

## CHAPTER 4: MATERIALS & METHODS

### 4.1 Experimental Design

The experiment was divided into Phase 1 (Figure 6) and Phase 2 (Figure 7). The moisture, protein, fat and ash content (%) of each egg ingredient were determined in Phase 1. The moisture content of the individual egg samples was applied to calculate the reconstitution formula of the sponge cakes (4.3.2.1). pH, coagulation temperature, foaming overrun and water holding capacity were also determined for all egg samples. The sponge cake samples were analyzed in Phase 2. Specific volume, index to volume and water activity were determined on Day 0. In addition, texture analysis (by instrument) were conducted every four days from Day 0 to Day 24, whereas the yeast and mould counts were determined every four days from Day 12 onwards. The sensory characteristics of sponge cakes were evaluated every four days from Day 0 to Day 16 only, due to positive yeast and mould counts found on Day 16.

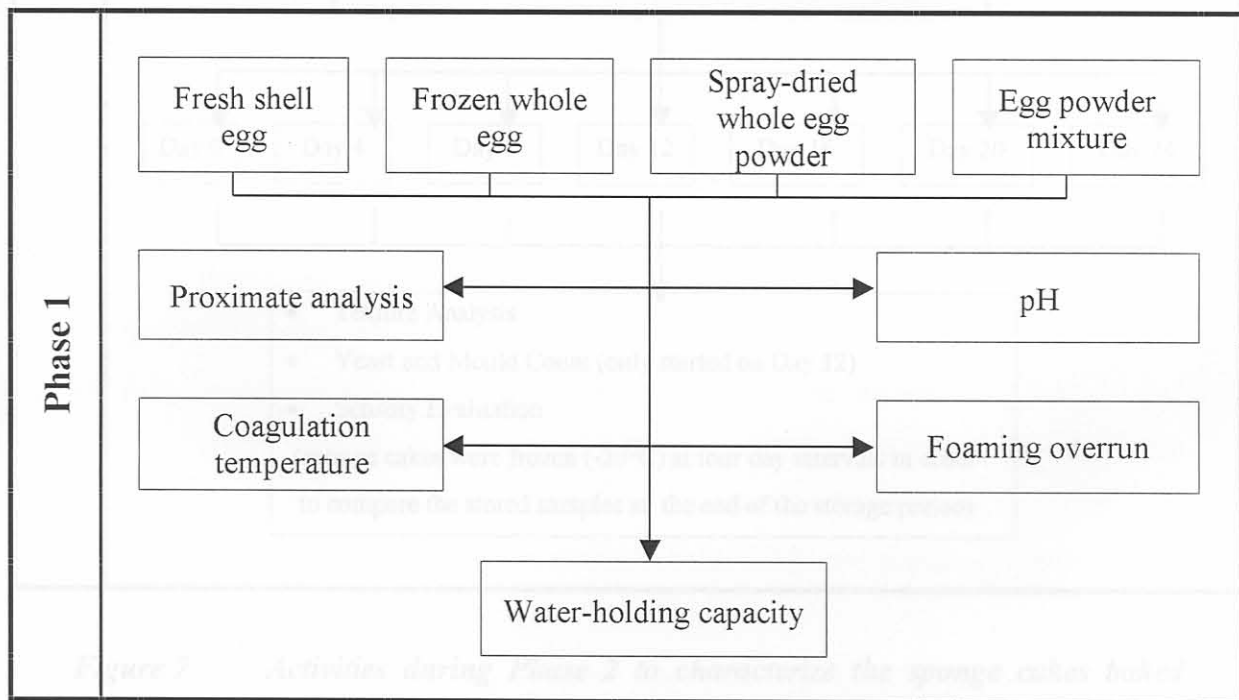
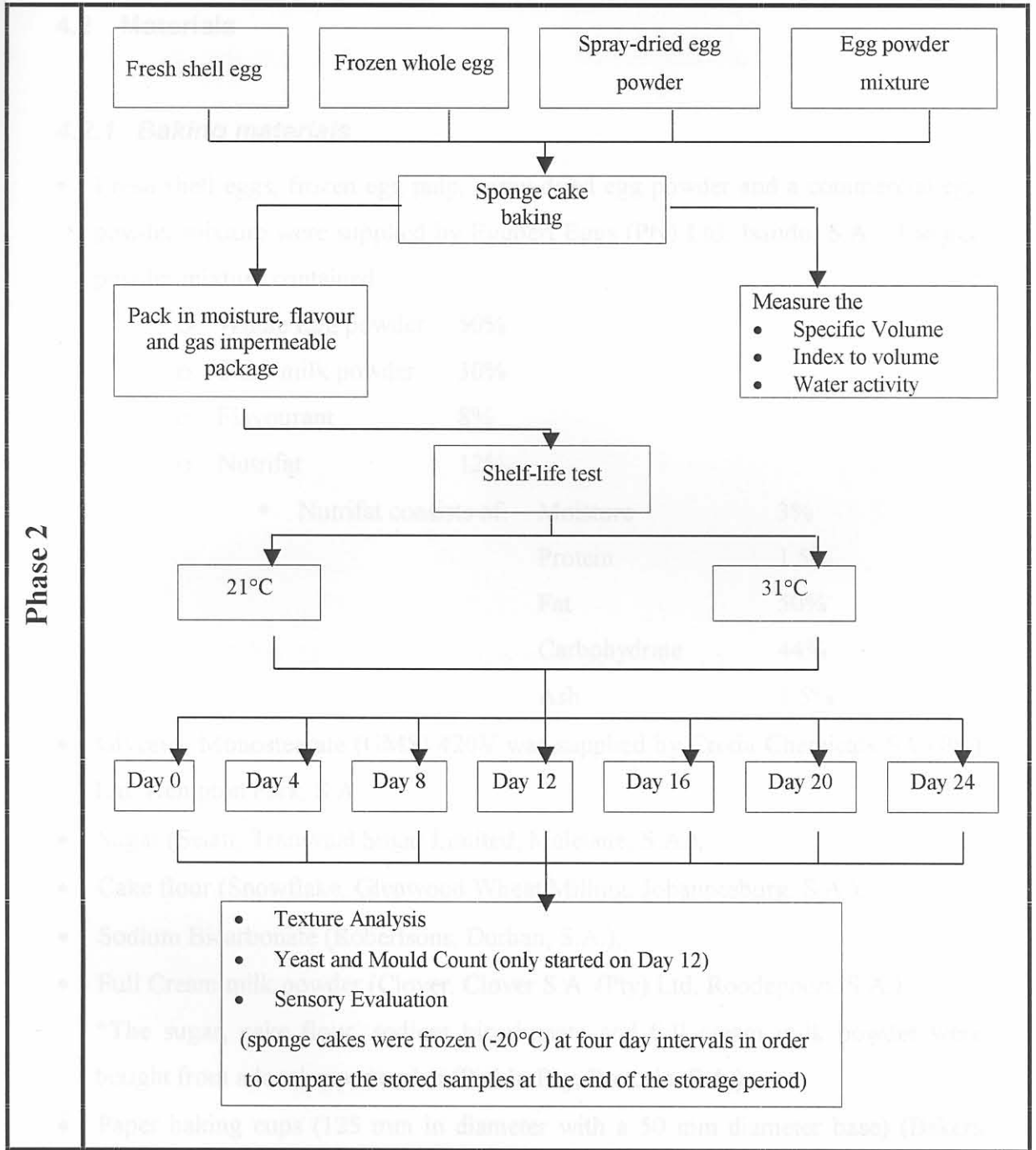


Figure 6 The measurements of Phase 1 to characterize the fresh shell egg, frozen egg pulp, spray-dried egg powder and egg powder mixture samples



*Figure 7 Activities during Phase 2 to characterize the sponge cakes baked from fresh shell egg, frozen egg pulp, spray-dried egg powder or egg powder mixture*

## 4.3 Methods

## 4.3.1 Phase 1 (Analyses of egg samples)

## 4.2 Materials

## 4.2.1 Baking materials

- Fresh shell eggs, frozen egg pulp, spray-dried egg powder and a commercial egg powder mixture were supplied by Eggbert Eggs (Pty) Ltd, Isando, S.A. The egg powder mixture contained:
    - Whole Egg powder 50%
    - Skim milk powder 30%
    - Flavourant 8%
    - Nutrifat 12%
      - Nutrifat consists of:
 

Moisture	3%
Protein	1.5%
Fat	50%
Carbohydrate	44%
Ash	1.5%
  - Glycerol Monostearate (GMS) 420V was supplied by Croda Chemicals SA (Pty) Ltd, Kempton Park, S.A.
  - Sugar (Selati, Transvaal Sugar Limited, Malelane, S.A.),
  - Cake flour (Snowflake, Glenwood Wheat Milling, Johannesburg, S.A.),
  - Sodium Bicarbonate (Robertsons, Durban, S.A.),
  - Full Cream milk powder (Clover, Clover S.A. (Pty) Ltd, Roodepoort, S.A.)
- \*The sugar, cake flour, sodium bicarbonate and full cream milk powder were bought from a local supermarket (Pick'n Pay, Pretoria, S.A.)
- Paper baking cups (125 mm in diameter with a 50 mm diameter base) (Bakers World, Pretoria, S.A.)
  - Moisture, air and flavour impermeable plastic package (130 x 145 mm) (Plastilon, Pretoria, S.A.)

### 4.3 Methods

#### 4.3.1 Phase 1 (Analyses of egg samples)

Three fresh shell eggs ( $\pm 51$  g each) were hand-broken and the white and yolk were mixed with a Kenwood Chef Excel mixer at speed 1 for 30 sec at  $21^\circ\text{C}$  ( $\pm 0.5^\circ\text{C}$ ). The frozen egg samples (2 kg batch size) were thawed by warming up to  $40^\circ\text{C}$  using a water bath and cooled to  $21^\circ\text{C}$  ( $\pm 0.5^\circ\text{C}$ ). The powder samples were reconstituted with  $45^\circ\text{C}$  distilled water in ratio 1:3 by stirring with a glass rod, followed by cooling to  $21^\circ\text{C}$  ( $\pm 0.5^\circ\text{C}$ ) for all the analyses except proximate analysis. All the analyses were done in triplicate.

##### 4.3.1.1 Proximate analysis of egg ingredients

###### 4.3.1.1.1 Moisture content

The AOAC Official Method 925.30 (Lebryk, 1995) vacuum method was used to determine the moisture content of the egg samples. Approximately 5 g of liquid samples (fresh shell egg and frozen egg pulp) and 2 g of dried samples (spray-dried egg powder and egg powder mixture) were weighed in covered tin dishes. These covered dishes were previously dried at  $98 - 100^\circ\text{C}$  in a force circulated oven (Labcon (Pty) Ltd, Model FSOE), cooled in a desiccator and then weighed after they were cooled to room temperature. The liquid samples were heated in a steam bath without covering the dishes for 1 hr to allow most of the water to evaporate from the sample. Then, all liquid and dried samples were covered and heated in a vacuum oven (VISMARA) for 5 hrs. All the samples were transferred to the desiccator after drying and weighed after they had cooled to room temperature.

**% Moisture =**

$$\frac{\text{Mass of sample before drying (g)} - \text{Mass of sample after drying (g)}}{\text{Mass of sample before drying (g)}} \times 100$$

#### 4.3.1.1.2 Protein content

A nitrogen analyzer (Leco® FP-528) was used to determine protein content. The instrument was calibrated with five blank samples and EDTA which contained  $9.57 \pm 0.04\%$  nitrogen. Approximately 0.25 g of samples was weighed into a tin capsule for liquid samples and tin foil cup for dried samples. Then, the tin capsule or tin foil cup was transferred to the equipment. The results of the samples were indicated on the screen and printed out after a few minutes.

#### 4.3.1.1.3 Fat content

The AOAC Official Method 925.32 (Lebryk, 1995) acid hydrolysis method was used to determine fat content. Approximately 3 g of liquid samples and 1 g of dried samples were weighed into Mojonnier fat-extraction tubes. Ten ml HCl was then added slowly with vigorous shaking. The tube was set in the water bath (BC model BTC-9090), which was heated to 70 °C and brought to boil for 30 min while shaking after every 5 min. Then, the lower bulb of the tube was filled with water, and cooled to room temperature. The sample was extracted by adding 25 ml ether and 25 ml redistilled petroleum ether followed by mixing. The mixture was left to stand until it separated into two separate layers. The upper layer (clear layer) was then drawn off in a beaker (which was dried in a conventional oven, cooled in a desiccator and weighed). The beaker was transferred to a conventional oven at 100°C for 90min for the moisture to evaporate. The beaker was then cooled in the desiccator to room temperature and weighed. The fat content was calculated as follows:

**% Fat =**

$$\frac{(\text{Mass of sample after drying (g)} - \text{Mass of beaker (g)})}{\text{Mass of sample before drying (g)}} \times 100$$

#### 4.3.1.1.4 Ash content

The AOAC Official Method 945.46 (Lebryk, 1995) was used to determine the ash content of egg samples. Approximately 5 g of liquid sample and 2 g of dried sample were weighed into a silica ashing crucible which had been previously ignited, cooled in a desiccator and weighed. The sample was incinerated in a muffle oven (Gallenkamp) until a light grey ash was obtained, then it was cooled in a desiccator and weighed. The ash content was calculated as follows:

$$\% \text{ Ash} = \frac{\text{Mass of crucible after ashing (g)} - \text{Mass empty crucible (g)}}{\text{Mass of sample before drying (g)}} \times 100$$

#### 4.3.1.2 pH

The pH of egg samples was measured by using the pH electrometer (Mettler DL 25 Titrator). Firstly, the pH meter was calibrated to pH 4 and 7 by dipping the electrode and stirrer in the calibration buffer solutions. Then, the electrode and stirrer was rinsed with distilled water followed by dipping them into the samples. The reading was taken when a stable value appeared.

#### 4.3.1.5 Water-holding capacity (WHC)

#### 4.3.1.3 Foaming overrun

Foams were formed from egg samples at room temperature ( $20 \pm 0.5^\circ\text{C}$ ) by using a Kenwood Chef Excel mixer at speed 3. The whipping period lasted for a total of 15 min with 5 min intervals. The foamed sample was scooped with a spatula and filled into a weighing spoon (60ml). The excess foam was scraped off the weighing

container by using a spatula to level the top of the foam to obtain a constant volume for each measurement (Phillips, German, O'Neill, Foegeding, Harwalkar, Kilara, Lewis, Mangino, Morr, Resentein, Smith & Kinsella, 1990). The foaming overrun (%) was calculated as follows:

$$\% \text{ Overrun} = \frac{(\text{Mass of 60ml egg sample (g)} - \text{Mass of 60ml foam (g)})}{\text{Mass of 60ml foam (g)}} \times 100$$

#### 4.3.1.4 Coagulating temperature

The coagulating temperature of the egg sample was measured using a Rapid Visco Analyzer (RVA) (Newport Scientific (Pty) Ltd, Australia). The temperature was increased by 1°C from 50°C to 85°C at 100rpm in 1 min intervals. Twenty-five grams of egg samples were placed in the RVA aluminum container. Then, a plastic paddle was put into the container and placed on the RVA stage. The paddle was clicked into place to ensure that it turned freely. The RVA stage was pressed down and as the process ran, a graph was plotted.

#### 4.3.1.5 Water-holding capacity (WHC)

The WHC was measured using the high-speed centrifugation method (Barbut, 1996). The egg samples were heated in a water bath (BC model BTC-9090) at 80°C for 40 min in 50 ml polycarbonate centrifuge tubes. The tubes were cooled in ice water immediately after the final temperature was reached followed by centrifuging the tube at 2000 rpm for 30 min with a centrifuge (MSE Super Minor Centrifuge, England,

model 5-80). The coagulated egg samples were measured after careful removal of liquid exudates (Handa *et al.*, 1998). The WHC was calculated as follows:

$$\text{WHC} = \frac{(\text{Mass of raw egg sample (g)} - \text{Mass of coagulated egg sample (g)})}{(\text{Mass of raw egg sample (g)})} \times 100$$

### 4.3.2 Phase 2 (Baking & analyses of sponge cakes)

#### 4.3.2.1 Reconstitution formula

A reconstitution formula was used to calculate the amount of water (Table 2) that was necessary to be added into the baking formula (Table 3). The moisture content of fresh shell egg was 75.76% (Watkins, 1995). Thus, the reconstitution formula for frozen egg pulp, spray-dried egg powder and egg powder mixture was calculated as follows to obtain the same moisture content as shell egg:

$x$  = the requirement of moisture content equal to fresh egg

$$\frac{x}{\text{Total solid} + x} = 0.76 \text{ (Moisture content of fresh egg)}$$

$$\text{Therefore, } x = \frac{0.76 * \text{Total Solid}}{(1 - 0.76)}$$

However, because there were moisture ( $y$ ) present in the egg samples already,  $x - y$  = water (g) to be added per 100g of egg samples.

The percentages of egg and water were in the sponge cake formula different whereas sugar, emulsifier, cake flour, sodium bicarbonate and milk powder were constant.

**Table 2** Calculation of egg % and water % to be added in baking formula

	Fresh shell egg	Frozen egg pulp	Spray-dried egg powder	Egg powder mixture
Moisture (y)	75.76 %	72.63 %	1.86 %	2.89 %
Total Solid	24.24 %	27.37 %	98.14 %	97.11 %
Fresh shell egg moisture * total solid = m		20.74 %	74.35 %	73.57 %
$1 - 0.76 = p$		0.24 %	0.24 %	0.24 %
$m / p = x$		86.41	309.79	306.54
$x - y = \text{water (ml) per 100g of egg sample (z)}$		13.78	307.93	303.65
$z / (z + 100) * 100 = \text{water \% to be added per 100g of egg sample (q)}$		12.11%	75.48%	75.23%
Fresh shell egg <sup>1</sup> (%) * (100 - q) = egg (%) in baking formula		20.77%	5.79%	5.85%
(Fresh shell egg <sup>1</sup> (%) * q) + fresh shell egg water <sup>1</sup> (%) = water (%) in baking formula		14.68%	29.66%	29.60%

<sup>1</sup> Used in baking formula (Table 3)

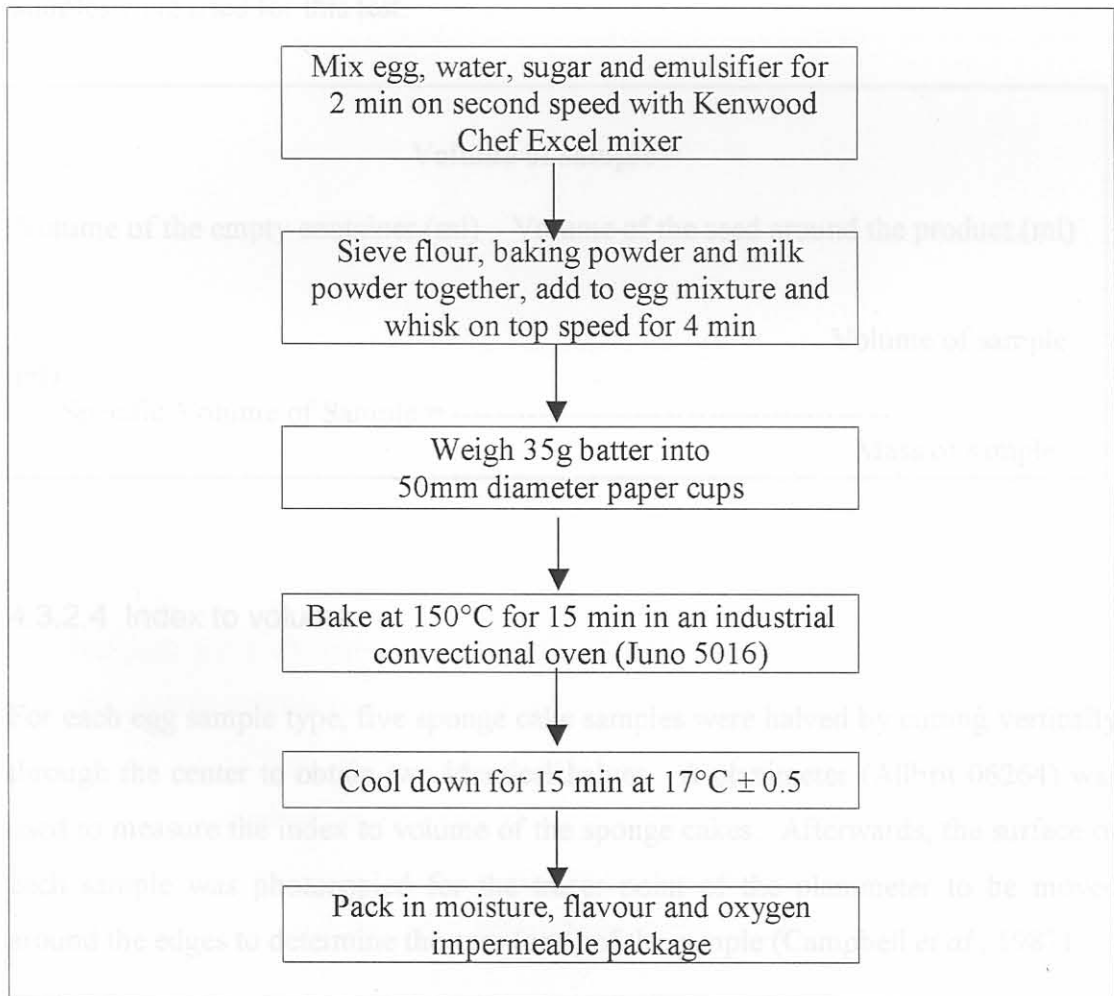
**Table 3** The baking formulas for sponge cakes with fresh shell egg, frozen egg pulp and dried egg powder (Adapted from Bennion & Bamford, 1997)

Ingredients	Fresh shell egg	Frozen egg pulp	Spray-dried egg powder	Egg powder mixture
Egg	23.63%	20.77%	5.79%	5.85%
Water	11.82%	14.68%	29.66%	29.60%
White sugar	32.67%	32.67%	32.67%	32.67%
Emulsifier (GMS)	1.69%	1.69%	1.69%	1.69%
Cake flour	28.20%	28.20%	28.20%	28.20%
Sodium bicarbonate	0.89%	0.89%	0.89%	0.89%
Full cream milk powder	1.09%	1.09%	1.09%	1.09%

4.3.2.2 Baking method

4.3.2.3 Specific volume

The sponge cup cake samples were prepared by the “all-in method” (Figure 8) (Bennion & Bamford, 1997). Water additions were adjusted in the formulations (Table 2) to account for the different moisture contents of the fresh shell egg and other egg samples. During the mixing of ingredients, the frozen egg pulp was warmed up to 40°C (AOAC Official Method, 1995), spray-dried egg powder and egg powder mixture were mixed with sugar and emulsifier, followed by mixing with 45°C distilled water. Two batches of each egg sample were baked which contained seventy-two cup cake size sponge cakes in each batch.



**Figure 8** The method used for sponge cake manufacturing (Adapted from Bennion & Bamford, 1997; Abellana et al., 1999)

4.3.2.3 Specific volume

The rapeseed displacement method was used for measuring the specific volume of the sponge cake samples. Rapeseeds were poured into a container until it overflowed. The seed was leveled by passing a ruler across the top of the pot once followed by using a measuring cylinder to measure the volume of seeds. Then, a sample was placed in the empty pot and seeds were poured into the pot until it overflowed. The seeds were leveled by passing a ruler across the top of the pot. The seeds were measured by measuring cylinder once again to obtain the volume of the seed around the product (Campbell, *et al.*, 1987). For each egg sample type, five sponge cake samples were used for this test.

$$\text{Volume of sample} = (\text{Volume of the empty container (ml)} - \text{Volume of the seed around the product (ml)})$$

$$\text{Specific Volume of Sample} = \frac{\text{Volume of sample (ml)}}{\text{Mass of sample}}$$

4.3.2.4 Index to volume

For each egg sample type, five sponge cake samples were halved by cutting vertically through the center to obtain two identical halves. A planimeter (Allbrit 06264) was used to measure the index to volume of the sponge cakes. Afterwards, the surface of each sample was photocopied for the tracer point of the planimeter to be moved around the edges to determine the area (cm<sup>2</sup>) of the sample (Campbell *et al.*, 1987).

### 4.3.2.5 Water activity

A thermoconstanter (Novasina TH-2) was used to determine the water activity ( $a_w$ ). The instrument was calibrated at an actual temperature of 25°C with SAL-53, SAL-90 and SAL-11. The setpoint temperature was set in a measuring chamber with a temperature preselector switch. Then, the value for the desired setpoint temperature was adjusted, which can be found in the table “TEMPERATURE/ SET”. A sample bowl was filled with crushed sponge cake and spread evenly. The sample bowl was put into the measuring chamber. After the lid was closed, it was left to equilibrate. The figure was displayed on the machine in terms of % equilibrium relatively humidity.

Equilibrium Relatively Humidity (%)

$$a_w = \frac{\text{-----}}{100}$$

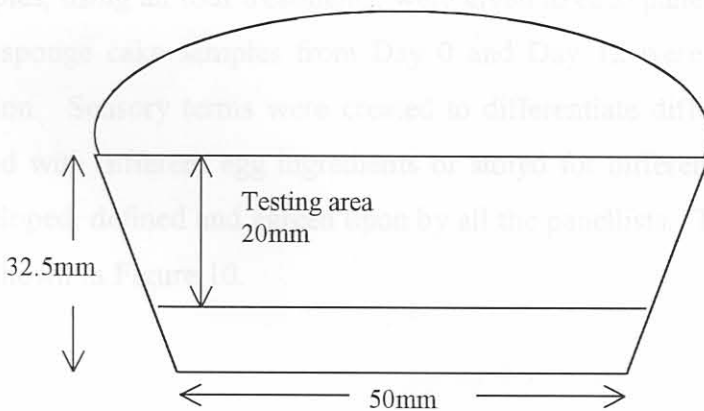
### 4.3.2.6 Yeast and mould counts

Potato Dextrose Agar was used for yeast & mould counts. The 10<sup>-1</sup> dilution was prepared by using 0.1% peptone dilution blank which was autoclaved (A U Tester 437- G) at 121 °C for 15 min before inoculating. Firstly, 10 g of sponge cake sample was weighed and 0.1% peptone water was added to obtain 1:10 dilution. Secondly, the mixture was homogenized in a stomacher (Art Medical Equipment (Pty) Ltd) for 2 min. Then, a sterilized pipette (Gilson) was used to measure 1 ml of diluted sample which was transferred to a sterilized petri dish (Biolab, Diagnostics (Pty) Ltd, Midrand, S.A). Twelve to 15 ml of liquefied agar was poured into each plate. This was followed by mixing the agar and diluted sample together in the petri dish by rotating the plate. The plate was left in place until the agar solidified. Afterwards, the plate was incubated in an incubator (LABex) at 22-25 °C for 5 days (Vanderzant & Splittstoesser, 1992).

#### 4.3.2.7 Texture analysis

The texture of the sponge cake samples was measured by the texture analyzer (TA-XT2®, SMS Stable Micro System). The crust of the sponge cake sample was removed prior to the testing. In addition, the Day 0 samples were analyzed after 1 hour from the packaging time, and Day 4 to Day 24 samples were analyzed within 3min after removing from the storage place. Four replications of each sponge cakes type were determined. The texture area (Figure 9) and settings of the test were as follows:

- Mode: Measure Force in Compression
- Option: Return to start
- Pre-Test Speed: 2.0 mm/s
- Test Speed: 1.0 mm/s
- Post-Test Speed: 10.0 mm/s
- Distance: 10mm
- Accessory: 35 mm cylinder probe
- Sample Preparation: Cut the sponge cake samples into 20mm thickness (Figure 9)



**Figure 9** Diagram to show the testing area of samples for texture analysis

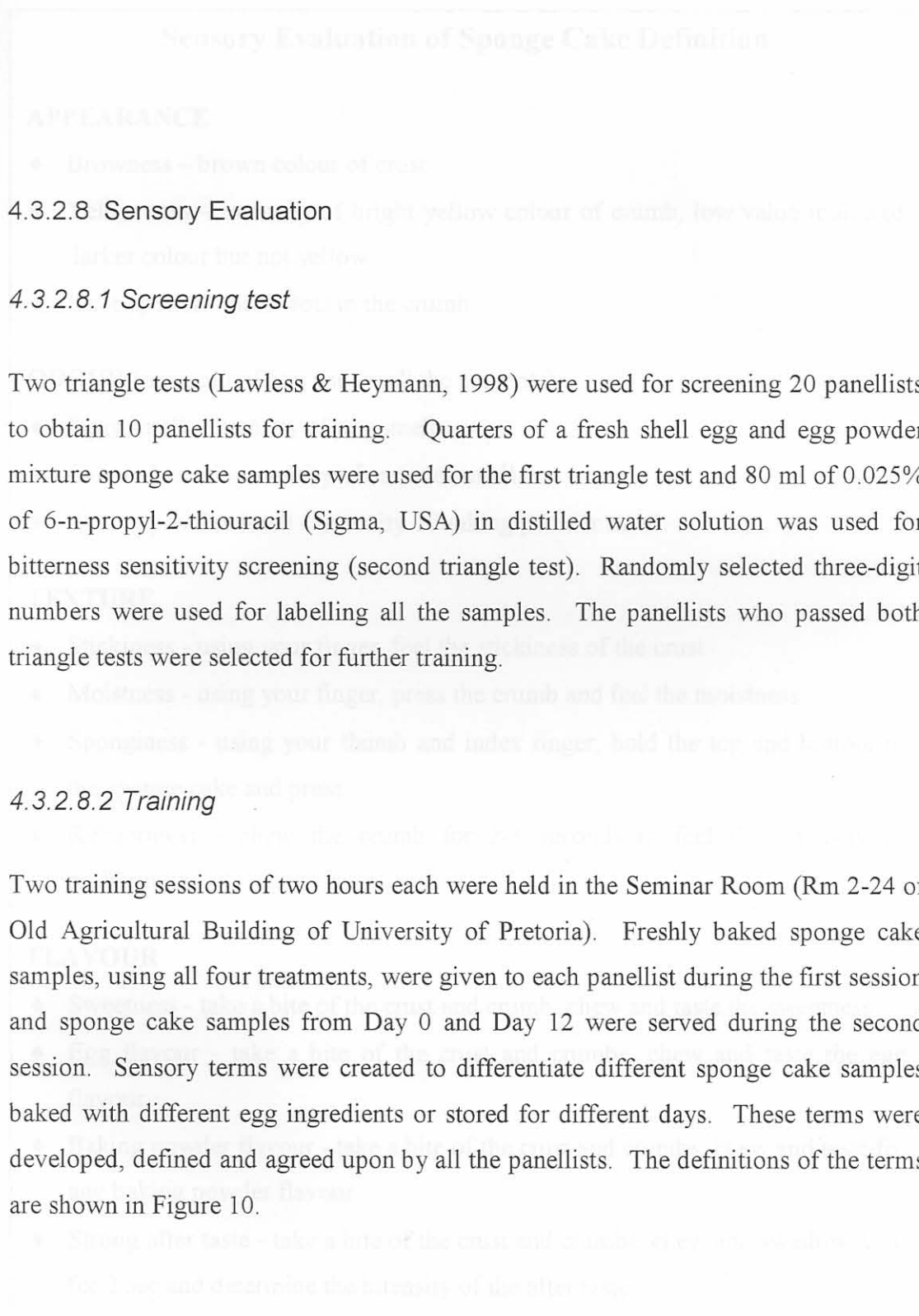


Figure 10 The definitions of sponge cake characteristics used for the sensory evaluation

## Sensory Evaluation of Sponge Cake Definition

### APPEARANCE

- ◆ Brownness – brown colour of crust
- ◆ Yellowness – intensity of bright yellow colour of crumb, low value indicated darker colour but not yellow
- ◆ Have specks - black dots in the crumb

### ODOUR (open zip of bag and smell the contents)

- ◆ Egg smell - intensity of egg smell
- ◆ Caramel smell - intensity of caramel smell
- ◆ Baking powder smell - intensity of baking powder smell

### TEXTURE

- ◆ Stickiness - using your finger, feel the stickiness of the crust
- ◆ Moistness - using your finger, press the crumb and feel the moistness
- ◆ Sponginess - using your thumb and index finger, hold the top and bottom of the sponge cake and press
- ◆ Rubberiness - chew the crumb for 2-3 seconds to feel the intensity of rubberiness

### FLAVOUR

- ◆ Sweetness - take a bite of the crust and crumb, chew and taste the sweetness
- ◆ Egg flavour - take a bite of the crust and crumbs, chew and taste the egg flavour
- ◆ Baking powder flavour - take a bite of the crust and crumbs, chew and taste for any baking powder flavour
- ◆ Strong after taste - take a bite of the crust and crumbs, chew and swallow, wait for 2 sec and determine the intensity of the after taste.

*Figure 10 The definitions of sponge cake characteristics used for the sensory evaluation*

#### 4.3.2.8.3 Testing period

The generic descriptive test method (Gillette, 1984) was used for evaluating the sensory characteristics of sponge cake samples. All the tests were held in the sensory evaluation laboratory (Rm 2-6 of Old Agricultural Building of University of Pretoria). Ten trained panellists were used. Sponge cakes samples were thawed at room temperature for one hour before tasting. For each panellist, a quarter of sponge cake sample was put into a zip seal plastic bag (80 x 100 mm) which was labeled with a random three-digit number. During each evaluation session, four sponge cake samples (from the same day of storage) which were baked from different egg ingredients, were served on the same tray for each panellist. Drinking water (175ml) was provided for each panellist to rinse his or her mouth before and in between tasting the samples. The evaluation form is shown in Figure 11.

#### 4.4 Statistical analysis of data

One-way analysis of variance (ANOVA) was used to test for the effect of treatment (egg ingredient) on protein content, fat and ash content, pH, coagulating temperature, foaming overrun and water holding capacity. The baking quality (index to volume and specific volume) of sponge cake samples which were baked from various egg ingredients were also compared by ANOVA. Two-way ANOVA was used to test for the effects of treatment and storage time as well as the relevant interaction effects on the sensory properties and texture analysis of the sponge cake samples. When significant differences ( $p < 0.05$ ) were found by ANOVA, the Least Significant Difference test (LSD-test) was used to identify the specific nature of the differences. All analyses were performed using Statistica ® 5.0 and Microsoft Excel 2000 for data capturing.

Figure 11 The sensory evaluation form for sponge cake samples

Name: \_\_\_\_\_ Date: \_\_\_\_\_ Set: \_\_\_\_\_

You have received four samples of sponge cakes. Mark, using a cross, the position of the intensity of the properties, which can either be on the points or between the points on the scale and put the code no. under the cross which you want to indicate

Remember to rinse your mouth with water before and in-between tasting the samples; refresh your breath with coffee bean before and in-between smelling the samples.

Sample no:

**Appearance**

Brownness \_\_\_\_\_  
not intense very intense

Yellowness \_\_\_\_\_  
not intense very intense

Have speckles \_\_\_\_\_  
not intense very intense

**Odour**

Eggy smell \_\_\_\_\_  
not intense very intense

Caramel smell \_\_\_\_\_  
not intense very intense

Baking powder smell \_\_\_\_\_  
not intense very intense

**Texture**

Stickiness (Crust) \_\_\_\_\_  
not intense very intense

Moistness \_\_\_\_\_  
not intense very intense

Rubberiness \_\_\_\_\_  
not intense very intense

Sponginess \_\_\_\_\_  
not intense very intense

Figure 11 The sensory evaluation form for sponge cake samples

