

Effects of eggshell powder supplementation on nutritional and sensory attributes of biscuits

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Abstract: Chicken eggshell, a poultry waste material, is a potential but poorly recognised source of calcium that can be used by humans to increase their dietary calcium intake by incorporating it into foods. This study was aimed at assessing the effects of eggshell powder supplementation at 5, 10, 15, and 20% levels on the chemical composition, sensory characteristics, and calorific value of the biscuits. Calcium absorption from supplemented biscuits was also determined. The inclusion of eggshell powder resulted in significant variations in the chemical composition of biscuits. It profoundly increased the mineral content, mainly calcium from 43.57 mg 100 g⁻¹ to 1 054.7, 2 186.7, 2 941.6, and 3 843 mg 100 g⁻¹ at 5, 10, 15 and 20% supplementation level, respectively. Substantial changes in the sensory quality of biscuits were also observed with corresponding rises in eggshell addition levels. Biscuits prepared with 5 and 10% supplementation levels were found acceptable in terms of sensory attributes. The *in vivo* study affirmed the absorption of calcium from eggshell powder and it was found highest (41.83%) at 5% supplementation level. Conclusively, supplementation of biscuits with eggshell powder might be an attractive source of dietary calcium intake without any significant adverse effects on biscuits quality up to 10% supplementation level.

Keywords: chicken eggshell; calcium supplementation; *in vivo* calcium absorption; calorific value

Chicken eggshells are waste materials from home, hatcheries and food industries that utilise eggs in their products. In recent years, the global per capita consumption of eggs has increased particularly in developing countries owing to the cost effectiveness and higher dietary acceptance. The rise in eggs consumption has led to an upsurge in their production. With an increase in the production of eggs by more than 150% in the past three decades (FAO 2020), the resulting eggshell waste which typically goes to landfills poses serious hazards of environmental pollution and health (Ajala et al. 2018). Although eggshell is a waste material, it can be used as a valuable product for human nutrition, which could alleviate its environmental burden (Faridi and Arabhosseini 2018; Waheed et al. 2020). The shell is mainly cal-

cium carbonate and has the potential to be used by humans as a calcium source (Waheed et al. 2019).

Calcium is an essential nutrient that is required for a variety of metabolic functions by the human body. The recommended intake of this mineral is important for bone growth and maintenance and to reduce the risk of osteoporosis and osteoporotic fracture (Ahmad et al. 2015). Calcium intake from dairy products such as milk, yoghurt and cheese, is an appropriate way to fulfil the daily requirement. The recommended intake (1 000–1 300 mg per day) is difficult to meet for those who do not take any dairy items in their routine diet. Hence alternate calcium sources are required.

Chicken eggshell is an inexpensive calcium source that is accessible at home. It is composed of 94% cal-

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cium carbonate, 1% magnesium carbonate and 1% of calcium phosphate. With 39% elemental calcium, it is a promising and effective source of dietary calcium (Murakami et al. 2007). According to Milbradt et al. (2015), half of an eggshell (2.7 g) can provide enough calcium to meet the daily requirement of adults.

The consumption of eggshell powder has positive effects on bone mass development in humans (Schaafsma and Pakan 1999). It greatly increases the femoral neck bone density (Schaafsma et al. 2002). In an investigation, Sakai et al. (2017) found eggshell calcium more efficient compared to calcium carbonate in increasing the bone mass in women, indicating its potential use as a calcium supplement in human nutrition.

Various foods have been used for calcium supplementation by using eggshell powder. Salem et al. (2012) determined the feasibility of using the eggshell powder in butter cake and concluded that fortification of the bakery items with eggshell powder might be suggested at 10% inclusion level. Hassan (2015) reported that eggshell powder supplementation up to 6% level did not produce any significant changes in the flavour of biscuits. In another study, it was suggested that white bread has the potential to be fortified with eggshell powder and is acceptable up to 2% fortification level (Platon et al. 2020). However, no data is present on *in vivo* calcium absorption from eggshell fortified products. The aim of the present study was to determine the effects of eggshell powder supplementation on the chemical composition, calorific value, and sensory properties of biscuits as well as *in vivo* calcium absorption from the prepared supplemented biscuits.

MATERIAL AND METHODS

Material. Commercially available wheat flour (72% extraction), chicken eggs and all other ingredients were obtained from the local market (Q. Mart, UAF) in Faisalabad, Pakistan.

Preparation of eggshell powder. Chicken eggshells were collected, demembrated, washed under running tap water, scrubbed with a household sponge, immersed in a sodium hypochlorite solution (10 drops per litre water), rinsed, dried, and ground to powder. After this, powdered eggshells were passed through 0.18 mm sieve and dried in an oven (UNB 100; Memmert, Germany) at 80 °C until a constant weight was obtained. The eggshell powder was sterilised in an autoclave (Labo Autoclave; Sanyo; Japan) at 134 °C for 15 min before use (Hassan 2015).

Proximate analysis of eggshell powder and wheat flour. Moisture, crude fat, crude fibre, crude protein,

and ash contents were determined in triplicate according to their relevant procedures as described in Approved methods of American Association of Cereal Chemists, AACC (2000).

Mineral analysis of eggshell powder and wheat flour. Minerals including calcium, phosphorus, magnesium, iron, and zinc were quantified after wet digestion using an atomic absorption spectrophotometer (Varian AA240; Varian Inc., Australia) according to their relevant methods described in Official Methods of Analysis AOAC (The Association of Official Analytical Chemists, 2006). Sodium and potassium were measured in a Model 410 flame photometer (Sherwood Scientific Ltd., UK) by following procedures described in AOAC (2006).

Preparation of eggshell powder supplemented biscuits. The eggshell powder was added to wheat flour at 0, 5, 10, 15, and 20% levels by substituting wheat flour (Table 1) and biscuits were prepared from supplemented flour according to the method (method No. 10-5-D) mentioned in AACC (2000).

Chemical (proximate composition and minerals) analysis of biscuits. Biscuit proximate composition (moisture, crude fat, crude fibre, crude protein, and ash content) and mineral content (calcium, phosphorus, magnesium, iron, sodium, potassium, and zinc) were calculated according to the methods described in AACC (2000) and AOAC (2006), respectively.

Calorific value. The calorific value of prepared biscuits was determined by using the Parr oxygen bomb calorimeter (C-200 basic; IKA®-WERKE; Germany). One gram of each sample of the prepared biscuits was loaded in the equipment, ignited with an electric spark and burnt in excess oxygen in the bomb. The maximum rise in the temperature of the bomb was measured with thermocouple and galvanometer system. Each sample was analysed in triplicate (Van Hal 2000).

Table 1. Treatment plan used in product development (%)

Treatments	Wheat flour 72% extraction	Eggshell powder
T ₀	100	0
T ₁	95	5
T ₂	90	10
T ₃	85	15
T ₄	80	20

T₀ – control, biscuits with 0% eggshell powder; T₁ – biscuits with 5% eggshell powder; T₂ – biscuits with 10% eggshell powder; T₃ – biscuits with 15% eggshell powder; T₄ – biscuits with 20% eggshell powder

Sensory analysis of biscuits. Biscuits were subjected to sensory evaluation of colour, odour, taste, texture, flavour, and overall acceptability by 20 trained panelists on a 9-point hedonic score system with individual scores: extremely liked – 9, very much liked – 8, moderately liked – 7, slightly liked – 6, neither liked nor disliked – 5, slightly disliked – 4, moderately disliked – 3, very much disliked – 2 and extremely disliked – 1 (Meilgaard et al. 2007).

In vivo calcium absorption. Young male albino rats (7–8 weeks old) provided by the Institute of Food Science and Technology, UAF, were fed a balanced food for rodents and water *ad libitum* according to the report of the American Institute of Nutrition (AIN-93). The experimental diets were prepared by mixing finely ground biscuits with casein and vitamin mixture (Hoffmann-La Roche, Ltd, Switzerland) in amounts of 60 and 1.5 g kg⁻¹, respectively, to meet the nutritional standards set for rats. CaCO₃ (3.5 g kg⁻¹) was added to the diet containing biscuits with 0% eggshell powder (control). Casein was added to fulfil the requirements for essential amino acids lacking in wheat flour such as lysine (Reeves et al. 1993).

Rats were divided into 5 groups (3 rats per group for each test diet) and fed the experimental diets for 4 days. On the fourth day, feed intake was measured for 24 h, and faeces were collected, air dried, weighed, finely ground, and stored in a freezer (Froster Labo 330; Kirsch; Germany) at –20 °C. The amount of calcium in the feed and faeces of 4th day was measured using an atomic absorption spectrophotometer (Varian AA240; Varian Inc., Australia) according to the method defined in AOAC (2006). The apparent calcium absorption was calculated as follows (Equation 1):

$$\text{Apparent absorption (\%)} = \frac{24 \text{ h Ca intake} - 24 \text{ h Ca excretion}}{24 \text{ h Ca intake}} \times 100 \quad (1)$$

Statistical analysis. Data obtained from each parameter was subjected to analysis of variance (ANOVA) with the SPSS software (V23; SPSS Inc., USA). To determine the significance level among control, 5, 10, 15, and 20% eggshell supplemented biscuits, Tukey's post hoc multiple comparison test was used ($P < 0.05$).

RESULTS AND DISCUSSION

Proximate composition of wheat flour and eggshell powder. The proximate chemical composition of commercial wheat flour (72% extraction) and eggshell powder is reported in Table 2. The wheat flour

Table 2. Proximate composition of chicken eggshell powder and wheat flour; 72% extraction (%; mean \pm SD; $n = 3$)

Components	Eggshell powder	Wheat flour
Moisture	0.81 \pm 0.05 ^b	12.16 \pm 0.36 ^a
Ash	94.69 \pm 2.47 ^a	0.88 \pm 0.03 ^b
Crude protein	2.11 \pm 0.06 ^b	10.85 \pm 0.15 ^a
Crude fat	0.04 \pm 0.01 ^b	1.09 \pm 0.13 ^a
Crude fibre	nd ^b	0.35 \pm 0.02 ^a

SD – standard deviation; means carrying different letters across the same row differ significantly ($P < 0.05$); ^aindicates the highest value group across the same row; nd – not detected

had the highest values of protein (10.85%) and the lowest values of ash content (0.88%). These results are consistent with those of Yaseen et al. (2007) and Ali and Halim (2013). In contrast to wheat flour, the eggshell powder had the highest ash content (94.69%) and the lowest crude fat content (0.04%). According to Ray et al. (2017), ash is an essential part of the eggshell (94.6%) followed by protein (3.92%) and water (0.46%). Ali and Badawy (2017) determined the constituents of white eggshell and mentioned that its components of ash, protein, fat and water were 96.70, 3.17, 0.06, and 0.95 g 100 g⁻¹, respectively.

Mineral composition of wheat flour and eggshell powder. Results for the mineral profile of eggshell powder and wheat flour are presented in Table 3. The obtained results indicated that eggshell powder was composed mainly of calcium (36 200 mg 100 g⁻¹) followed by magnesium, phosphorus, sodium, potassium, iron and zinc. The lowest contents of these minerals were found in wheat flour compared to eggshell powder. Several workers have determined the mineral content of the eggshell powder.

Table 3. Mineral composition of chicken eggshell powder and wheat flour; 72% extraction (mg 100g⁻¹; mean \pm SD; $n = 3$)

Minerals	Eggshell powder	Wheat flour
Ca	36 200 \pm 712 ^a	56.54 \pm 3.69 ^b
Mg	240 \pm 44.65 ^a	74.68 \pm 4.85 ^b
P	106 \pm 23.18 ^b	124.63 \pm 6.99 ^a
Na	80 \pm 16.24 ^a	48.29 \pm 6.44 ^b
K	60.20 \pm 9.40 ^b	206.27 \pm 9.57 ^a
Zn	0.67 \pm 0.03 ^a	0.59 \pm 0.16 ^a
Fe	11.47 \pm 0.87 ^a	0.92 \pm 0.17 ^b

SD – standard deviation; means carrying different letters across the same row differ significantly ($P < 0.05$); ^aindicates the highest value group across the same row

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Table 4. Proximate composition of biscuits (%; mean \pm SD; $n = 3$)

Treatments	Components				
	moisture	ash	crude protein	crude fat	crude fibre
T ₀	3.52 \pm 0.28 ^e	0.35 \pm 0.03 ^e	6.61 \pm 0.22 ^a	24.77 \pm 1.02 ^a	0.16 \pm 0.02 ^a
T ₁	5.66 \pm 0.2 ^d	2.04 \pm 0.08 ^d	6.55 \pm 0.22 ^a	24.53 \pm 0.69 ^a	0.14 \pm 0.01 ^{ab}
T ₂	6.76 \pm 0.14 ^c	4.52 \pm 0.07 ^c	6.54 \pm 0.10 ^a	24.26 \pm 0.57 ^a	0.13 \pm 0.02 ^b
T ₃	7.51 \pm 0.25 ^b	6.40 \pm 0.15 ^b	5.46 \pm 0.08 ^b	23.80 \pm 0.43 ^b	0.12 \pm 0.03 ^b
T ₄	8.02 \pm 0.14 ^a	8.58 \pm 0.39 ^a	5.43 \pm 0.11 ^b	23.40 \pm 0.57 ^b	0.12 \pm 0.02 ^b

SD – standard deviation; mean values with different superscripts in the same column are significantly different ($P < 0.05$); ^aindicates the highest value group across the same column; T₀ – control, biscuits with 0% eggshell powder; T₁ – biscuits with 5% eggshell powder; T₂ – biscuits with 10% eggshell powder; T₃ – biscuits with 15% eggshell powder; T₄ – biscuits with 20% eggshell powder

According to Brun et al. (2013), eggshell comprises fundamentally of calcium (382 mg g⁻¹), sodium (5.1 mg g⁻¹), phosphorus (4.4 mg g⁻¹), and potassium (1.4 mg g⁻¹). Likewise, Bartter et al. (2018) calculated the calcium content of eggshell from three different studies and found it ranging from 360 to 400 mg g⁻¹ of eggshell.

Effect of eggshell powder on the proximate chemical composition of biscuits. The proximate chemical composition of prepared biscuits presented in Table 4 indicated that with an increase in the level of supplementation, the ash content of the biscuits increased. Since the ash value determines the mineral value of the product, it indicated that eggshell

powder addition increased the mineral content of biscuits, as shown in this study. No significant effect was observed on crude protein and crude fat contents up to 10% level (T₁ and T₂). However, a significant decrease was observed at 15% (T₃) and 20% level (T₄). Chilek et al. (2018) also reported that eggshell addition increased the ash content of the product without effecting its protein and fat contents, like the current findings. Eggshell powder supplementation also resulted in a significant reduction in the calorific value of the biscuits (Figure 1).

Effect of eggshell powder on the mineral composition of biscuits. Data presented in Table 5 shows

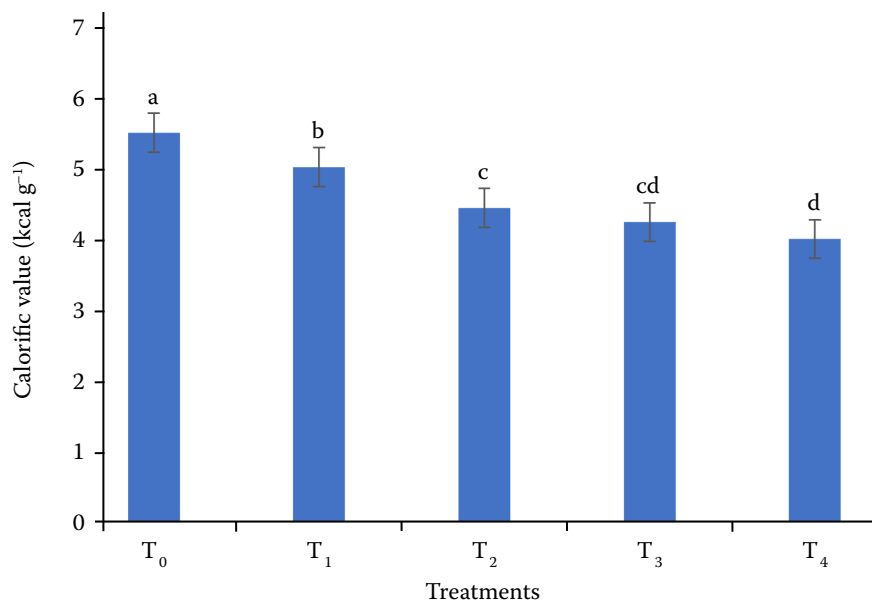


Figure 1. Calorific value of biscuits (kcal g⁻¹) (mean \pm SD; $n = 3$)

SD – standards deviation; T₀ – control, biscuits with 0% eggshell powder; T₁ – biscuits with 5% eggshell powder; T₂ – biscuits with 10% eggshell powder; T₃ – biscuits with 15% eggshell powder; T₄ – biscuits with 20% eggshell powder; different letters indicate a significant difference ($P < 0.05$), with 'a' representing the highest caloric value group

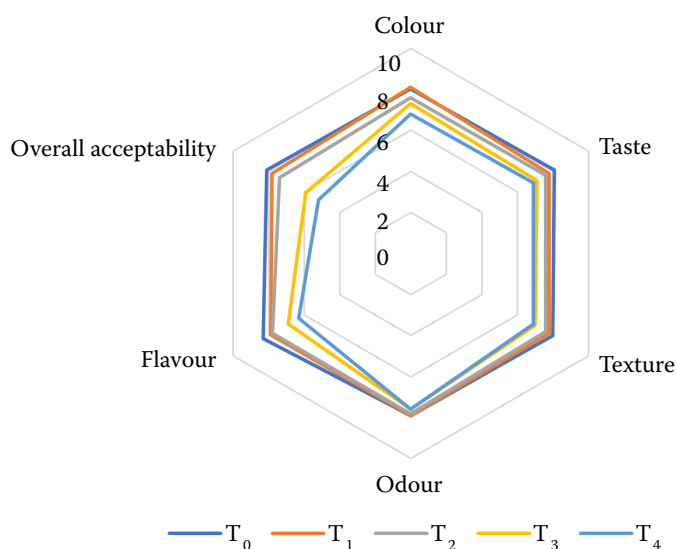
Table 5. Mineral composition of biscuits (mg 100 g⁻¹; mean \pm SD; $n = 3$)

Treatments	Minerals						
	Ca	P	Mg	Na	K	Fe	Zn
T ₀	43.56 \pm 1.23 ^e	73.33 \pm 8.96 ^a	19.42 \pm 0.81 ^d	79.00 \pm 2.10 ^d	160.32 \pm 11.92 ^a	1.10 \pm 0.36 ^{bc}	0.08 \pm 0.01 ^e
T ₁	1 054.70 \pm 3.92 ^d	71.50 \pm 7.78 ^{ab}	25.60 \pm 0.72 ^d	119.67 \pm 2.14 ^c	149.66 \pm 12.00 ^{ab}	1.12 \pm 0.09 ^c	0.20 \pm 0.03 ^d
T ₂	2 186.70 \pm 12.50 ^c	67.67 \pm 4.51 ^{abc}	33.92 \pm 0.27 ^c	139.00 \pm 2.08 ^b	139.00 \pm 18.03 ^{abc}	1.26 \pm 0.10 ^c	0.34 \pm 0.03 ^c
T ₃	2 941.60 \pm 5.54 ^b	61.00 \pm 5.05 ^{bc}	42.96 \pm 0.07 ^b	156.66 \pm 2.52 ^a	133.00 \pm 14.00 ^{bc}	1.54 \pm 0.16 ^{ab}	0.50 \pm 0.02 ^b
T ₄	3 843.00 \pm 17.77 ^a	59.33 \pm 4.51 ^c	55.71 \pm 0.42 ^a	159.00 \pm 4.26 ^a	120.33 \pm 18.00 ^c	1.75 \pm 0.08 ^a	0.64 \pm 0.05 ^a

SD – standard deviation; mean values with different superscripts in the same column are significantly different ($P < 0.05$); ^aindicates the highest value group across the same column; T₀ – control, biscuits with 0% eggshell powder; T₁ – biscuits with 5% eggshell powder; T₂ – biscuits with 10% eggshell powder; T₃ – biscuits with 15% eggshell powder; T₄ – biscuits with 20% eggshell powder

the mineral profile of prepared biscuits. Supplementation of eggshell powder increased the biscuit contents of calcium, sodium, magnesium, iron, and zinc, while it decreased the potassium and phosphorus contents. The most profound effect was observed in the calcium level that increased from 43.57 mg 100 g⁻¹ in T₀ (control) to 1 055.71, 2 186.70, 2 941.60, and 3 843 mg 100 g⁻¹ in T₁, T₂, T₃ and T₄, respectively. This led to an increase in the calcium to phosphorus ratio which is in favour of increased calcium utilisation in humans (Makai and Chudáček 1991). Such data coincide with Ray et al. (2017) and Bradauskiene et al. (2017) studies as they also found a great increase in the calcium level of bakery products upon eggshell addition.

Sensory characteristics of biscuits. Sensory scores of the biscuits supplemented with 5 and 10% eggshell powder (T₁ and T₂) showed similarity to the control (T₀) (Figure 2) whereas at 15 and 20% (T₃ and T₄) levels significant changes in taste, flavour, and texture were observed. The scores for taste decreased with an increase in the level of supplementation because of sandy taste. Compared to previous studies, Chilek et al. (2018) also reported the presence of sandy taste upon eggshell addition in higher quantities. The overall acceptability of biscuits tended to be until 10% supplementation level. These outcomes are consistent with those reported by Salem et al. (2012), yet not with Hassan (2015), who suggested 6% inclusion in biscuit formulation. This inconsistency might be the conse-

Figure 2. Radar chart of sensory characteristics of biscuits (mean \pm SD; $n = 20$)

SD – standards deviation; T₀ – control, biscuits with 0% eggshell powder; T₁ – biscuits with 5% eggshell powder; T₂ – biscuits with 10% eggshell powder; T₃ – biscuits with 15% eggshell powder; T₄ – biscuits with 20% eggshell powder

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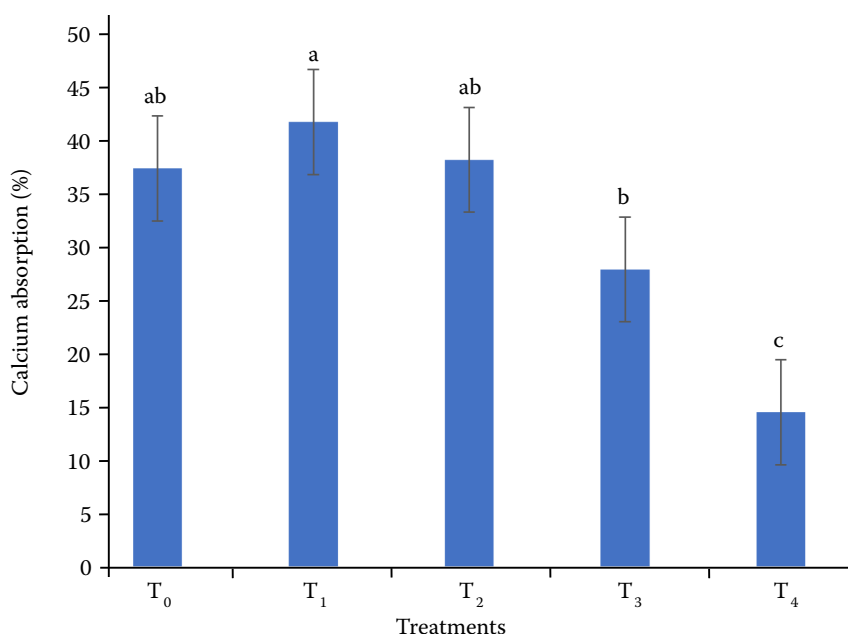


Figure 3. *In vivo* calcium absorption from biscuits (%) (mean ± SD; n = 3)

SD – standards deviation; different letters indicate a significant difference ($P < 0.05$), with 'a' representing the highest absorption value group; T₀ – control, biscuits with 0% eggshell powder; T₁ – biscuits with 5% eggshell powder; T₂ – biscuits with 10% eggshell powder; T₃ – biscuits with 15% eggshell powder; T₄ – biscuits with 20% eggshell powder

quence of differences in the particle size of eggshell powder used.

***In vivo* calcium absorption from biscuits.** Results for apparent calcium absorption are shown in Figure 3. Calcium absorption in T₀, T₁, T₂, T₃ and T₄ was found as 37.47, 41.83, 38.27, 27.95, and 14.51%, respectively. Biscuits with 5% eggshell powder supplementation (T₁) exhibited a higher rate of absorption. A significant decrease in absorption rate was observed at 15 and 20% supplementation levels (T₃ and T₄). This reduction in absorption rate by increasing the supplementation level might be the result of a high calcium level that surpasses the calcium requirement of rat's diet (Reeves et al. 1993). Eggshell calcium is highly bioavailable. Chang (2003) analysed the calcium bioavailability from eggshell and calcium carbonate in rats and found a more noteworthy increase in the calcium content of the femur for eggshell than for calcium carbonate. Likewise, in human studies eggshell powder consumption has shown constructive impacts on bone mineral density (Schaafsma et al. 2002; Sakai et al. 2017), indicating eggshell is a better source of bioavailable calcium.

CONCLUSION

This study illustrated that eggshell powder supplementation led to a considerable increase in the minerals, mainly in the calcium content of biscuits, with a sig-

nificant reduction in the calorie value. The 5% inclusion in biscuits resulted in a higher calcium absorption rate. Moreover, the eggshell powder did not produce any undesirable sensory changes up to 10% supplementation level. These results affirm the potential benefits of eggshell powder utilisation to increase the dietary calcium intake of individuals by using biscuits as a supplementation vehicle. Furthermore, the application of eggshells in food could provide additional means of reducing the huge waste of generated eggshells.

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