The Influence of Eggshell Powder on the Physicochemical and Sensorial Attributes of Gluten-Free Mung Bean Biscuits

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Abstract. The extensive production of chicken eggshell (ES) waste in the egg industry and kitchens necessitates finding sustainable solutions. ES is known for its high amount of calcium. While previous studies have explored the physicochemical and sensorial attributes of ES addition in gluten-containing foods like muffins, white bread, and bread strips, to the best of our knowledge, research on the impact of ES addition in gluten-free foods has not been reported. Therefore, this study aimed to evaluate the physicochemical properties and sensorial acceptance of gluten-free mung bean biscuits (MBB) with the addition of ES. Textural, moisture, water activity, and sensory (ranking test) analyses were conducted for MBB with varying ES amount (0 to 9%). Notably, the addition of ES led to significant increases in maximum force required to break MBB (36 to 45 N). In contrast, the ES addition reduced the moisture content (6.47 to 4.87%) and water activity (0.21 to 0.17) of MBB. The sensorial ranking test showed no significant preference differences between MBB containing 0% and 9% ES. This research provides valuable insights into the potential of ES addition for producing calcium-enriched, gluten-free MBB for people with non-celiac gluten sensitivity. These findings suggest that future research should explore the broader applicability of ES in various gluten-free products.

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1 Introduction

Chicken eggs are a staple food used in both domestic and commercial settings worldwide. Egg production generates a by-product, the eggshell (ES), which makes up about 10% of the egg. The ES contains two main components: water (2% by weight) and dry matter (98% by weight) [1]. ES contains 94% calcium carbonate with approximately 40% calcium, and this compound can cause an increase in dough consistency when heated and cooled, hence, ES is useful in the food manufacturing industry, including biscuit-baking industry [2-4].

In Malaysia, biscuits are a very popular food due to their convenience and are among the top ten most frequently consumed foods by Malaysian adults [5]. A total of 16.3% of Malaysians were found to consume an average of five (5) biscuits per day [6]. One of the most famous biscuits in Malaysia is the Mung bean biscuit (MBB) [7].

The main ingredient in MBB is mung beans (*Vigna radiata*) flour. This makes MBB a healthier snack for young children and pregnant women [8]. The addition of ES powder into food offers significant benefits and turn them into calcium-fortified products [4]. By the addition of the ES powder, the food becomes a more nutritious option, providing consumers with an easily absorbable and natural source of calcium.

Various research works have been conducted to study the effect of ES addition on the food products containing gluten, such as muffins [9], white bread [10] and bread strips [11]. However, limited investigation has been done on the effect of ES addition on gluten-free foods, such as MBB. In addition, the demand for gluten-free foods has increased in recent years, especially among gluten-intolerant individuals (1 in every 100) and healthy lifestyle advocates [12, 13]. Thus, in this study, the influence of ES powder on the physicochemical and sensorial attributes of gluten-free mung bean biscuits was investigated.

2 Materials and methods

The chicken ES used in this study were sourced from a flatbread store in Kuala Pilah. The store owner purchased Omega Plus Lutein eggs (Gred B) from a local supermarket and the ES waste was collected by the owner in a designated container. Mung bean (MB) (Econsave Brand) and sugar (CSR brand) were purchased from a local supermarket in Kuala Pilah, Negeri Sembilan, Malaysia

2.1 Preparation of ES powder

Firstly, the ES was cleaned using distilled water. Next, inner membranes were carefully removed from the ES manually. Then, the membrane-free ES was sterilised in water at 100°C for 20 minutes. After sterilisation, the ES was broken into small pieces and dried in a cabinet dryer at 60°C for 6 hours. Finally, the dried ES were ground using a grinder (Mill power, RT-02, Taiwan) and passed through a 30 µm mesh. Finally, the fine ES powder was placed in a high-density polyethylene (HDPE) bag and stored at room temperature (23°C to 26°C) for future use [14].

2.2 Preparation of the biscuits

The ingredients including MB flour, sugar, ES powder, and water were weighed using an analytical balance (Adam, PW254, UK). The formulations are shown in Table 1. The ingredients for each formulation were then mixed and moulded separately. The moulded biscuits were baked in an oven (Two Thousand, TT-0199, China) at 150°C for 20 minutes. Subsequently, the biscuits were allowed to cool before being transferred into airtight containers. The eggshell mung bean biscuit (ESMBB) were stored in airtight container for future analysis.

Table 1: Formulations of MBB with different amount of ES powder (0 to 9%)

Sample	Mung Bean Flour (%)	Powdered Sugar (%)	Water (%)	ES powder (%)
ESMBB (0%)	66	30	4	0
ESMBB (3%)	63	30	4	3
ESMBB (6%)	60	30	4	6
ESMBB (9%)	57	30	4	9

2.3 Texture analysis

The Texture Analyser (TA-XT Plus, Stable Micro Systems, Godalming, Surrey, UK) with Exponent software (configured for measuring the hardness and fracturability of shortbread and ginger nut biscuits) was used to analyse the texture of the ESMBB. The texture analyser was calibrated with a 2 kg load cell using the following parameter: a test speed of 10 mm/s, post-test speed of 10 mm/s, and a test distance of 15 mm. The SMS P/2N needle puncture probe was employed, and the maximum force required to break the samples was recorded [15].

2.4 Moisture and water activity analyses

The moisture content of the sample was determined using a moisture analyser (ADAM, PMB-53, UK), Approximately 2 g of the sample was evenly distributed in the sample pan, and the moisture analyser heated (120°C to 130°C) for about 13-15 minutes until a stable reading was obtained. The moisture content was then recorded.

Water activity analysis of MBB was conducted using a water activity meter (AQUA LAB, 4TE, USA). Approximately 5 g of sample was placed in the vessel and inserted into the meter. The temperature during the analysis ranged from 24°C to 27°C. The duration for each analysis varied between 15 and 30 minutes.

2.5 Ranking test

The ranking preference test of MBB samples was conducted using Commercial (purchased from a local shop selling traditional cookies), ESMBB (0%), and ESMBB (9%) were performed. Thirty partially trained panellists were provided with three (3) MBB samples, each labelled with a random three-digit number. The panellists assessed the sensory qualities of the samples and ranked them from 1 (least preferred) to 3 (most preferred). During the testing session, panellists were required to rinse their mouths before each tasting session and between sampling different products. Additionally, panellists were instructed to taste the samples from left to right. All scores provided by the panellists were compiled, and both the total and average scores were calculated [16].

2.6 Statistical and data analysis

Each sample was tested in triplicate for all the analyses. Statistical evaluations of the results were performed using the Statistical Package Social Sciences (SPSS) software. Turkey's test was used to analyse the significance differences of the data.

3 Results and discussion

The ESMBB with different levels of ES addition (0%, 3%, 6%, and 9%) were measured using texture analyser, the maximum force values required to break the ESMBB samples are reported in **Error! Reference source not found.**.

Table 2: Maximum force values required to break the ESMBB ((0%, 3%, 6%, and 9%) samples

Analysis	Sample	n	Mean
Maximum Force (N)	ESMBB (0%)	3	36 ± 1 ^{b}
	ESMBB (3%)	3	38 ± 1 ^{b}
	ESMBB (6%)	3	43 ± 2 ^{a}
	ESMBB (9%)	3	45 ± 2 ^{a}

Note: Different lowercase superscripts (a, b) indicate significant differences ($p<0.05$) between samples within the same column

The results showed that the maximum force increased with higher concentrations of ES powder in the samples. 6% and 9% ESMBB samples were significantly different from the 0% and 3% samples but not from each other. This suggests that the addition of ES powder enhances the structural integrity and firmness of the gluten-free ESMBB samples. Although ESMBB is gluten-free, this finding is similar to previous research that reported an increase in the hardness of gluten-containing bread with the addition of ES [17].

The moisture content and water activity of the ESMBB are shown in Table .

Table 3: Moisture and water activity of ESMBB (0 to 9%)

Sample	n	Moisture (%)	Water activity (a_w)
ESMBB (0%)	3	6.47 ± 0.53 ^{a}	0.21 ± 0.01 ^{a}
ESMBB (3%)	3	5.98 ± 0.35 ^{a}	0.19 ± 0.01 ^{a,b}
ESMBB (6%)	3	4.94 ± 0.23 ^{b}	0.16 ± 0.01 ^{b}
ESMBB (9%)	3	4.87 ± 0.36 ^{b}	0.17 ± 0.01 ^{b}

Note: Different lowercase superscripts (a, b) indicate significant differences ($p<0.05$) between samples within the same column

The moisture content of ESMBB samples ranged from 4.87% to 6.47%. The highest moisture content was observed in the ESMBB (0%) sample (6.47 ± 0.53%), while the lowest was in the ESMBB (9%) sample (4.87 ± 0.36%). The data show that the moisture content of the samples decreased as the ES powder amount of ES powder increased. A significant reduction in moisture was observed in the 6% (4.94 ± 0.23%) and 9% (4.87 ± 0.36%) ESMBB samples.

Previous studies have demonstrated similar observations. A study on the development of high-calcium chicken nuggets with added ES powder highlighted that a higher quantity of ES powder resulted in a reduced water content within the nugget dough, leading to nuggets with decreased overall moisture content [18]. This finding also aligns with another study, which identified that ES powder possesses properties that hinder water absorption. Consequently, the addition of ES powder to wet noodles reduced the water content of the final product [19].

Controlling water activity in biscuits is crucial for maintaining their quality and stability, as water activity is related to the availability of water for microbial growth and chemical reactions in food products. Biscuits are typically dry, low-moisture products with low water activity, usually in the range of 0.2 - 0.6 [20]. According to **Error! Reference source not found.**, the ESMBB (0%) sample, which serves as the control without any ES powder addition, shows a low water activity value (0.21 ± 0.01). Water activity values followed a similar trend, decreasing with higher levels of ES powder. Among the samples with ES, the ESMBB (9%) sample exhibits the lowest water activity value (0.17 ± 0.01). Since the addition of ES powder lowers water activity, it creates an unfavourable environment for microbial growth and thereby extending the ESMBB shelf life [21].

The observed decrease in moisture content and water activity with increasing ES powder concentration suggests that ES powder addition may play a crucial role in reducing the free water available in the ESMBB samples. The results suggest that incorporating ES powder into gluten-free formulations such as ESMBB not only improves structural integrity but also contributes positively to product stability.

However, this finding contradicts to previous studies on the addition of duck ES nano calcium to beef sausage. That study reported that as the level of duck ES increased, there was a corresponding rise in the water activity of the beef sausage, indicating an elevation in the free water content within the sausage [22]. In addition to that study, another study examined the effect of fortification with ES powder on injera quality, no significant impact on water activity was observed. The authors reported that ES powder addition did not significantly affect the injera's quality [23]. These results from previous studies indicate that the effect of ES powder fortification on water activity is inconsistent, and there is no clear justification for its impact on water activity across different food products.

Sensorial acceptance of ES powder addition is one of the most important aspects to consider in food product development. It has been reported that the grittiness of ES powder in bread and gingerbread can significantly affect consumer acceptance during chewing [17, 24]. However, controlling the amount of ES incorporation, such as adding 3% ES powder to gingerbread, did not significantly affect its sensorial acceptance [24]. A sensory test using the preference ranking method comparing Commercial, ESMBB (0%) and ESMBB (9%) was conducted, and the results are shown in Table 4.

Table 4. Rank sum of the preference ranking method between Commercial, ESMBB (0%), and ESMBB (9%) samples

Sample	**Commercial**	**ESMBB (9%)**	**ESMBB (0%)**
Rank sum	47^b	66^a	67^a

Average rank	1.57	2.20	2.23

Note: Values with different letter were statistically different ($p<0.05$) for each preference ranking test of the samples

The Commercial sample had the lowest rank sum (47) with the lowest average rank, this indicated it was the least preferred sample among the panellists. The rank sums for the 9% and 0% ESMBB samples were 66 and 67, respectively, with corresponding average ranks of 2.20 and 2.24. Interestingly, the consumer preference ranking test showed that the addition of 9% ES in the ESMBB sample has no significant differences to the ESMBB (0%) sample. These findings indicate that the incorporation of ES up to 9% in ESMBB does not significantly alter the sensory appeal of the product as perceived by panellists, making it a viable option for gluten-free MBB formulation without compromising consumer acceptability. Nevertheless, a note of caution is due here since the panellists were exclusively undergraduate students of the Universiti Teknologi MARA, Kuala Pilah Campus. Future studies should include more diverse panel, including both younger and older participants to enhance the reliability of the sensory evaluation.

A few studies have explored fortification of calcium with the addition of ES in gluten-free food to improve nutritional quality and sensory acceptability. For instance, a study found that addition of ES powder in gluten-free cake enhanced calcium content but reduced overall sensorial acceptability [25]. However, another finding reported the overall acceptability of the optimum gluten-free cake with ES powder fortification was statistically higher than the control gluten-free cake [26]. Compared to these studies, our ES-fortified MBB showed improved hardness while maintaining sensory acceptability, and likely with improve calcium content. Our findings highlight the potential of ES powder as a functional fortifying ingredient for gluten-free products.

In summary, the addition of ES powder improved textural firmness and reduced the moisture content and water activity of the MBB without significantly affecting consumer preference or acceptance. These findings highlight the potential of using ES powder to enhance calcium content in gluten-free products while maintaining desirable sensory quality. ESMBB may offer a sustainable and nutritious option for individuals with gluten sensitivity. In the future, researchers may explore the potential of using ES powder in other gluten-free food matrices to validate its versatility and benefits.

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