ENHANCED KNOWLEDGE IN SCIENCES AND TECHNOLOGY
e-ISSN: 2773-6385

EKST

Vol. 4 No. 2 (2024) 613-622
https://publisher.uthm.edu.my/periodicals/index.php/ekst

# Extraction of Chicken Bone as Alternative Source of Calcium Incorporated in Bread

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DOI: https://doi.org/10.30880/ekst.2024.04.02.066

**Article Info**

Received: 27 December 2023
Accepted: 12 January 2024
Available online: 12 December 2024

**Keywords**

Extracted chicken bone, calcium, bread

**Abstract**

The composition of the inorganic matter in the chicken bone includes calcium phosphate, calcium carbonate, and magnesium phosphate. Calcium is essential for numerous vital bodily processes adults have a recommended daily calcium intake (RDI) of 1,000 milligrams (mg). In this study, the calcium contained in the chicken bone was extracted by using an alkaline treatment. Then the extracted chicken bone was analysed for its physicochemical and mineral contents. The treated chicken bone was added to the bread to enhance the calcium contain in the bread. The effect of bread that has been fortified with chicken bone powder was analysed further to determine the sensory, texture, colour and nutritional content. This study showed the calcium content from treated chicken bone was higher than untreated chicken bone which was 11555.36mg/100g. Besides that the pH and moisture for treated chicken bone were lower compared to the untreated chicken bone powder. There are significant differences detected in the texture of the bread with Extracted Chicken Bone Powder (ECBP) compared to common bread by using a texture analyzer to determine the firmness and springiness of the bread. There are no significant difference for bread with extracted chicken bone powder in terms of color for L* and a* value but there are significant differences with common bread for b* value. 50 panellists has been chosen to conduct the sensory analysis of bread and the panellists preferred to the bread with extracted chicken bone powder. For the proximate analysis of bread with extracted chicken bone powder, there are significant differences for moisture and ash content compared to the common bread. Overall results show that the extracted chicken bone powder has an impact to the properties of bread and the yield value for the calcium content of the powder was higher. This shows that the extracted chicken bone powder can be used as a calcium source for enrichment in food.

## 1. Introduction

The chicken bone is a type of waste material that is often underutilized or only minimally employed in the production of animal feed, pet food, and fertilizers. The chicken bone's chemical makeup contained 2.9% nitrogen, equivalent to approximately 15.6% protein, 9.5% fat, 14.7% mineral, and 57.5% moisture [1]. Chicken bones constitute a significant constituent of chicken flesh and are a valuable reservoir of essential nutrients such as calcium, phosphorus, magnesium, and iron. Chicken bones vary in composition based on the age of the chicken, the breed of the bird, and the food of the chicken. Typically, the composition of chicken bones comprises approximately 70% inorganic matter and 30% organic matter. The composition of the inorganic matter comprises calcium phosphate, calcium carbonate, and magnesium phosphate. The composition of organic matter comprises collagen, proteins, and lipids [2]. Due to the higher of inorganic compounds in the chicken bone especially calcium this material was chosen as a sample for this study.

Calcium is an essential nutrient that is required for the proper functioning of muscles and nerves. However, many individuals do not consume enough calcium through diet. This can result in calcium deficiency, which can have numerous adverse health effects. Insufficiency in calcium can also cause muscle cramps, restless legs syndrome, and excessive blood pressure [3]. Previous researchers [4] used gravimetric and atomic absorption spectrometry to compare calcium and phosphate levels in chicken and duck bones. The study found that chicken bones contain higher calcium (24.9%) than duck bones (20.8%), suggesting their potential as a dietary source for individuals with low calcium intake [4].

Bread has been a staple food in the Middle East for centuries, and its consumption is among the highest in the globe. Therefore, fortifying wheat flour with essential vitamins and minerals is regarded as one of the most efficient and costly methods for enhancing diet. Fortification of flour and other cereals has played a significant role in providing essential vitamins and minerals to individuals in industrialized nations. This has contributed to the elimination of nutritional deficiencies, the improvement of health, and the reduction of infant and maternal morbidity and mortality [5]. It is found that calcium-fortified bread emerged as a promising product with high calcium bioavailability and consumer acceptance, providing an effective means to enhance calcium intake and promote bone health while addressing environmental concerns associated with eggshell disposal [7].

Therefore, this study aimed to evaluate the physicochemical properties and trace mineral content of extracted chicken bone powder and to analyze the sensory acceptance of bread fortified with it. Additionally, the study investigated the effects of chicken bone powder on the bread's color, texture, and nutritional profile.

## 2. Materials and methods

The instruments that were used in this study are a drying oven (TR 240 Germany), balance (AS 60-220.X2), sieving machine (911MPESM), grinder (HOM-100), moisture analyzer (MX-50 A&D JAPAN), pH meter (INE-PHSJ-3F), Atomic Absorption Spectrophotometer (AAS320), furnace (ELF1106-230SN United Kingdom), Kjedahl (UDK 149), Soxhlet (K-5-3 SERIES), furnace (CDF 15-1C), colorimeter (NS8XX) and texture analyzer (TA.XTplusC Stable Micro System Ltd., Goldaming, United Kingdom).

The extraction of calcium from the chicken bone was done by using alkaline treatment [7]. The chicken bone was collected at the market area around the local supplier. The chicken bone was deboned and cleaned thoroughly. The skin and meat from the bone were removed. The bone was washed and rinsed. The chicken bone was separated into two parts which are untreated and treated chicken bone powder. The chicken bone powder would then be analyzed for its moisture content, pH level, and trace mineral content. Afterward, the treated chicken bone was incorporated into bread, and the bread's sensory acceptability was assessed using sensory analysis, texture analysis, and color analysis.

### 2.1 Preparation of chicken bone powder

The cleaned chicken bone was dried in a drying oven at 65℃ for 10 hours. After the drying process finished the untreated chicken bone was grinded and sieved to become a fine powder. Following a previous study by Kettawan *et al.,*2002 [7] the extraction of calcium from chicken bone was conducted by using alkaline treatment. 100 g of chicken bone was first dried in a drying oven (TR 240 Germany) at 65°C for 10 hours. 3% sodium hydroxide (NaOH) was then added to the dried bone at a ratio of 1:3 (w/v), and the mixture was boiled for 1 hour. The treated bone was separated using a filter cloth and washed twice with 1% HCl and deionized water until neutrality was achieved. Finally, the neutralized bones were dried in a drying oven (TR 240 Germany) at 100°C for 2 hours, ground by a grinder (HOM-100), and sieved (911MPESM) through a 100-mesh sieve.

## 2.2 Physicochemical properties of treated chicken bone powder

The physicochemical analysis has been analyzed to study the effect of alkaline treatment on the chicken bone. The pH and moisture of chicken bone powder were analyzed after the treatment was completed. 5 g of the test sample was weighed and transferred into a 125 ml glass stoppered flask. 100ml of distilled water was then added, and the mixture was shaken for a minute. After settling for one hour, the pH meter (INE-PHSJ-3F) was calibrated. The clear aqueous solution from the flask was carefully transferred into the beaker, and the pH value was measured. The moisture content was analyzed using a moisture analyzer (MX-50 A&D JAPAN) at a temperature of 105°C and 2 g of chicken bone powder was used [7].

## 2.3 Trace mineral analysis for treated chicken bone powder

The analysis was done to determine the effect of alkaline treatment on the trace mineral of chicken bone. The determination of trace minerals in chicken bone powder involves standard solution preparation, a dry ashing technique for chicken bone powder, and Atomic Absorption Spectroscopy (AAS320) analysis. 5 grams of extracted chicken bone powder were weighed and placed in a crucible for dry ashing. The sample was charred over a hotplate (MHK-4) until smoking ceased. The charred sample was then placed in a furnace (CDF 15-1C) and incinerated at 525°C for 3-4 hours. The ashing process was monitored, and if incomplete, the sample was cooled, moistened with water or diluted acid, and returned to the furnace for further ashing until white or grey ash was obtained. The furnace was turned off, and the temperature was allowed to drop to 250°C before the crucible was removed and cooled in a desiccator (PYREX 12-3051). 10ml of HCl was added to dissolve the ash, with rinsing of the dish. 2ml of HCl was added, and the dish was covered with a watch glass. The sample was heated until boiling, and then 20 mL of distilled water was added, washing down the watch glass. The solution was filtered through filter paper (Hawach) into a 50- or 100-mL volumetric flask and diluted to the mark [7]. All the samples and stock solution were analyzed using atomic absorption spectrophotometry. The wavelengths of calcium and potassium were 422.7 nm and 766.5 nm, respectively.

## 2.4 Preparation of treated chicken bone powder bread

All the ingredients were weighed according to the recipe provided by Khan *et al.,*2017 [8] in Table 1. Then it was mixed thoroughly for dough making. The dough was kneaded with hands for 5 – 10 min and was left for fermentation of 3 hours. Then the dough was baked in an oven (Berjaya) at 200°C for 20 minutes.

**Table 1** *Ingredients of bread*

| Ingredients | Weight of ingredients | | | |
|---|---|---|---|---|
| | White bread 0% | White bread 1% | White bread 2% | White bread 3% |
| Wheat flour (g) | 300 | 285 | 270 | 255 |
| Salt (g) | 9 | 9 | 9 | 9 |
| Water (ml) | 180 | 180 | 180 | 180 |
| Yeast (g) | 5 | 5 | 5 | 5 |
| Sugar (g) | 20 | 20 | 20 | 20 |
| Oil (ml) | 10 | 10 | 10 | 10 |
| Extracted chicken bone added (g) | 0 | 3 | 6 | 9 |

## 2.5 Colour and texture of bread

The analysis was done to determine the effect of chicken bone powder on the colour and texture of the bread. The texture analysis used a Texture Analyzer (TA.XTplusC Stable Micro System Ltd., Goldaming, United Kingdom) interfaced with a computer as equipment to help identify the results. The bread was sliced at the exact dimensions (10 mm x 6 mm x 1 mm, length x width x thickness). The Texture Expert 1.05 (Stable Microsystem) was used for the data analysis. Triplicate measurements were taken for every formulation and the mean value. The color of the bread was analyzed using a colorimeter (NS8XX). The colorimeter was calibrated first, and then the bread's color was analyzed. The color attributes such as hunter lightness (L*), redness (a*) and yellowness (b*) values will be recorded. The bread was analyzed at different locations on the surface of the bread [9].

## 2.6 Proximate analysis of bread

This analysis was done to determine the effect of chicken bone powder on the nutritional composition of bread. The moisture, ash, protein, fat and carbohydrate content of each sample were analyzed. All the analysis has been done by using the AOAC method except for moisture which was done by using a moisture analyzer at a temperature of 105°C [9].

## 2.7 Sensory analysis

This analysis was done to predict the acceptance of the bread incorporated with chicken bone in the community. The sensory evaluation was done using a 9-hedonic scale point by a group of 50 panelists. The bread was evaluated for color, taste, texture, aroma and overall acceptability with a score of 1-9, where 1 represented extremely disliked and 9 extremely liked, respectively [10].

## 2.8 Statistical analysis

This analysis was done to ensure the result of each analysis was acceptable to be used. All samples were analyzed in triplicates, and the results will be averaged. The statistical analysis will be assessed using Minitab Statistical Software. Furthermore, significant differences between the mean values will be determined by using the analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) post hoc test was conducted at a significance level of $p<0.05$ [10].

# 3. Results and Discussion

## 3.1 Physicochemical properties of calcium powder extracted from non-treated and alkaline-treated chicken bone

### 3.1.1 pH analysis

The pH of the chicken bone powder is shown in Table 2. There is a significant difference between each sample, with the Extracted Chicken Bone Powder having a higher acidity (6.93 ± 0.07) level as compared to ordinary chicken bone powder. The chicken bone powder without any treatment has more than the natural pH (6.35 ± 0.07).

This result was aligned with the study by Wang *et al.,*2022 [11]. According to Wang *et al.,*2022 [11], chicken bone powder demonstrates an optimal pH below neutrality (pH 7). Conversely, calcium tends to precipitate as calcium phosphate when the pH is either neutral or slightly alkaline (around 7), leading to a decline in calcium concentration in the extract. Under acidic conditions, typically within a pH range of 2-5, there is an increased dissolution of calcium salts. This heightened dissolution is attributed to the protonation of carboxylic groups in collagen and other binding molecules, diminishing their ability to retain calcium ions. Consequently, the extract exhibits an elevated concentration of calcium. Based on this study it can be observed that the suitable pH for the chicken bone powder was acidic to ensure the calcium concentration did not reduce.

**Table 2** *pH of chicken bone powder*

| Sample | pH |
|---|---|
| Untreated chicken bone powder | 6.35 ± 0.03 $^{a}$ |
| Treated chicken bone powder | 5.93 ± 0.07 $^{b}$ |

Data represent the mean ± standard deviation (n=3); a & b characterize significant difference ($P \leq 0.5$) with in the column

### 3.1.2 Moisture content analysis

An analysis of the results in Table 3 indicates the moisture content of chicken bone powder. There is a significant difference between the extracted chicken bone powder and normal chicken bone powder. The moisture content of chicken bone powder is higher compared to the extracted chicken bone powder. The extraction method is successful in removing moisture, most likely by techniques such as oven drying, which contributes to increased shelf stability and a lower risk of microbial decomposition. This 15% decrease emphasizes the effectiveness of the extraction process. Reducing the moisture content inhibits the growth of microorganisms, which in turn increases the longevity of the extracted powder and decreases the likelihood of spoiling. Elevated moisture levels typically result in a shorter shelf life since they promote heightened microbial proliferation and deterioration [12].

**Table 3** *Moisture of chicken bone powder*

| Sample | Moisture (%) |
|---|---|
| Untreated chicken bone powder | 7.33 ± 0.017 [a] |
| Treated chicken bone powder | 6.243 ± 0.30 [b] |

Data represent the mean ± standard deviation (n=3); a & b characterize significant difference (*P* ≤ 0.5) with in the column

### 3.1.3 Mineral content analysis

As shown in Table 4 chicken bone powder (7739.2mg/100g) contains less amount of calcium compared to the extracted chicken bone powder (11555.4mg/100g). From this result, it highlights there are larger differences between the two methods. The extraction method for the chicken bone powder was successful. According to the Malaysia recommendation nutrient intake for calcium is 1000mg/day. By referring to the results of the extraction of chicken bone from Table 4 it can be calculated 1g of extracted chicken bone contains approximately 115.55mg of calcium [13]. This result is aligned with the study by Khan *et al.,*2017 [8], which found 13345.13mg/100g of calcium from extracted chicken bone. Hence, it can be concluded that adding extracted chicken bone powder to the bread would help to increase the calcium content of the bread.

In this study, the potassium contained in chicken bone powder (243.91mg/100g) is higher compared to the extracted chicken bone powder (162.97mg/100g) as shown in Table 4. According to the Malaysia recommendation nutrient intake for potassium is 4700mg/day. By referring to the results of the extraction of chicken bone, it can be calculated that 1g of extracted chicken bone contains approximately 1.63mg of potassium [13]. By comparing the results to other studies, they found 0.735mg/g of potassium [11]. Besides that, *Khan et al.*,2017 [8] do not detect the presence of potassium in the chicken bone powder. Referring to the past study and the tabulated result in Table 4 the potassium content in the chicken bone powder was significantly lower compared to the calcium content. Therefore, this evidence indicates the current method was not suitable for the potassium content because it was more focused on the calcium content only.

**Table 4** *Trace minerals in chicken bone powder*

| Sample | Calcium (mg/100g) | Potassium (mg/100g) |
|---|---|---|
| Untreated chicken bone powder | 7739.2 | 243.91 |
| Treated chicken bone powder | 11555.4 | 162.97 |

## 3.2 Physicochemical properties of bread incorporated with calcium powder from alkaline-treated chicken bone

### 3.2.1 Texture analysis

The firmness of the bread is highlighted in Table 5, bread with 1% Extracted Chicken Bone Powder (ECBP) having the highest firmness (570.1 ± 24.8) compared to other bread samples. Bread with 3% of ECBP has the lowest firmness (349.2 ± 19.2) compared to the other bread sample. All the samples of the bread had a significant difference in texture of the firmness. According to He and Hoseney 1990 [14], the firmness of the bread is influenced by the time the bread was stored normally for bread that had been stored below 5 days the 500g. Possible factors for the outcome could be attributed to inadequate dough manipulation, insufficient utilization of high-grade packing materials, or variations in the composition of ingredients [15].

As depicted at Table 5 the bread with 3% of ECBP has the highest springiness (65.23 ± 1.67) compared to other bread samples. Bread with 1% of ECBP contains the lowest springiness compared to other samples. Apart from that, the bread with 2% of ECBP and 3% of ECBP has a significant difference with control but it did not have a significant difference with the bread with 1% of ECBP. Springiness in bread is indicated by the freshness, while bread with low values is correlated with crumb brittleness [16]. According to Tóth *et al.,*2022 [17], fresh bread normally contains 90% of springiness. Based on the results the highest springiness is 65.23% this outcome may be different due to the different of raw materials that had been used.

**Table 5** *Texture analysis of extracted chicken bone powder bread (ECBP)*

| Sample | Firmness (g) | Springiness (%) |
|---|---|---|
| Control | 419.69 ± 9.50 [a] | 61.10 ± 1.88 [a] |
| Bread 1% of ECBP | 570.1 ± 24.8 [b] | 58.23 ± 2.75 [a b] |
| Bread 2% of ECBP | 487.41 ± 8.92 [c] | 58.93 ± 2.06 [b] |
| Bread 3% of ECBP | 349.2 ± 19.2 [d] | 65.23 ± 1.67 [b] |

Data represent the mean ± standard deviation (n=3); a - d characterize significant difference ($P \leq 0.5$) with in the column

### 3.2.2 Color analysis

The findings outlined in Table 6 showcase that bread with 1% of ECBP has the highest L* value (30.67 ± 1.01) compared to other samples and bread with 3% of ECBP contain lowest L* value (26.61 ± 0.41). There is no significant difference in presence between each sample. According to Mohd *et al.,*2008 [18], the difference between the L* value (lightness to darkness) is due to the direct heat penetration during the baking process. Commercial bread usually contains below 30 L* values [18].

As demonstrated in Table 6 the sample with the highest a* value is bread with 2% of ECBP (2.90 ± 0.46) and the sample with the lowest a* value is bread with 3% of ECBP (2.16 ± 0.23). There is no significant difference between each sample and all the samples show the redness color. This condition may be associated with the process of caramelization and the maillard reaction that occurred during the creation of a crust. These two significant actions that occurred during baking are accountable for converting reducing sugars into other substances and altering the colors of materials in the presence of heat [19].

From Table 6 the highest sample with b* value is bread with 1% of ECBP (0.069 ± 0.58) and the lowest sample with b* value is bread with 2% of ECBP (-3.31 ± 0.57). There is a significant difference between bread with 1% and 3% of extracted chicken bone powder with the other two samples. All the sample has a more tendency to the blueish in colour except for bread with 1% extracted chicken bone powder which is more yellowish in colour. Based on the study by Mohd *et al.,*2008 [19], the color for commercial bread normally more to yellowish. The different between the color of the commercial bread and our bread may due to the raw material that had been used.

**Table 6** *Colour analysis of extracted chicken bone powder bread (ECBP)*

| Sample | L* | a* | b* |
|---|---|---|---|
| Control | 30.19 ± 7.04 [a] | 2.68 ± 0.70 [a] | -2.85 ± 1.38 [b] |
| Bread 1% of ECBP | 30.67 ± 1.01 [a] | 2.40 ± 0.17 [a] | 0.69 ± 0.58 [a] |
| Bread 2% of ECBP | 26.61 ± 0.41 [a] | 2.90 ± 0.46 [a] | -3.31 ± 0.57 [b] |
| Bread 3% of ECBP | 29.89 ± 0.74 [a] | 2.16 ± 0.23 [a] | -0.06 ± 0.23 [a] |

Data represent the mean ± standard deviation (n=3); a & b characterize significant difference ($P \leq 0.5$) with in the column

### 3.2.3 Sensory analysis

From Table 7 the bread with 3% of ECBP had highest color score (7.11 ± 1.50) compared to other samples and the bread with 2% of ECBP had lowest color score (6.67 ± 1.57). There is no significant difference present from each sample but difference between the highest and lower scores were 6%. Figure 1 shows the color of bread with 3% of ECBP wider than other samples. According to the study [8], the panellists more likely to the color for control bread compared to the ECBP bread. Aside from that, some factors must be considered such as raw materials and the age of the panelist must be considered for these results. The decrease in color intensity could be attributed to the interaction between amino acids and reducing sugars [20].

Based on Table 7 the aroma of bread as control and 3% of ECBP has the same highest score (6.38 ± 1.55) and the bread with 1% of ECBP had the lowest score (6.39 ± 1.85). There is no significant difference between each sample but by referring to Fig. 1 it has been noted that the control and bread with 3% of ECBP had the widest area compared to the other sample. According to Sittikulwitit *et al.,*2004 [10], the average for aroma score of the chicken bone bread was 6.10. This factor may be influenced by the bone flavor from the extracted chicken bone.

As displayed in Table 7 the texture of bread with 3% of ECBP (6.63 ± 2.07) was the highest and bread with 2% of ECBP (6.11 ± 1.94) was the lowest compared to other samples. There is no significant difference present in these results but from Figure 1 it can be deduced that the bread with 3% of ECBP has wider area compared to other sample scores. The result was aligned with the research from Khan *et al.,*2017 [8] there is some increase in terms of texture of the bread with extracted chicken bone. For bread with 2% of ECBP some factors influence the texture of bread such kneading process of the bread.

By referring to Table 7 the taste of control bread was higher (6.80 ± 1.53) and bread with 2% of ECBP was lower (6.07 ± 1.66) compared to other samples. There is no significant difference that has been detected for each sample but by referring to Figure 1 it is clear that the control sample has wider area compared to other samples. The result was aligned with the study from Khan *et al.,*2017 [8] the bread sample with extracted chicken bone had the lowest taste score compared to the control sample.

As tabulated in Table 7 the overall acceptance for bread with 3% of ECBP was highest (6.72) and bread with 2% of ECBP was lowest (6.13) compared to other samples. There is no significant difference that was present in Table 7 but referring to Figure 1 it is noticeable the bread with 3% of ECBP has wider area compared to other samples. Therefore, this result highlighted the panelist most referred to the bread with 3% of ECBP compared to other samples. These results were aligned with the study from Khan *et al.,*2017 [8] the higher the percentage of foreign material the higher the overall acceptance level from the panelists.

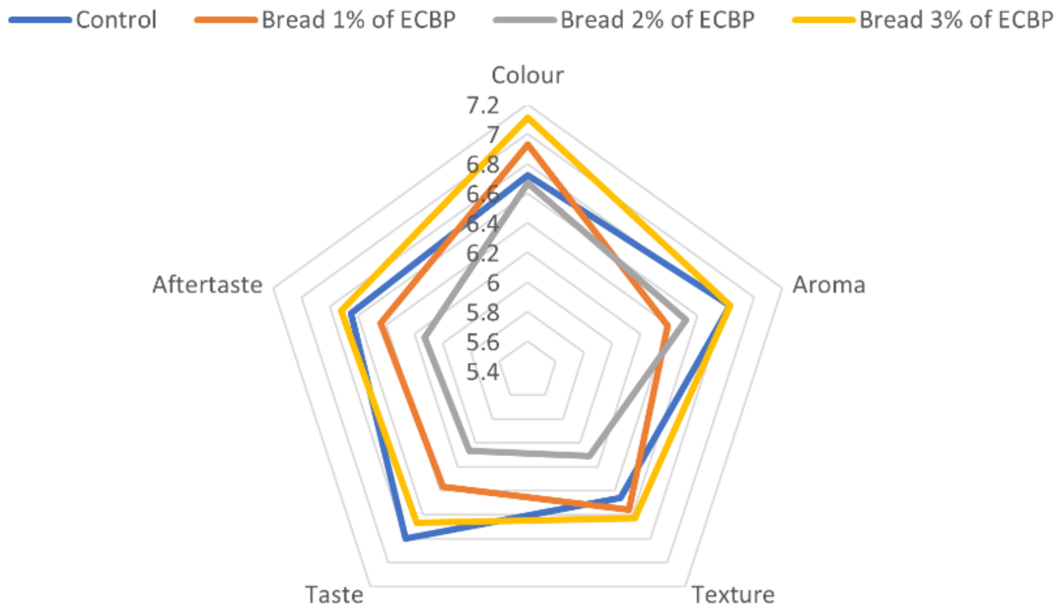


**Fig. 1** *Sensory analysis of extracted chicken bone powder bread (ECBP)*

**Table 7** *Sensory analysis of extracted chicken bone powder bread (ECBP)*

| Sample | Colour | Aroma | Texture | Taste | Overall acceptance |
|---|---|---|---|---|---|
| Control | 6.72 ± 1.55 [a] | 6.83 ± 1.40 [a] | 6.46 ± 1.66 [a] | 6.80 ± 1.53 [a] | 6.65 ± 2.02 [a] |
| Bread 1% of ECBP | 6.93 ± 1.46 [a] | 6.39 ± 1.85 [a] | 6.56 ± 1.88 [a] | 6.37 ± 1.91 [a] | 6.44 ± 2.05 [a] |
| Bread 2% of ECBP | 6.67 ± 1.57 [a] | 6.52 ± 1.51 [a] | 6.11 ± 1.94 [a] | 6.07 ± 1.66 [a] | 6.13 ± 1.80 [a] |
| Bread 3% of ECBP | 7.11 ± 1.50 [a] | 6.83 ± 1.55 [a] | 6.63 ± 2.07 [a] | 6.67 ± 1.71 [a] | 6.72 ± 1.65 [a] |

Data represent the mean ± standard deviation (*n*=3); a characterize significant difference ($P \leq 0.5$) with in the column

### 3.2.4 Proximate analysis

As highlighted in Table 8 the moisture content for control bread has the highest value (42.67 ± 0.14) and bread with 3% of ECBP sample has the lowest moisture content (36.86 ± 0.08). There is a significant difference between each sample except for bread with 2% of ECBP, this sample does not have a significant difference with bread with 1% and 3% of ECBP. This result was aligned with the research from Khan *et al.,*2017 [8] as increase the chicken bone powder percentage the moisture of the bread decreased. The decrease in moisture content may be attributed to the dry form of these calcium sources. The moisture content of the bread supplemented with calcium carbonate dropped as the dry matter content increased [21].

The ash content for each sample of bread has been displayed in Table 8 and it shows a significant difference. The control sample has the lowest ash content (1.51 ± 0.22) and the bread with 3% of ECBP has the highest ash content (9.48 ± 0.20). this result shows as increasing the ECBP the ash content also increases. This data was aligned with the other study by Khan *et al.,*2017 [8], which shows the ash content for unleavened and leavened bread increased as the chicken bone and eggshell powder increased. Another study by Mashayekh *et al.,*2008 [22] shows the increased ash content of whole bread as an increase in the defatted soy percentage. Therefore, it can be detected as increased the ECBP the ash content of the bread increases.

As depicted in Table 8, the protein content for bread with 3% of ECBP was highest (7.8%) and bread with 2% of extracted chicken bone powder was lowest (7.2%). By referring to Table 8 it clearly shows that there are no large differences in the protein content for each sample. All the sample with ECBP was increased compared to the control sample. According to Khan *et al.,*2017 [8], this situation could be related to the baking process, which substantially reduced the amino acid concentration of the bread.

Based on Table 8, the control sample has the highest fat content (5.9%) and bread with 2% extracted chicken bone powder has the lowest fat content (5.3%). By referring to Table 3.7 the fat content was reduced as increased the ECBP percent. According to Kettawan *et al.,*2002 [8], the extracted chicken bone content was 6.71g/100g of fat. Therefore, it is reasonable to deduce that the extracted chicken bone powder has the lowest fat content to help increase the fat content in the bread.

As tabulated in Table 8, bread with 3% of ECBP has the lowest carbohydrate content (40.16%) and bread with 1% of ECBP contain the highest carbohydrate content. Based on the result from Table 3.7 the carbohydrate content for bread with ECBP was higher compared to control bread except for bread with 3% of ECBP. This result is due to the influence of the highest amount of extracted chicken bone powder that is present in the bread. According to Noroul-Asyikeen *et al.,*2018 [23], the high carbohydrate level in bread is a result of the high carbohydrate content in flour. Carbohydrates are essential in a diet, and it is indicated that wheat flour is the primary source of carbohydrates in bread.

**Table 8** *Proximate analysis of extracted chicken bone powder bread (ECBP)*

| Sample | Moisture | Ash | Protein | Fat | Carbohydrates |
|---|---|---|---|---|---|
| Control | 42.67 ± 0.14 [a] | 1.51 ± 0.22 [d] | 7.3 | 5.9 | 42.62 |
| Bread 1% of ECBP | 39.31 ± 1.13 [b] | 3.53 ± 0.32 [c] | 7.6 | 5.5 | 44.66 |
| Bread 2% of ECBP | 38.97 ± 1.48[bc] | 4.70 ± 0.15 [b] | 7.2 | 5.3 | 43.83 |
| Bread 3% of ECBP | 36.86 ± 0.08 [c] | 9.48 ± 0.20 [a] | 7.8 | 5.7 | 40.16 |

Data represent the mean ± standard deviation (n=3); a -d characterize significant difference ($P \leq 0.5$) within the column

## 4. Conclusion

In the conducted study, chicken bones were utilized as a calcium source for fortifying bread, with the extraction process employing alkaline treatment. This method was chosen for its cost-effectiveness and the ability to yield a higher calcium content compared to alternative approaches. The complete study looked at the mineral content of the chicken bone powder that was extracted and what happened when it was added to bread. This was done by looking at the bread's taste, color, and texture. Additionally, proximate analysis was conducted to scrutinize the nutritional effects of chicken bone powder on the bread. The findings revealed a significantly higher calcium content in the extracted chicken bone powder compared to normal chicken bone powder, showcasing its potential as a suitable ingredient for calcium-fortified products aimed at enhancing daily calcium intake. Notably, the bread fortified with 3% of extracted chicken bone powder exhibited distinct characteristics, including increased firmness, reduced springiness, a lighter color, and higher overall acceptance in sensory evaluations. Proximate analysis further highlighted significant differences, particularly in the ash and moisture content of the fortified bread. The bread with extracted chicken bone powder exhibited a lower moisture content and a higher ash content, contributing to its unique nutritional profile. In conclusion, the study suggests that incorporating extracted chicken bone powder has a notable impact on the nutritional composition and sensory attributes of bread. Future investigations focusing on food safety, shelf life, and a deeper exploration of mineral content may expand the scope of understanding. Additionally, further exploration of the potential utilization of other chicken waste products, such as eggshells and feathers, could contribute to global waste reduction initiatives and broaden the spectrum of sustainable practices in food production.

## Acknowledgment

The authors gratefully acknowledge the Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia, Pagoh for providing necessary facilities for the successful completion of this research work.

## Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

## Author Contribution

***Study conception and design:*** *Muhammad Asyraf Abdul Kadir, Syazwan Hanani Meriam Suhaimy;* ***data collection:*** *Muhammad Asyraf Abdul Kadir;* ***analysis and interpretation of results:*** *Muhammad Asyraf Abdul Kadir, Syazwan Hanani Meriam Suhaimy, Mohammad Arif Budiman Pauzan, Saliza Asman, Zalilah Murni Yunus;* ***draft manuscript preparation:*** *Muhammad Asyraf bin Abdul Kadir, Syazwan Hanani Meriam Suhaimy, Mohammad Arif Budiman Pauzan, Saliza Asman, Zalilah Murni Yunus, Mohd Hanafi Mohd Hafidzal. All authors reviewed the results and approved the final version of the manuscript.*

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