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DEVELOPMENT OF WHITE BREAD FORTIFIED WITH CALCIUM DERIVED FROM EGGSHELL POWDER

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ABSTRACT

Bread is commonly used mostly around the world which obtained from grains, legumes, tubers and other fortified ingredients. Eggshell makes up from 9-12% of the total egg weight that consists largely of calcium carbonate (94%) with some magnesium carbonate and calcium phosphate deposited in the organic matrix. The objectives of this study were to produce white bread fortified with calcium derived from the eggshell powder and to study the physicochemical changes, microbiological status and sensory evaluation of the product. The main analyses were done on specific volume, texture, colour, proximate analysis, calcium determination by ICP-MS and microbiological test for the determination of shelf life. Besides, acceptance test was also carried out. The result showed that increasing of the eggshell did not have any effect on the specific volume, springiness, cohesiveness and colour but affect the hardness of the bread. Furthermore, addition of the eggshell significantly increased ($p<0.05$) the moisture content, ash, fibre but decreased the carbohydrate. However, there were no significantly different between sample in terms of fat and protein. The bread with addition of eggshell have potential to be produced and accepted by the consumer especially the bread with fortification of 2% egg shell powder.

Key words: Product development, white bread, fortified with calcium, eggshell powder

INTRODUCTION

Over the past 10 years, annual per capita egg consumption in New Zealand has increased from 204 eggs in 1995 to 222 eggs in 2005 (Gerber, 2015) and it was one of the highest in the world. According to Fururuwa (2013), every 5 g of the eggshells can provide daily calcium requirement which is around 1000-1200 mg of human body. This means that, if properly processed, the chicken eggshell can be used as calcium supplement. Furthermore, calcium is the most abundant mineral in the body and makes up to 1.9% of the body by weight where nearly all (99%) of this in the skeleton. The remainder is in the teeth (0.6%), the soft tissues (0.6%), the plasma (0.03%) and the extracellular fluid (0.06%). Calcium provides a "structural role" in providing rigidity (structure and strength) to the skeleton. This function is provided with a form of calcium phosphate that is generally crystals which are embedded in collagen known as hydroxyapatite fibrils (Khor & Chee, 2005).

Studies have shown that calcium is important nutrients for bone health and its maintenance. Sufficient intake of calcium is important for healthy bone and prevention of osteoporosis. This was supported by findings that adequate calcium intake was associated with lower risk of osteoporotic fracture (Ahmad *et al*., 2015). Moreover, as stated by Ahmad *et al*. (2015), calcium is especially important for post-menopausal women who are prone to bone loss. High intake of vitamin D was found to lower the risk of hip fracture in post-menopausal women. Similarly, sufficient in-take of calcium was proven to reduce the risk of osteo-porosis in postmenopausal women. In addition, it was found that the best way to use chicken eggshell as a calcium dietary supplement is by adding the powdered supplements to bread, pizza or spaghetti (Hassan, 2015). Breads are products of grains, legumes, and tubers ground into meals, moistened and usually combined with a leavening agent, kneaded, shaped into loaves and baked. Globally,

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bread has been a central constitution in diets of most populations for thousands of years. For example, bread provides approximately 30% of daily requirement of calories and protein in Russian diet (Samsonov & Petrasov, 1993).

Nowadays, there are so many wastes obtained from either domestic or industry especially the eggshell. People used egg everywhere for example in a household, bakery and restaurant. Moreover, the egg is used as a basic ingredient for cooking and baking both in-house and bakeries. Thus, there is a waste of eggshell everywhere and uncontrollable. This situation leads to the serious environmental problem. However, although eggshell is a waste, it contains a high amount of calcium carbonate that provides calcium sources. According to King O'ri (2011), one whole medium sized eggshell makes about one teaspoon of powder, which yields about 750-800 mg of elemental calcium plus another microelement.

People need calcium in their daily diet especially children and old folks for bone development. People who lack calcium intake will have a possibility to get a disease such as osteoporosis, osteopenia, and calcium deficiency or hypocalcemia (Natalie, 2016). Statistically, more than 75% of Americans are calcium deficient and more than one in 10 Americans either has, or is at risk of developing, osteoporosis or other bone diseases (Dee, 2016). Moreover, according to Ahmad *et al.* (2015) in Malaysia, the Chinese has the highest incidence of osteoporosis, followed by the Malay and Indians and the total hip fracture cases reported in 1996 were 1353 cases in Chinese, 424 cases in Malay, 294 cases in Indians and 95 cases in other races. Meanwhile, in 1997, the hip fracture cases for Chinese, Malay, Indians, and other races were reported to be 1442, 478, 80 and 94 cases, respectively. This shows that the cases increasing by the year.

Calcium is important in our life for bone development by keeping them healthy, help in blood clotting, sending of messages by the nerves and muscle contraction. About 99% of the calcium in our bodies is in our bones and teeth. Eggshell contained a lot of calcium and apart from treating eggshell as a waste, it can be used as one of the calcium sources in preventing a lack of calcium intake in the body and at the same time reducing the environmental problem. This present study is therefore undertaken due to there is still no study on the use of calcium derived from an eggshell powder in bread. Moreover, there already widely uses of calcium from eggshell such as the study conducted by Hassan (2015) in using calcium from eggshell in biscuit. Thus, by doing this study, the use of eggshell powder can be varied. In addition, the amount of eggshell as a waste can be reduced and at the same time environmental problem related to this will be solved.

MATERIALS AND METHODS

The eggshell was obtained from Batu Enam market at Kuala Nerus, Terengganu. The whole eggshell was used to produce eggshell powder. Raw material for bread making such as flour, salt, leavening agent, sugar and other ingredients were purchased from the Mydin Mall at Gong Badak, Kuala Terengganu.

Preparation of eggshell powder

The eggshell was washed before processing to remove the dirt on the eggshell. Then, the eggshell was boiled in water for 30 minutes to kill the bacteria. After that, the eggshell was dried in the oven at 80°C for 2 hours. The drying process could make the grinding process easier. Then, the sample was ground and sieved before storage. The eggshell powder was stored in a jar at room temperature.

Determination of calcium content in the eggshell flour

The calcium content was measured using ICP-MS (Perkin Elmer, Elan 9000).

Preparation of bread

Firstly, high protein flour and yeast were weighed and mixed using dough mixer with ladle followed by other dry ingredients such as salt, sugar and milk powder. Water was added into the mixer and lastly followed by shortening. The dough was continuously stirred until it mixed well. Then, the dough was proofed about 20-25 min using a dough proofer. The dough was then shaped into smooth, round ball. After that, the dough was rested on bench for 10-15 min before the dough was put into the mould that had been oiled. After that, the second proofing was done and lastly, the bread was baked in oven at 175-190°C for about 20 minutes. After baking, the bread was stored for evaluation. This method was modified based on Wayne (2015) as shown in Table 1.

Physical analysis

Determination of texture

Firmness test was conducted for texture determination. Firmness was defined as the maximum force obtained during compression. The bread firmness was determined using a texture analyser (TA-XT Plus, Stable Micro Systems, Godalming, Surrey UK) which was calibrated for a load cell of 30 kg. Bread loaves were sliced mechanically into 10 mm × 40 mm × 40 mm thickness using knife and the two end slices of the

Table 1. Table of formulation of white bread

Ingredient	Weight (gram)				
Bread flour	100	98.0	96.0	94.0	92.0
Eggshell flour	0.00	2.00	4.00	6.00	8.00
Yeast	1.30	1.30	1.30	1.30	1.30
Water	60.0	60.0	60.0	60.0	60.0
Salt	2.50	2.50	2.50	2.50	2.50
Sugar	3.75	3.75	3.75	3.75	3.75
Milk powder	5.00	5.00	5.00	5.00	5.00
Shortening	3.75	3.75	3.75	3.75	3.75
Total	176	176	176	176	176

Modified, Wayne (2015).

loaf was discarded (Abboud & Charles, 2012). The bread firmness was measured with a probe 25 mm in diameter and at 40% strain. Bread firmness, hardness, and springiness was measured using American Association of Cereal Chemists (AACC) Standard Method 74-09 (Purna *et al*., 2011).

Determination of specific volume

Bread loaves was weighted about 20 minutes after baking using a laboratory scale and the readings was recorded in grams (Hamzah & Lian, 2012; Haslina, 2008; Purna *et al*., 2011). The formula used to calculate the specific volume was:

Specific volume (cm^3/g) = volume of loaf/ weight of bread

Determination of colour

The colour of the sample was analysed by exposing two slices of bread arranged horizontally to the incident of light on a spectrophotometer Minolta Chroma Meter CR300 (Konica Minolta, Inc, Tokyo, Jepun.) with three replicates.

Chemical analysis

Proximate analysis of white bread fortified with calcium was determined using AOAC method (AOAC, 2000) for moisture, ash, crude fat, crude fibre, protein and carbohydrate content. Besides, the amount of calcium in the eggshell powder and in the fortified bread was also determined.

Determination of moisture content

The moisture content was determined using standard methods of analysis (AOAC, 2000).

Determination of ash content

The ash content was determined using standard methods of analysis (AOAC, 2000).

Determination of ash content

The crude protein content using Kjeltec method was determined (AOAC, 2000).

Determination of fat content

Soxhlet method was used to determine the fat content in the bread sample based on AOAC (2000).

Determination of fibre

The crude fibre content by using Kjeltec method was determined using standard methods of analysis (AOAC, 2000). Elimination process of fat and carbonate was done for food samples with fat content more than 10% or carbonate content more than 5%.

Determination of carbohydrate

Carbohydrate content was calculated by using formula:

% carbohydrate = 100% – % moisture – % fibre – % protein – % fat – % ash

Determination of calcium

The calcium content of the bread and the eggshell sample was determined according to a standard method of analysis (AOAC, 2015). Two grams of sample was weighted in crucible and dried in conventional oven at 135°C for 2 h. Then, the sample was heated again at 450-550°C in muffle furnace for overnight. Two millilitres of concentrated HNO_3 was added into sample and evaporated to dryness on warm hot plate. After that, the sample was heated at 550°C in muffle furnace for 1 h to obtain clean carbon. Ten millilitres of 1N HCL was added into sample and heated continuously on hot plate to dissolve the ash. Finally, sample was diluted in volumetric flask until 50 ml with deionized water.

Microbiological profile of bread

Aseptically, the bread was cut into small pieces and 25 g of sample was weighed. Then, the sample was transferred into a stomacher bag and aseptically diluted the sample by adding 225 ml of sterile saline into the stomacher bag and homogenized by using the stomacher. Then, a serial of dilution was prepared.

Total plate count using PCA plate was based on Keyser *et al*. (2008) while yeast and mould count according to Nagy *et al*. (2017). Microbial load estimation for the bread samples were carried out for fresh as well as 4 days old samples. In addition, Salmonella was detected using Standard ISO 6579, 2002.

Sensory evaluation

Acceptance test was conducted and a total of 30 panels of Universiti Malaysia Terengganu (UMT) students were chosen randomly as panellists. The panellist need to evaluate five samples. The panels must evaluated four samples of white bread fortified with eggshell powder. Hedonic scale was used for the affective test. An evaluation was performed on a seven points scale with ranged score 1 (dislike extremely) and score 7 (like extremely). Six attributes were evaluated such as colour, odour, appearance, firmness, sandy taste, and overall acceptance. Samples used in the sensory test were sealed in a transparent plastic bag individually and labeled with specific code (See *et al*., 2008).

Statistical analysis

All data obtained were analysed using MINITAB statistical software package. The data obtained from physical and chemical analysis was subjected to one way analysis of variance (ANOVA). The data was stated as mean value ± standard deviation with three replications.

RESULTS AND DISCUSSION

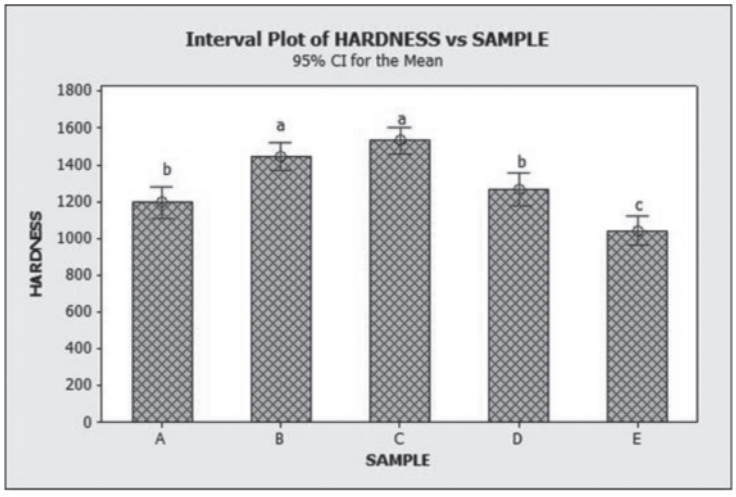
Development of white bread fortified with calcium derived from egg shell powder

Bread for formulation A showed the highest specific volume with 2.97 cm^3/g followed by bread B, E, C and D with 2.90 cm^3/g, 2.86 cm^3/g, 2.86 cm^3/g and 2.81 cm^3/g, respectively. According to the hypothesis made by Minitab 14, there was no significantly different between the samples since the p value > 0.05. It can be concluded that adding eggshell in the sample did not influence the volume of the bread. Loaf volume is regarded as the most important bread characteristic since it provides a quantitative baking performance and consumer desired breads that appear to be light and not so dense.

Physicochemical properties of the white bread

Texture

Hardness can be defined as the maximum force during the first cycle of compression (Elzabeita & Kunizka, 2011). Figure 1 shows the hardness of the bread sample according to the formulation. Bread C had the highest hardness with mean of 1533.2g followed by bread B, D, A and E with 1446.5g, 1268.0g, 1196.0g and 1043.0g, respectively. Since the hardness of the bread fluctuated, it may be due to the thickness of the bread which was not uniformly cut during the analysis. Furthermore,



[a-c] means value with different letter were significantly different ($p<0.05$), where formulation of white bread (eggshell powder, bread flour) for A = (0%, 100%), B = (2%, 98%), C = (4%, 96%), D = (6%, 94%), E = (8%, 92%)

Fig. 1. Graph of hardness against sample.

according to data that analysed by Minitab 14, the *p* value obtain was 0.00 ($p<0.05$), thus there were a significant difference between samples in terms of hardness and it can be concluded that adding the eggshell powder in the bread did affect the hardness of the bread. In addition, the hardness decreased as the percentage of eggshell powder increased due to the water absorption decreased and the movement of moisture from starch to gluten by diffusion was prevented (Leal-Calderon *et al*., 2007).

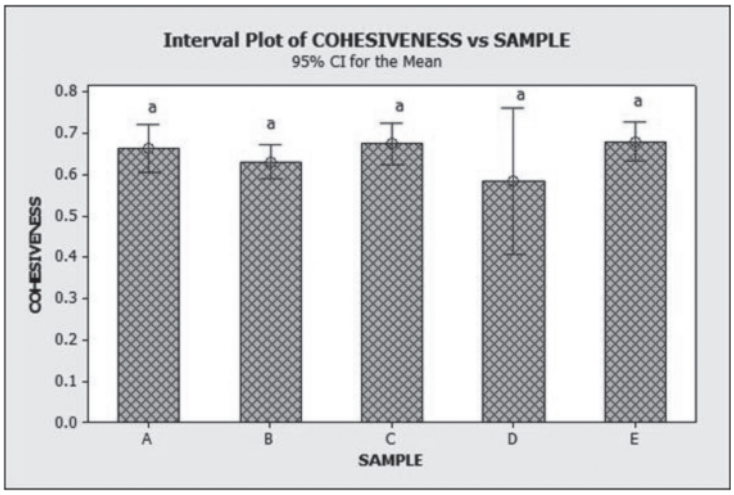
Cohesiveness is the characteristic of the forces of internal bonds which hold the product in one piece. Cohesiveness is measures by taking the ratio of the areas beneath the graphs of the first and second compression of the sample (Koh=W2/W1) (Elzabeita & Kunizka, 2011). Figure 2 shows the cohesiveness of the bread at different formulation. Sample E had the highest cohesiveness with 0.6791 followed by sample C, A, B and D with 0.6741, 0.6613, 0.6304 and 0.5838, respectively. Furthermore, there was no significant different between samples in terms of cohesiveness since p value obtain was 0.434 ($p>0.05$). Thus, it can be concluded that adding eggshell does not affect the cohesiveness of the bread.

Springiness can be defined as the degree of recovery of the initial form and it is the quotient of sample deformations during the first and second compression (Spr=L2/L1) (Elzabeita & Kunizka, 2011). Figure 3 shows the springiness of bread sample at different formulation. Formulation A had the highest springiness followed by formulation E, C, B, and D with 0.975, 0.944, 0.936, 0.930 and 0.829, respectively. In addition, since the p value is 0.323 ($p>0.05$), there was no significant difference between the sample in terms of springiness. Thus, it can be concluded that adding eggshell does not affect the springiness of the bread.

Colour

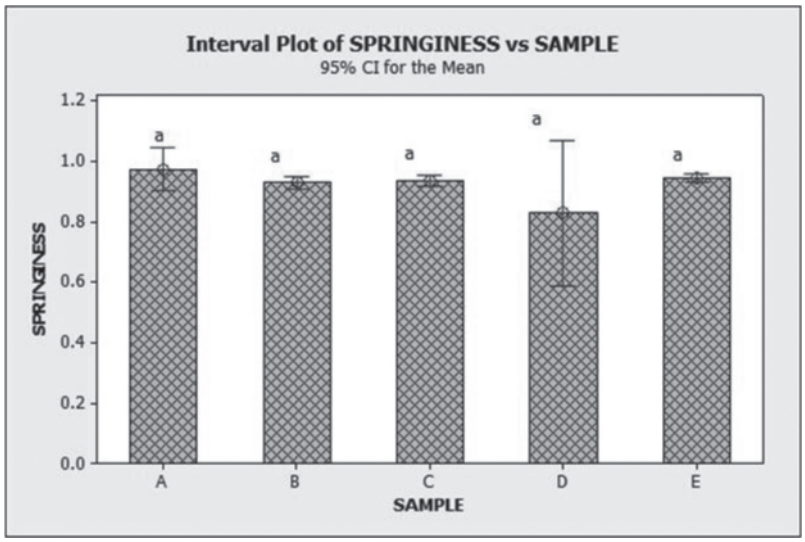
Figure 4 shows the reading of lightness (L*), greenness (a*) and yellowness (b*) of bread sample using colorimeter. Lightness also known as value or tones is a representation of variation in the perception of a colour or colour space's brightness. Baked foods such as bread often present a challenge in colour measurement due to their highly textured and non-uniform consistency. Sample C had the highest reading for lightness with 74.411 and the lowest was sample E with 64.671. Furthermore, the p value obtain was 0.00 ($p<0.05$). It can be concluded that there was a significant difference in terms of lightness between the samples and since white bread is an opaque food, it shows a high reading of lightness. However, there are many other factors that influence the bread whiteness, such as wheat pigment, grain content, and grain fineness and among those vary factor, the flour particle size is the most important (Rózyło *et al*., 2015). The variant reading of the lightness may be due to the different amount of eggshell used and the lowest lightness was the sample that has highest eggshell powder in it.

The 'a' value refers to the index of redness/greenness of the bread which + represent redness and – represent the greenness of the sample. Since the value of 'a' obtains in this study was +, the sample was more to redness instead of greenness. Sample A has the highest reading and sample C has the lowest reading. The reading increases from sample C (0.5978) to sample B (0.7867) and followed by sample D, E with same reading (0.7656) and lastly



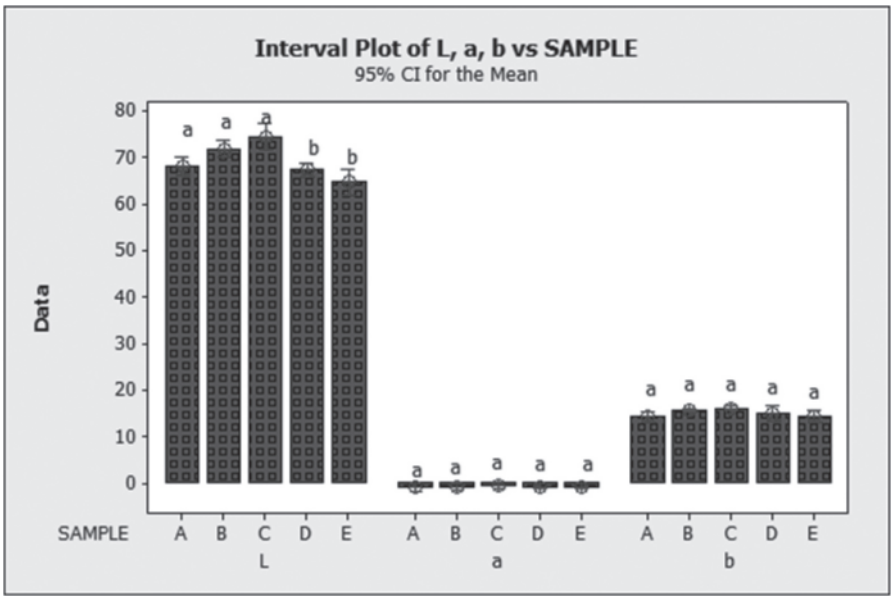
[a] means value with different letter were no significantly different ($p>0.05$), where formulation of white bread (eggshell powder, bread flour) for A = (0%, 100%), B = (2%, 98%), C = (4%, 96%), D = (6%, 94%), E = (8%, 92%)

Fig. 2. Graph of cohesiveness against sample.



[a] means value with different letter were no significantly different (p>0.05), where formulation of white bread (eggshell powder, bread flour) for A = (0%, 100%), B = (2%, 98%), C = (4%, 96%), D = (6%, 94%), E = (8%, 92%)

Fig. 3. Graph of springiness against sample.



L*= [a–b] means value with different letter were significantly different (p<0.05), a*= [a] means value with different letter were significantly different (p>0.05), b* = [a] means value with different letter were significantly different (p>0.05), where formulation of white bread (eggshell powder, bread flour) for A = (0%, 100%), B = (2%, 98%), C = (4%, 96%), D = (6%, 94%), E = (8%, 92%)

Fig. 4. Lightness (L*), Greenness (-a*), Yellowness (b*) of the bread samples.

sample A with highest reading (0.9700). However, since the p value was 0.946 (p>0.05), there was a significant difference between the sample. Furthermore, the + or – value of 'b' refers to the index of the yellowness / blueness of the bread. Sample C has the highest reading with 15.984 while sample A had the lowest reading with 14.168. However, according to the p value obtain, the sample has a significant difference since p = 0.04 (<0.05). In addition, as the development of colour occurs classically during later stages of baking, it can be used to judge the completion of baking process. Surface colour might depend on physicochemical characteristics of the raw dough for example the water content,

Table 2. Proximate analysis results for each samples

Sample	Moisture	Ash	Fat	Protein	Fibre	Carbohydrate
A	64.28,± 0.33^{a}	2.02,±0.14^{b}	1.15 ±0.17^{a}	24.3, ±0.29^{a}	1.67 ±0.43^{b}	6.53 ±0.61^{a}
B	64.70, ±0.69^{a}	2.76, ±0.22^{b}	1.96 ±1.34^{a}	22.6 ±1.78^{a}	2.53±0.84^{b}	5.37 ±1.48^{a}
C	63.38, ±0.55^{ab}	3.23, ±0.24^{ab}	2.28 ±0.84^{a}	24.41 ±1.26^{a}	3.37±0.13^{ab}	3.23±0.24^{ab}
D	61.40,±1.67^{b}	4.52, ±1.06^{a}	1.86, ±0.43^{a}	23.07 ± 1.51^{a}	3.12 ±0.20^{a}	6.01 ±1.81^{a}
E	65.15 ±0.44^{a}	4.47 ±0.43^{a}	3.80±1.81^{a}	21.14±2.33^{a}	3.82 ±0.15^{a}	1.69 ±0.30^{b}

Moisture = $^{a-b}$ in same column means value with different letter were significantly different ($p<0.05$), Ash = $^{a-b}$ in same column means value with different letter were significantly different ($p<0.05$).
Fat = ^{a} in same column means value with different letter were no significantly different ($p>0.05$).
Protein = ^{a} in same column means value with different letter were no significantly different ($p >0.05$).
Fiber = $^{a-b}$ in same column means value with different letter were significantly different ($p<0.05$), Carbohydrate = $^{a-b}$ in same column means value with different letter were significantly different ($p<0.05$).
Where formulation of white bread (eggshell powder, bread flour) for A = (0%, 100%), B = (2%, 98%), C = (4%, 96%), D = (6%, 94%), E = (8%, 92%).

pH, reducing sugars, and amino acid content and condition during baking such as temperature, air speed and relative humidity.

Chemical properties of white bread

Proximate analysis

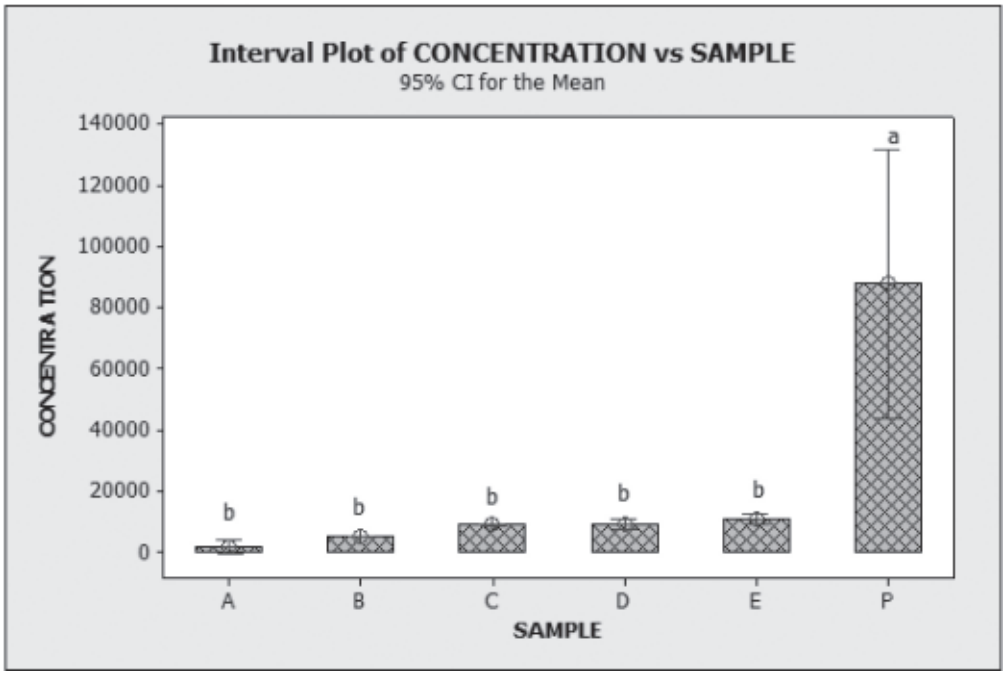
The percentage of ash content increased with the increasing of eggshell powder in the bread where sample E with the highest amount of eggshell powder showed the highest percentage of ash content. Since ash content determined the mineral content in the product, it proved that the adding eggshell powder could increase the mineral content of the bread as showed in this study. There was a significant different between sample in terms of ash content with $p = 0.03$ ($p<0.05$).

Sample C obtained the highest protein content (24.41%) compared to sample E with the lowest protein content (21.13%). However, there was no significant different between each sample in terms of protein content since $p = 0.146$ ($p>0.05$). Thus, it can be concluded that adding egg shell powder in the bread did not affect the protein content. Vijole *et al*. (2017) reported that the protein obtained in their study was 14% which was less than the protein obtained in this study. This may due to the difference of flour used in the formulation. Since bread flour had 13% protein more than wheat flour, the percentage of protein obtained was slightly higher than previous studies. Moreover, the major protein in the baking industry is gluten, a protein found in flour. Gluten plays a very important role in the world of baking. When mixing begins, the protein takes in water and swells under the absorption of the moisture and then formation of gluten forms a protein matrix that comprises the structure and support within the dough, giving elastic and extensible properties of dough (Bakerpedia, 2017).

There was no significant difference of fat content with value $p=0.122$ ($p>0.05$). The highest fat content obtained in this study was 3.8% in sample E and the lowest was 1.15% in sample A. In contrast, for both fibre and carbohydrate, there was significantly different between the sample for fibre and carbohydrate ($p<0.05$). The highest fibre content obtained in sample E was 3.83%. The percentage of fibre increased from sample A to sample C and decreased at sample C to sample D before increased again from sample D to sample E. Moreover, according to Angioloni (2011), a number of health benefits are linked to dietary fibre, among them are promoting healthier bowel function, decreasing cholesterol levels in the body and controlling blood glucose levels. Moreover, dietary fibre increases the volume of stools as well as softening them, thereby preventing constipation and at the same time, maintaining bowel health.

Sample A contained the highest carbohydrate content (6.53%). Compared to the study done by Vijole *et al.* (2017), the carbohydrate obtained was 13% which was higher than in this study. Between bread flour used in this study and the wheat flour used in previous study, bread flour had less carbohydrate content than wheat flour with 23 g and 79 g, respectively (USDA, 2017). In addition, carbohydrate supplies the body's cells with glucose, which is the basic unit of carbohydrates and an important energy source. One gram of carbohydrate gives 16 kJ or 3.75 calories of energy. At least half the energy in our diets should come from carbohydrate, mostly as complex carbohydrates.

Figure 5 shows the concentration of calcium in the bread and in the eggshell powder (P). The amount of calcium in eggshell powder was 87910 mg/ml. The amount of calcium increased as the percentage of eggshell powder added increased in bread. The amount of calcium increased from 1639 mg/ml in formulation A to 5109 mg/ml, 8885



[a–b] means value with different letter were significantly different ($p<0.05$) where formulation of white bread (eggshell powder, bread flour) for A = (0%, 100%), B = (2%, 98%), C = (4%, 96%), D = (6%, 94%), E = (8%, 92%), P= Eggshell powder

Fig. 5. Concentration of bread samples and egg shell powder.

mg/ml, 9275 mg/ml and 10772 mg/ml in formulation B, C, D, and E, respectively. It showed that the concentration of calcium was directly proportional with the percentage of eggshell powder. However, there were no significant difference in the concentration of calcium in the bread ($p>0.05$).

The amount of calcium in the sample was decreased after the eggshell had been added into the bread. This means that, there was calcium lost during the baking process. However, from this study, the calcium contains exceeds the recommended intake. The recommended intake of calcium is minimum 200 mg and maximum 1300 mg according to National Institute of Health (2016). Since the calcium in control sample had reached 1639 mg in this study, it showed that the original ingredient of the bread such as milk already contributes to the calcium in the bread. In milk, there were 912 mg of calcium and 0.51% of calcium can be found in the bread flour (USDA, 2017). Thus, it showed that the ingredient itself already consist enough calcium. However, as the control had enough calcium, by subtracting the calcium content from sample B with the control, the calcium supplied by the eggshell was 3470 mg which was still higher and exceed from the recommended intake. However, in previous study by Vijole *et al*. (2017), they found that the best quality bread was the bread with addition of 5 g of eggshell powder. According to Hassan (2015), he suggested that the best way to use chicken eggshell as dietary calcium supplement was powdered to biscuit up to 6% eggshell fortification. Thus, by conducting this study, the eggshell powder was not suitable to be added in this type of food, and the eggshell amount added was too high. However, for future study using eggshell powder in white bread, the percentage of the eggshell can be reduced or completely substituted the other calcium source such as milk.

Microbiological analysis of white bread

Table 3 shows that the bacteria were detected in all samples. Total plate count was detected between 2.3×10^3 CFU/g to 4.0×10^3 CFU/g at 0 days and then increased up to 3.5×10^6 CFU/g at 4 days. It is similar to the yeast and mould count where at 0 days showed the count between 6.1×10^1 CFU/g to 3.5×10^3 CFU/g and then increased to 3.5×10^6 CFU/g at four days. However, Salmonella was not detected in all samples. Eggshell powder which was fortified in the making of bread found to be free from bacteria. The bacteria present in the sample may be due to cross contamination from laboratory apparatus and analyst. However, Claudia, (2016) stated that the presence of yeast in the bread due to the used of yeast in the bread processing. Beside no addition of preservative, high moisture content and high nutrient content of sample also contributed to the growth of yeast and mould on four days. The shelf life of the bread can be prolonged by adding

Table 3. Total Plate Count, Yeast and Mould Count, and Detection of *Salmonella*

Sample	Total Plate Count (CFU/g)		Yeast And Mould (CFU/g)		*Salmonella* Detection
	Day 0	Day 4	Day 0	Day 4	
A	2.3 x 10^3	1.1 x 10v	7.5x 10^2	>3.0 x 10v (3.5x 10v est.)	N.D
B	2.3x 10^2	>3.0 x 10v (3.5x 10v est.)	6.7 x 10^1	>3.0 x 10v (3.5x 10v est.)	N.D
C	4.0x 10^2	>3.0 x 10v (3.5x 10v est.)	1.3x 10^2	>3.0 x 10v (3.5x 10v est.)	N.D
D	4.0x 10^3	>3.0 x 10v (3.5x 10v est.)	3.5x 10^3	>3.0 x 10v (3.5x 10v est.)	N.D
E	2.3x 10^2	>3.0 x 10v (3.5x 10v est.)	3.2x 10^2	>3.0 x 10v (3.5x 10v est.)	N.D
Eggshell powder	<1.0x 10^1	<1.0x 10^1	<1.0x 10^1	<1.0x 10^1	N.D

N.D indicates not detected
*means value with different letter were no significantly different (p>0.05) where formulation of white bread (eggshell powder, bread flour) for A = (0%, 100%), B = (2%, 98%), C = (4%, 96%), D = (6%, 94%), E = (8%, 92%).

Table 4. Sensory acceptance test for bread samples

Sample	Color	Odor	Appearance	Firmness	Sandy taste	Overall acceptability
A	5.62±0.90^a	4.89 ±1.58^a	5.55±0.87^a	4.89±1.39^a	5.41±1.52^a	5.44± 0.78^a
B	5.43±1.00^a	5.06±1.17^a	5.23±0.97^a	5.26±1.25^a	4.16±1.76^b	4.76±1.50 ^{ab}
C	5.36±1.12^a	4.56±1.33^a	7.50±12.98^a	5.16±1.31^a	3.63±1.47^b	4.43±1.30 ^{ab}
D	5.06±1.14^a	4.96±1.40^a	4.83±1.21^a	4.96±1.29^a	3.06±1.46^b	4.00±1.55^b
E	4.83±1.34^a	4.60±1.42^a	5.06±1.38^a	5.00±1.55^a	3.00±1.39^b	3.90±1.37^b

Colour = ^{a} with same column means value with different letter were no significantly different (p >0.05).
Odour = ^{a} with same column means value with different letter were no significantly different (p>0.05).
Appearance = ^{a} with same column means value with different letter were no significantly different (p>0.05), Firmness = ^{a} with same column means value with different letter were no significantly different (p >0.05), Sandy Taste = $^{a-b}$ with same column means value with different letter were significantly different (p<0.05), Overall Acceptability = $^{a-b}$ with same column means value with different letter were significantly, different (p<0.05) where formulation of white bread (eggshell powder, bread flour) for A = (0%, 100%), B = (2%, 98%), C = (4%, 96%), D = (6%, 94%), E = (8%, 92%).

of preservative such as calcium propionate, the bread can be stored in dark and cool place, tightly sealing the bread and freeze the bread (Saranraj, 2016).

Sensory acceptability of white bread

There was no significantly different colour between the samples (p>0.05). Although the acceptance of colour by the panellist, the colour of samples were decreased as the increasing of eggshell powder fortified to the formulation. Compared to the physical analysis, the lightness (L*) of the bread increased in formulation A to C and then decreased in formulation C to E. This means that the panellist was unable to differentiate the colour of the bread produced from the control, 2%, 4%, 6%, and 8% (Mashayekh, 2008). Majority of the panellist stated that colour of the bread crumb for all formulations had no difference in which the colour of bread crumb was white.

In this study, the odour attribute also showed no significant difference between the sample since p value was 0.541 (> 0.05). The highest score for the odour was sample B with 5.06 and sample C scored the lowest number with 4.56. This also means that the panellist unable to differentiate the odour of the samples. However, there were a few panellist stated that the odour quite strong with a sour smell. This was due to over fermentation of the dough during the fermentation (Mashayekh, 2008).

In terms of appearance, sample C had the highest score given by the panellist (7.50) followed by sample A, B, E and D with 5.55, 5.23, 5.06 and 4.83, respectively. There was also no significant difference between the sample in terms of appearance since p = 0.416 (>0.05). This showed that the panellist unable to differentiate the appearance of the bread with different formulations. It also indicated that adding eggshell in the bread did not affect the porosity of the bread.

There was no significant difference between the sample in terms of firmness since p = 0.830 (> 0.05). Sample B had the highest score for the appearance with 5.267 while the sample A scored the lowest with 4.89. Comparing the texture among samples, sample C had the highest reading for hardness. Texture of the bread can be identified by touching, the degree to which it was rough or smooth, hard or soft (Sanful, 2011). The texture was mainly influenced by the protein content and quality which

was used in the evaluation as an indicator of food quality (Mashayekh, 2008).

There is a significant different between sample since p = 0.00 (<0.05) for the attribute of sandy taste. Sample A obtained the highest score from panellist with 5.41 followed by sample B, C, D and E with 4.16, 3.63, 3.06 and 3.00, respectively. The scored of acceptance obtained from the panellist in terms of sandy taste decreased as the percentage of eggshell added increase. Compared to previous study by Vijole *et al*. (2017), their study showed that there was no grainy taste, however, in this study the score obtained quite low and the grainy taste quite appealing. This was due to the size of the powder was not small enough. Since eggshell functioning as a container for the egg, providing protection of the contents and a unique package for a valuable food (Hunton, 2005), the structure of eggshell was quite hard and need to be grind into very small size to avoid the grainy taste.

Lastly, for overall acceptability, there was a significant difference between the sample since p = 0.00 (< 0.05). Sample A obtained the highest score from the panellist with 5.44 followed by B, C, D and E with 4.76, 4.43, 4.00 and 3.90, respectively. This showed that the score obtains decreased as the eggshell added increased and this showed that panellist preferred the sample A with 0% of eggshell powder compared to the previous study done by Vijole *et al*. (2017) and Hassan (2015) that the acceptability of the sample was at addition with 5g and addition of 6% of eggshell powder respectively.

CONCLUSION

White breads fortified with calcium derived from the eggshell were developed successfully with the different percentage of eggshell (0%, 2%, 4%, 6% and 8%). Physical properties (specific volume, texture and colour), chemical properties (moisture, ash, fat, protein, fibre, carbohydrate, and calcium content) and shelf life of the bread were determined. The result showed that increasing of the eggshell added did not have any impact on the specific volume of the bread. However, the addition of eggshell affected the hardness of the bread but not the springiness and cohesiveness of the bread. The addition of the eggshell also affects the L* but not a* and b*. Furthermore, the addition of the eggshell significantly (p<0.05) increased the moisture content, ash, fibre and significantly decreased the carbohydrate. However, there were no significantly different between the sample in terms of fat and protein. Calcium content increased significantly as the percentage of the eggshell powder increased and the shelf life of the bread was 4 days. Lastly, there was no significant difference in the acceptance of the colour, odour, appearance and firmness of the samples, but for the sandy taste and overall acceptability, the panellist most preferred the sample with 0% eggshell (control) than other formulation. The results showed that bread fortified with eggshell has potential to be produce and accepted by the panellist especially formulation B with addition of 2% of eggshell powder. The diversification usage of eggshell will reduce waste and thus ensure the environmental cleanliness.

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