremely and 7 = like extremely) were used in Sensory evaluation of bread samples.

3.7 Statistical analysis

Results were statistically analyzed by using analysis of variance technique (ANOVA). Level of significance within means was calculated by using the Tukey's Test. Minitab (version. 16.2.1) statistical software (Minitab Inc., PA, USA). Three replications were used for all experiments.