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PHYSICOCHEMICAL AND SENSORY PROPERTIES OF BREAD ADDED WITH CHICKEN EGGSHELL POWDER

(Fizikokimia dan Sifat Sensori Roti Ditambah dengan Serbuk Kulit Telur Ayam)

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Abstract

The purpose of this study was to determine the physicochemical properties and acceptability of bread with added chicken eggshell powder at 2%, 4% and 6%. It was found that the eggshell powder consisted ash (94.4%), fibre (50.77%) and protein (3.66%). Additionally, the eggshell powder had high calcium content, which was 27074.50 mg/100 g. The crude protein and ash content in bread increased as eggshell powder supplemented increased. The addition of eggshell powder in bread increased the calcium content present in bread significantly at 219.97 mg/100 g, 418.93 mg/100 g and 646.7 mg/100 g at 2%, 4% and 6%, respectively. Volume of bread added with eggshell powder was significantly higher compared to control. Crumb colour was observed to be increasingly yellowish while the crust appears darker with increasing level of supplementation. There was increased hardness of bread after addition of eggshell powder according to the result obtained through texture profile analysis. Sensory panellists preferred bread with 2% eggshell powder added compared to others based on overall acceptability attribute.

Keywords: bread, calcium, sensory properties, chicken eggshell

Abstrak

Tujuan kajian ini adalah untuk menentukan komposisi kimia kulit telur, sifat fizikokimia roti dan penerimaan roti yang ditambah dengan serbuk kulit telur pada 2%, 4% dan 6%. Analisis yang dilakukan pada serbuk kulit telur adalah abu, serat, protein mentah, lemak mentah dan kandungan mineral. Telah didapati bahawa kulit telur mengandungi tinggi kandungan abu iaitu sebanyak 94.40%, serat 50.77% dan diikuti oleh protein 3.66%. Serbuk telur ayam mengandungi kalsium sebanyak 27074.50 mg/100g. Bagi analisis roti, kandungan protein dan abu mentah meningkat dengan ketara seiring dengan peningkatan serbuk telur yang ditambah. Penambahan serbuk kulit telur meningkat dalam kandungan kalsium iaitu 219.97, 418.93 dan 646.7 mg/100g masing-masing pada 2%, 4% dan 6%. Bagi analisis fizikal roti, isipadu roti yang ditambah dengan kulit telur lebih tinggi berbanding dengan sampel kawalan. Warna serbuk ke arah kekuningan manakala warna kerak ke arah gelap dengan peningkatan tahap suplemen. Terdapat peningkatan terhadap kekerasan roti selepas penambahan serbuk kulit telur menurut hasil analisis profil tekstur. Panelis deria memilih roti dengan penambahan serbuk kulit telur sebanyak 2% adalah yang terbaik berbanding yang lain.

Kata kunci: roti, kalsium, sifat sensori, kulit telur ayam

Introduction

Chicken egg is considered a cheap resource that contain nutrients such as protein, fat and vitamin. The eggshell structure is composed of inner and outer shell membrane, mammillary layer, palisade layer, vertical crystal and cuticle. Weight wise the eggshell is 9-12% of the total weight of egg and majority of that is made of 94-96% calcium carbonate and other trace microelements [1]. As such, the eggshell can be a source of calcium to serve as a form of human nutrition by adding into calcium-fortified food. Additionally, it was shown that calcium carbonate ($CaCO_3$) derived from eggshell in the form of powder is easier absorbable compared to commercial $CaCO_3$ in small intestine of rat [2]. Since eggshells are not usually consumed as part of diet, which only includes egg white and yolk, the eggshells that are typically considered as waste would be an excellent source of calcium, which can be converted, and serve as a beneficial contribution to human nutrition.

Until recently, calcium deficiency in the human diet remains a common problem. Calcium is the mineral needed by human body that maintains the bone tissue. Calcium will combine with other mineral such as phosphorus to provide the structural component of bone and teeth [3]. As a probable solution to the problem, a study has shown positive feedback where an increase value of bone density to osteoporotic women was observed with a diet supplemented with eggshell powder together with vitamin D and magnesium [4]. In food application, it was suggested that the eggshell powder could be added in breads and pizzas as it was found that the powder has minimal effect on the texture while not affecting the flavour [5].

Bread is a bakery product that is made from moistened wheat flour, then undergoes kneading and baking. Bread is a popular food that widely consumed all over the world especially in the Mediterranean and Europe region [6]. Moreover, according to Malaysian Adult Nutrition Survey, the baked products consumption particularly biscuit and bread appeared in the list of top ten daily consumed although rice and noodles are the main dish to consume during breakfast, lunch and dinner in Malaysia. Due to its high consumption, incorporating eggshell powder into bread could be a good potential as a model diet in meeting calcium requirements. Hence, the objectives of this study were to determine the physicochemical properties and acceptability of bread added with eggshell powder at 2%, 4% and 6%, respectively. Analysis conducted on eggshell powder was ash, fibre, crude protein, crude fat, moisture and mineral content. Physicochemical properties needed to characterise the bread added with eggshell powder, meanwhile, acceptability test can be used to evaluate the sensory attributes of bread incorporated with eggshell powder.

Materials and Methods

Preparation of eggshell powder

Chicken eggshells used as raw materials was obtained from café at Dataran Cendikia UiTM Shah Alam. The other ingredients that was used in the bread production was obtained from Two Ply, Bakery supply store, Klang, Selangor. The chicken eggshell was washed twice and boiled using deionised water for 30 minutes. Then, the chicken eggshell was dried in cabinet drier for 24 hours at 60 °C. The dried eggshell was grounded into powder by using blender. Lastly, ground eggshell powder was passed through 120 µm sieve before use [1].

Production of bread

Bread was prepared by using previous method described by Makinde & Akinoso [6]. The ingredients together with eggshell powder in different compositions of 2%, 4% and 6% was mixed for 5 minutes in a mixer. Then, the mixture was rested for about 30 minutes in order to relieve residual stress occurred during mixing. After resting, the mixture dough was moulded into cylindrical shape to fit into an aluminium container. The prepared dough was proofed in baking pans for 40 minutes at 35 ^{o}C. The dough was then transferred into oven and baked at 180 ^{o}C for 30 minutes. Similarly, a control was prepared using the same method but without the addition of eggshell powder.

Microbial evaluation of chicken eggshell powder

Microbial evaluation was conducted according to method described by Umar et al. [7]. The eggshell powder was diluted serially. One gram of eggshell powder was weighed and placed in a test tube containing 9 mL buffered peptone water. The solution was mixed in order to make a dilution 1:10 (10^{-1}). Then 1 mL from the 1:10 was transferred to the other test tube containing 9 mL buffered peptone water to make dilution of 1:100 (10^{-2}). This procedure was repeated until a dilution 1:1000 (10^{-3}) was obtained. Thereafter, 0.1 mL from each dilution solution was inoculated onto the *Salmonella-Shigella* agar (SS agar). The inoculum was streaked on the media and incubated in inverted position at 30 ^{o}C for 72 hours. Then, the number of colonies was counted. This dilution procedure was conducted in three independent replicates.

Chemical composition

Moisture, crude protein, crude fat, ash and fibre were determined according to the standard methods described by AOAC 2000 [8].

Mineral composition

The mineral content was determined according to Kumaravel & Alagusundaram [9]. In wet digestion, 0.5 g of sample was added with 10 mL concentrated nitric acid ($HN0_3$) and the mixture was left overnight. Then, the mixture was digested on hotplate until red fumes evolve from the mixture start to ease. Next, 1 mL of hydrogen peroxide (H_2O_2) was added to the mixture and the heating was resumed. The mixture was heated until a clear solution was obtained. The mixture was then filtered and marked up to 50 ml distilled water. Multi-element standard was prepared by diluting stock standard to desired concentration, which was 0.2, 0.4, 0.6, 0.8 and 1.0 mg/L with deionized water. Minerals content of the bread and eggshell powder which are calcium (Ca), magnesium (Mg), sodium (Na), potassium (K) and Strontium (Sr) was determined by using Inductively Couple Plasma (ICP).

Specific volume of bread

Specific volume was measured by using small seeds displacement method described previously by Al-Saleh & Brennan [10]. Rapeseed was poured into a container of known volume until the bottom was covered. The loaf was placed inside the container and then seed was filled to the top. The extra rapeseeds, which equals to the loaf volume was measured in a graduated cylinder.

Colour measurement

The colour of bread was measured by using method explained by Makinde & Akinoso [6]. The crust and crumb colour were measured using chroma meter CR-400 (Konica Minolta, Japan). The sample was cut into cubes 2 x 2 x 2 cm and on the chroma meter. The colour attributes L, a and b was recorded using the Spectramagic NX software (CM-S100w).

Texture profile analysis

Texture profile analysis of the bread was conducted by using the Stable Micro System Texture Analyser (Model TA.XT2 Plus Texture Technologies Corp., Scarsdale, New York and U.S.A). The compression was performed by using a 75 mm cylindrical probe and resulting deformation curve was analysed for hardness, springiness, gumminess, cohesiveness and chewiness parameter [8].

Sensory analysis

Sensory analysis on the acceptability of bread was carried out using 30 untrained panellists. The freshly baked bread with four coded sample from different formulations was served to the panellists. The panellists were asked regarding their acceptance to the product in the terms of aroma, softness, taste, colour and overall acceptability. The score was evaluated based on nine points hedonic scale where the value of "9" indicates extremely like and "1" indicates extremely dislike [11].

Statistical analysis

The statistical analysis was determined by using the Statistical Package for the Social Sciences (SPSS, version 25) software. The data analysis was carried out using analysis of variance (ANOVA) and significant differences at (p <0.05) which was determined by Duncan's multiple range test (DMRT).

Results and Discussion

Characterisation of eggshell powder

Table 1 showed the result of chemical composition of eggshell powder. There were significant differences between all the parameters. From the results obtained, the eggshell powder contained ash (94.40%), fibre (50.77%) and protein (3.66%). The ash content from this study was similar from a previous study [12]. Meanwhile, previously, the ash content recorded in eggshell powder was 94.28% and 90.2% [1]. From Table 1, the results showed the mineral content had significant differences between each element. From all elements, calcium recorded that highest value which was 27074.50 mg/100 g. It was followed by magnesium, sodium, potassium and strontium with values of 334.57, 55.64, 33.04, 11.36 mg/100 g, respectively. The high amount of calcium was contributed by the composition of eggshell that was majorly composed of calcium carbonate at about 96% [13]. Additionally, another study reported that 3700 mg/100 g (Ca) and 500 mg/100 g (Mg) were found to be contained in eggshell powder [14]. Elemental analysis in eggshell powder is highly important as it defines which element is present in abundance that can be utilised in food application, in this research was bread particularly. This utilisation can target to minimise calcium deficiency among Malaysians, if the bread production is taken up to large scale to meet the demand for human consumption.

As for microbial analysis of eggshell powder, the bacterial count was done on SS agar. The total bacterial counts on SS agar showed negative results for the eggshell powder after boiling treatment was carried out. This finding agrees with literature [1], in that different treatments done to the eggshell powder (boiling, auto clave, microwave, oven) showed negative results on microbial growth on SS agar.

Characterisation of bread added with chicken eggshell powder

Based on the results showed in Table 2, the protein content had significant differences between all the formulations. The protein and ash content in bread significantly increased with the increased level of supplementation of eggshell powder. Both protein content (10.65%) and ash content (6.45%) were recorded the highest compared to other formulations in 6% of eggshell powder supplementation.

The mineral content in supplemented bread increased for all mineral elements measured and demonstrated significant differences between all formulations, which also meant that the amount of mineral significantly increased. The amount of calcium greatly increased at 2% supplementation compared to control, which was from 31.82 mg/100 g to 219.97 mg/100 g. This increase was consistently observed for 4% and 6% eggshell powder addition, which was to 418.93 mg/100 g and 646.71 mg/100 g, respectively. The observed increase of calcium content in this study agrees with literature [12], where a similar increase in calcium content was observed in chocolate cakes from 504.5, 816.8 and up to 1364.5 mg/100 g when supplemented with 3%, 6% and 9% eggshell. Based on the results obtained, it can be seen that the calcium content in bread was greatly influenced by high level of calcium in eggshell powder.

Then, the amount magnesium in bread increased in the range of 30.58 mg/100 g to 64.99 mg/100 g from control up to 6% supplementation of eggshell powder. Besides calcium and magnesium, sodium was high in supplemented bread where the 6% addition of eggshell powder recorded 408.21 mg/100 g compared to 126.33 mg/100 g in control formulation. For potassium content, the control bread showed 81.25 mg/100 g then increased to 108.83 mg/100 g after addition of 2% of eggshell powder. Then it increased to 110.50 mg/100 g and further to 116.77 mg/100 g in 4% and 6% supplementation, respectively. Strontium content in control also increased from 0.56 mg/100 g to 1.68 mg/100 g after 6% supplementation of eggshell powder. Similar higher levels of calcium, magnesium and potassium was also found previously in leavened bread with 1545.3%, 192.76% and 141.63%, respectively after added with 1.5% eggshell powder [15].

Specific volume showed that there were significant differences between all formulations of breads (Table 3). The specific volume of bread increased from control to 6% supplementation of eggshell powder, which ranged from 3.1 cm^3/g to 3.36 cm^3/g. The results agree with previous study that indicated the volume of cake

increased as the amount of eggshell powder added increased [12]. Naturally, the increase of loaf volume might be due to water level and high-water absorption in the dough [16]. However, protein content present in the dough could also affects water absorption where the increasing protein quality and quantity can result in high water absorption, which leads to higher loaf volume [17].

Table 3 also showed the result of breadcrumb and crust in the terms of L*, a* and b*, respectively. In terms of crumb colour, b* value was not significantly different between control and 2% supplementation of eggshell powder. However, b* values increased when 4% of eggshell powder was added compared to 2% which was from 13.05 to 15.00 then followed by 18.6 for addition of 6% eggshell powder in formulation. The increased b* values indicated the crumb was towards yellowness in colour. It agrees with previous findings [6], that the b* values was increased in crumb colour due to colour of black sesame flour. As for the crust colour, the L* values of supplemented bread with eggshell powder showed significantly lower values compared to the control. The lower L* values means that the crust is in darker colour after supplementation. The increased in darkness could be influenced by the protein content of bread and thereby affects the Maillard reaction as a result caused by the reaction between protein added and sugar. Therefore, formulation containing more protein can trigger the Maillard reaction resulting in brown colour seen on crust [18]. On the other hand, the a* values (redness) of the bread crust is higher than control formulation which was contrarily with b* values (yellowness), in that the supplemented formulation was seen to decrease from control. The surface colour depends on the physico-chemical characteristic of dough in terms of water content, pH, reducing sugar and amino acid [19]. In addition, the operating conditions during baking (temperature, humidity) could also affects the colour.

The results of texture profile analysis showed in Table 3, where the supplementation of eggshell powder significantly affects the textural properties of bread in terms of hardness, gumminess and chewiness. The hardness of bread significantly increased with increasing addition of eggshell powder in bread formulation. The hardness of bread increased from 4.58 N in control to 5.27 N, 5.42 N and 5.69 N in 2%, 4% and 6% supplementation, respectively. The increased hardness may be due to high ash content. The hardness also might be due to interaction between gluten and fibrous material [11]. The chewiness for bread supplemented with 6% eggshell powder was significantly higher compared to other formulations. Gumminess is energy needed to disintegrate a semisolid and ready to swallow while chewiness is energy needed to chew until ready to swallow [20]. Addition of natural calcium interferes with the protein-protein interaction or protein solubility thereby affecting the gumminess and chewiness [21].

Sensory evaluation of bread added with chicken eggshell powder

Table 4 indicated the summary of scores of hedonic sensory evaluations for colour, aroma, texture taste and overall acceptability. The colour of 6% supplementation had significant difference with control and 2% addition of eggshell powder. This might be due to the contrast in the yellowish colour of breadcrumb present in bread added with eggshell powder while the control demonstrated whitish in colour in which the whitish colour was more familiar to the panellists (Table 3). In the term of aroma, the bread with added eggshell powder had no significant differences with each other but was significantly different with control formulation. The higher calcium added resulted in stronger fishy smell to the product [2].

There were significant differences between all formulations in terms of texture, taste and overall acceptability. The texture of bread was significantly decreased from 2% to 4% followed by 6% of eggshell powder supplementation which was 7.20, 6.03 and 5.03, respectively. The texture maybe greatly affected by the grittiness of eggshell powder that distracted the sensation while chewing. Then, in terms of taste, the trend was similar with texture where a decrease in the mean score was observed from control up to 6% eggshell powder addition in bread formulation. As for overall acceptability, the 2% eggshell powder bread formulation had the significantly higher score (7.37)

compared to other bread formulations. This showed that the addition of eggshell powder in bread gave negative effect generally to the sensory as in agreement with study by [22], where it was stated that the addition of $CaCO_3$ should not exceed a certain level as the it will negatively affect the organoleptic properties. This was further supported in another study where 1.2% addition of chicken eggshell powder in a biscuit formulation revealed better overall acceptability in sensory evaluation among panellists [23]. Meanwhile, overall acceptability and taste attribute for leavened bread was higher when added with 1.5% chicken eggshell powder compared to other treatments such as 1.0% and 0.5% [15].

Table 1. Chemical composition of eggshell powder

Parameter (%)	**Eggshell powder (ESP)**
Ash	94.40 ± 0.03^{a}
Crude Protein	3.66 ± 0.10^{c}
Crude Fat	0.02 ± 0.10^{e}
Moisture	0.55 ± 0.02^{d}
Crude Fiber	50.77 ± 0.12^{b}
Elements (mg/100g)	**Eggshell powder (ESP)**
Ca	27074.50 ± 0.50^{a}
Mg	334.57 ± 0.62^{b}
Na	55.64 ± 0.44^{c}
K	33.04 ± 0.14^{d}
Sr	11.36 ± 0.08^{e}

*Mean values with different with different superscript letter(s) within each row differ significantly (p <0.05).

Table 2. Chemical composition of bread for all formulations

Parameter	**Eggshell powder addition (%)**			
	Control	**F1 (2%)**	**F2 (4%)**	**F3 (6%)**
Protein	9.87 ± 0.04^{d}	9.99 ± 0.08^{c}	10.39 ± 0.06^{b}	10.65 ± 0.05^{a}
Ash	1.29 ± 0.07^{d}	3.13 ± 0.01^{c}	4.65 ± 0.02^{b}	6.45 ± 0.02^{a}
Elements	**Minerals (mg/100g)**			
	Control	**F1 (2%)**	**F2 (4%)**	**F3 (6%)**
Ca	31.82 ± 0.10^{d}	219.97 ± 0.78^{c}	418.93 ± 0.50^{b}	646.7 ± 0.36^{a}
Mg	30.58 ± 0.28^{d}	40.14 ± 0.40^{c}	50.24 ± 0.50^{b}	64.99 ± 0.33^{a}
Na	126.33 ± 1.53^{d}	296.7 ± 1.74^{c}	332.77 ± 0.06^{b}	408.21 ± 0.69^{a}
K	81.25 ± 0.35^{d}	108.83 ± 0.42^{c}	110.50 ± 0.42^{b}	116.77 ± 0.12^{a}
Sr	0.56 ± 0.00^{d}	0.98 ± 0.01^{c}	1.29 ± 0.01^{b}	1.68 ± 0.02^{a}

*Mean values with different with different superscript letter(s) within each row differ significantly (p <0.05). (F1= 2% addition eggshell powder, F2 addition eggshell powder, F3= 6% addition eggshell powder).

Table 3. Physical composition of bread for all formulations

Parameter	Eggshell Powder Addition (%)			
	Control	**F1 (2%)**	**F2 (2%)**	**F3 (3%)**
Specific volume	3.13 ± 0.01^{d}	3.27 ± 0.01^{c}	3.39 ± 0.05^{a}	3.36 ± 0.04^{b}
L* value (crumb)	73.25 ± 0.12^{b}	73.80 ± 0.13^{a}	72.18 ± 0.03^{c}	73.12 ± 0.02^{b}
L* value (crust)	60.36 ± 0.03^{a}	53.41 ± 0.02^{c}	55.60 ± 0.15^{b}	55.35 ± 0.02^{b}
a* value (crumb)	-0.84 ± 0.01^{a}	-0.65 ± 0.00^{c}	-0.53 ± 0.01^{b}	0.62 ± 0.01^{d}
a* value (crust)	15.62 ± 0.17^{c}	17.22 ± 0.01^{a}	17.21 ± 0.06^{a}	16.11 ± 0.06^{b}
b* value (crumb)	13.12 ± 0.01^{c}	13.05 ± 0.02^{c}	15.00 ± 0.18^{b}	18.26 ± 0.01^{a}
b* value (crust)	37.32 ± 0.09^{a}	36.04 ± 0.29^{b}	35.55 ± 0.06^{c}	35.60 ± 0.02^{c}
Hardness (N)	4.58 ± 0.01^{d}	5.27 ± 0.01^{c}	5.42 ± 0.00^{b}	5.69 ± 0.00^{a}
Springiness (m)	0.92 ± 0.00^{a}	0.93 ± 0.00^{a}	0.92 ± 0.01^{a}	0.92 ± 0.00^{a}
Cohesiveness	0.83 ± 0.01^{a}	0.88 ± 0.00^{a}	0.76 ± 0.00^{b}	0.85 ± 0.04^{a}
Gumminess (N)	3.76 ± 0.00^{d}	4.74 ± 0.00^{a}	4.26 ± 0.00^{b}	4.11 ± 0.01^{c}
Chewiness (J)	3.45 ± 0.00^{c}	3.37 ± 0.01^{d}	3.58 ± 0.01^{b}	3.75 ± 0.01^{a}

*Mean values with different with different superscript letter(s) within each row differ significantly (p <0.05). (F1= 2% addition eggshell powder, F2 addition eggshell powder, F3= 6% addition eggshell powder).

Table 4. Sensory evaluation of all bread formulations

Formulation	Control	F1 (2%)	F2 (4%)	F3 (6%)
Colour	7.43 ± 0.90^{a}	7.23 ± 0.89^{ab}	6.70 ± 1.21^{bc}	6.63 ± 1.38^{c}
Aroma	7.40 ± 1.04^{a}	7.17 ± 1.02^{ab}	6.80 ± 1.06^{b}	6.60 ± 1.22^{b}
Texture	7.93 ± 0.94^{a}	7.20 ± 1.19^{b}	6.03 ± 1.27^{c}	5.03 ± 1.40^{d}
Taste	8.00 ± 1.14^{a}	7.33 ± 1.09^{b}	6.47 ± 1.20^{c}	5.33 ± 1.35^{d}
Overall acceptability	8.13 ± 0.94^{a}	7.37 ± 1.00^{b}	6.43 ± 1.19^{c}	5.70 ± 1.24^{d}

*Mean values with different with different superscript letter(s) within each row

**(F1= 2% addition eggshell powder, F2 addition eggshell powder, F3= 6% addition eggshell powder).

Conclusion

Based on results, the calcium content showed highest value present in the chicken eggshell powder, which was 27074.50 mg/100g. As for bread, the highest amount of elements recorded was calcium, which increased from 31.82 mg/100g (control) to 646.7 mg/100g (6%). In the analysis of texture profile analysis, there were significance differences between all formulations in the term of hardness, gumminess and chewiness. In terms of sensory acceptability in bread, among all the formulations, 2% of eggshell powder supplementation was considered as the best amount to be added as it could provide better nutritional values in bread while still acceptable in sensory evaluation. The findings from this study can further be explored for further fortification of calcium in bread products, which can be up-scaled to minimise the calcium deficiency problem in Malaysia.

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