BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 15, Number 1, April 2026 | Pages: 1021-1025 | DOI: 10.14421/biomedich.2026.151.1021-1025 ISSN 2540-9328 (online)

Optimization of Calcium- Enriched Cookies Incorporating Eggshell and Shiitake Powder

Roshan Kunwar, Bishal Thagunna*, Smriti Paudel, Anjana Lamichhane, Apekshya Bhattarai, Sandhya Subedi
Pokhara Bigyan Tatha Prabidhi Campus, Affiliated to Tribhuvan University, Nepal.

Corresponding author
bisu.thaguna123@gmail.com

Manuscript received: 17 March 2026. Revision accepted: 12 June 2026, Published: 18 June 2026.

Abstract

Eggshells from chickens can serve as a source of dietary calcium. This research examined the impact of incorporating eggshell powder on the proximate, mineral, and sensory characteristics of cookies. The cookies were made using wheat flour, enhanced with eggshell powder in proportions of 3, 5, 7, and 9%, along with shitake mushroom powder in amounts of 3, 4, 5, and 6%. The samples were labeled as A, B, C, D and E. The dough was prepared using a multi-stage mixing technique, like the creaming method. After that, it was shaped into round cookies, which were baked in a preheated oven at 180 ^{o}C for 25 minutes. The results obtained showed that incorporating eggshell powder significantly raised the calcium levels in the cookies, measuring 1085.05, 1505.20, 1975.22, and 2450.11 mg/100g at concentrations of 3%, 5%, 7%, and 9%, respectively. Additionally, there is a slight alteration in the texture and sensory characteristics of the cookies. The iron content of cookies containing shitake mushroom powder at 3, 4, 5, and 6 % were 3.50, 3.41, 3.36, and 2.28 mg/100g respectively. The proximate analysis revealed that adding eggshell and shiitake mushroom powder increased the protein content from 8.83% to 14.67%, the ash content from 1.56% to 4.59%, and the fiber content from 0.92% to 2.88%. Simultaneously, it reduced the moisture content from 5.78% to 4.69% and the fat content from 19.03% to 15.93%. To summarize, adding eggshell powder and shiitake mushroom could improve the nutritional profile of traditional cookies, particularly in terms of calcium, ash, fiber, and protein levels.

Keywords: Eggshell; Calcium; Shitake mushroom; Cookies; Multi-stage mixing.

INTRODUCTION

It is projected that the global population will hit 10 billion around the year 2050, and meeting the nutritional needs of everyone with the existing food resources seems unfeasible. Consequently, it becomes crucial to reduce food and nutrient loss and waste, as well as to make the most efficient use of our nutritional resources (FAO, 2019). One third of all greenhouse gas emissions globally are the result of food waste. Food is lost at every point in the supply chain, from production to distribution, which raises greenhouse gas levels and contributes to climate change and global warming, negatively affecting living organisms (Ray et al., 2017; Waheed et al., 2019). The shells of chicken eggs are considered agricultural waste that can cause environmental pollution if not disposed of correctly (Khan et al., 2021; Aditya et al., 2021). The decomposition of eggshell waste leads to environmental pollution due to the unpleasant smell that draws in flies, posing a challenge for its disposal (Oliveira et al., 2013; Khan et al., 2021). Chicken eggs are regarded as an economical source of nutrients, including protein, fat, and vitamins. The structure of the eggshell consists of an inner and outer shell membrane, the mammillary layer, the palisade layer, vertical crystals, and a cuticle. In terms of weight, the eggshell constitutes about 9-12% of the egg's total weight, primarily composed of 94-96% calcium carbonate along with various trace microelements (Hassan, 2015). The eggshell can act as a source of calcium, which can be included in calcium-fortified foods for human nutrition. Moreover, research indicated that eggshell-derived calcium carbonate (CaCO3) in powdered form is more easily absorbed in the small intestine of rats compared to standard commercial CaCO3 (Swiatkiewicz et al., 2015). Up until now, a lack of calcium in human diets has been a widespread issue. Calcium is an essential mineral for the human body that supports the maintenance of bone tissue. Calcium works in conjunction with other minerals like phosphorus to form the structural foundation of bones and teeth (Zulkeflee et al., 2020).

Edible mushrooms are consumed in numerous countries around the world, serving as a vital food source due to their significant contributions to human health, including medicinal properties and nutritional enhancement. These mushrooms are abundant in proteins, vitamins, and minerals, placing them in a category between meat and vegetables (Toan and Thu,

2018). Additionally, edible mushrooms are low in carbohydrates, calories, and fats, and they play a protective role in preventing chronic diseases (Maria et al., 2015). The health benefits of various mushroom species include reducing cholesterol levels, lowering blood pressure, boosting the immune system's defense against illnesses, fighting tumors, and improving liver function (Bilal et al., 2010). In comparison, there is a limited amount of research focused on using eggshell for calcium enrichment. This study aimed to explore the incorporation of eggshell powder and shiitake mushrooms in the creation of functional cookies and to assess their sensory acceptability.

MATERIALS AND METHOD

Preparation of egg shell powder

Eggshells are sourced from the market, cleansed with water, and boiled in deionized water for 30 minutes before the shell membrane is discarded. The eggshells are then placed in a hot air oven at 80°C for 2 hours, after which they are ground into a fine powder. The eggshell powder that passes through a 90 μm sieve is utilized in the study (Afzal et al., 2020).

Preparation of shiitake mushroom flour

The fruiting bodies of Shiitake mushrooms were harvested and rinsed with distilled water to eliminate dirt, sand, soil, damaged areas, and any other unwanted materials prior to use. After cleaning, the mushrooms were cut into smaller pieces with a knife and arranged on a tray lined with wax paper, then dried in an oven at 65°C for 24 hours. The dried mushrooms were subsequently blended into a powder and sifted through a 70 mesh (212 μm) screen in the laboratory. The Shiitake mushroom powder was stored in sealed polyethylene bags at a temperature of 4°C until it was used for analysis and cookies preparation (Toan and Thu, 2018).

Preparation of wheat, egg shell and shitake mushroom based cookies

Batter type cakes were made using multi-stage mixing technique, such as the creaming method (Wilderjans et al., 2013). A pastry bag was utilized for shaping and forming the cookies into rounds. The shaped round cookies were then baked in a preheated oven at 180 °C for 25 minutes. Finally, the baked cookies were cooled to room temperature and stored in low-density polyethylene pouches (Kshetri, 2017). For the preparation of cookies different formulation of wheat flour, egg shell powder, shitake mushroom and other ingredients shown in **Table 1**.

Table 1. Formulation of cookies.

Raw materials	A	B	C	D	E
Wheat flour	100 g	91 g	90 g	89 g	88 g
Eggshell powder	0	3 g	5 g	7 g	9 g
Shiitake mushroom powder	0	6 g	5 g	4 g	3 g
Sugar	40 g	40 g	40 g	40 g	40 g
Butter	40 g	40 g	40 g	40 g	40 g
Egg albumin	1 ml	1ml	1ml	1ml	1 ml
Salt	0.5 g	0.5 g	0.5g	0.5 g	0.5 g
Baking powder	1.5 g	1.5g	1.5 g	1.5 g	1.5 g
water	40 ml	40 ml	40 ml	40 ml	40 ml

Determination of proximate composition

Proximate analysis of the cookies was carried out using the method of Association of Official Analytical Chemist (AOAC 2005).

Determination of mineral composition

AOAC (2005) methods were used to determine the mineral compositions of the samples.

Sensory evaluation

The sensory analysis for overall quality was carried out by semi-trained panelists, which consisted of teachers and students of Pokhara Bigyan Tatha Prabidhi Campus. The parameters for sensory evaluation are appearance, aroma, taste, texture and overall acceptability. Sensory evaluation was performed according to the 9- Point Hedonic Scale.

Statistical analysis

Sensory score data was statistically analyzed using SPSS (version 20.0). LSD was done by using same software. Other physical, chemical and ultimate analyses data was analyzed by ANOVA using Microsoft Excel 2016.

RESULT AND DISCUSSION

Proximate composition of developed cookies

The proximate composition moisture, fat, protein, ash, fiber and carbohydrate of five different cookies were analyzed. The value of moisture, fat, protein, ash, fiber

and carbohydrate content of the cookies ranged from 4.69-5.78 %, 15.93-20.83 %, 8.83-14.67 %, 1.56-4.59 %, 0.92-2.88 %, and 65.88-57.87 % respectively as shown in Table 2 below.

Table 2. Proximate composition of developed cookies.

Cookies	Moisture %	Fat %	Protein %	Ash %	Fiber %	Carbohydrate %
A	5.78	19.03	8.83	1.56	0.92	64.20
B	5.54	20.83	14.67	3.27	2.88	53.04
C	5.41	19.36	12.68	3.72	2.20	57.55
D	5.17	17.51	11.82	3.59	1.86	60.80
E	4.69	15.93	9.70	4.59	1.29	64.22

Moisture content refers to the quantity of water contained in a material or substance (Animashaun et al., 2016). It was noted that there was a significant difference in moisture content between the fortified cookies and the control sample. The cookies made entirely from wheat had the highest moisture content at 5.78%, while the sample containing 9% eggshell powder had the lowest at 4.69%. This indicates that the fortified cookies possess greater shelf stability. It was noted that as the concentration of eggshell increased, the moisture content decreased (Afzal et al., 2020). The aim of the fortification process was to increase protein levels and enhance storage stability by lowering moisture content, thereby improving the product's shelf stability characteristics (Adebayo et al., 2010). The fortification of shiitake mushrooms raises the fat content, while a higher amount of eggshell powder reduces it. The fat content of the control sample was measured at 19.03%, whereas sample B, which contained the highest percentage of shiitake mushrooms, exhibited the highest fat content at 20.83%. Conversely, sample E had the lowest fat content at 15.93%, attributed to the greater proportion of eggshell powder used. A similar fluctuation was found by the (Ermiş et al., 2020). According to Afzal et al. (2020), the fat content in muffins enhanced with eggshell decreased as the quantity of incorporated eggshell powder increased. According to the data presented in **Table 2**, there were significant differences in protein and ash content among all the formulations. The levels of protein and ash in the cookies notably increased with higher amounts of supplementation from shitake mushroom and egg shell powder (Rosli, 2013). The highest protein (14.67%) and ash content (4.59%) were observed in this formulation compared to the others. An increase in protein and ash content may be due to the high mineral presence in both egg shell and shitake mushroom. These findings are consistent with the results reported by Krystyjan et al. (2015) and Silva et al. (2018). The information shown in **Table 2** demonstrates that adding shiitake mushroom powder to the mixtures results in an increased crude fiber content, with the highest level reaching 2.88% in sample B, which includes 6% shiitake mushroom powder. Sample B exhibited the greatest crude fiber content, attributed to the higher levels of crude fiber present in shiitake mushrooms in comparison to the other flours (USDA, 2019). According to Van and Thu (2018), the biscuits made from shiitake mushrooms contained a fiber content of 0.9%.

Mineral composition of cookie samples

Table 3. The different mineral compositions of unfortified and fortified cookies.

Cookies	Iron mg/100g	Calcium mg/100g
A	3.20	29.54.13
B	3.50	1085.05
C	2.41	1505.20
D	2.36	1975.22
E	2.28.	2450.11

The data indicated that cookies made with 9% egg shell powder had the highest calcium content at 2450.11. Likewise, the cookie containing 6% shiitake mushroom exhibited the highest iron content at 3.50mg/100g. The minerals content iron and calcium contents of the supplemented cookies with 3, 5, 7, and 9 % egg shell powder and 3, 4, 5, and 6 % shitake mushroom powder recorded highest than the control one. Numerous studies have been carried out to assess the potential of using eggshells as a food ingredient for calcium (Daengprok et al., 2002). This resulted in a higher calcium-to-phosphorus ratio in the enriched cookies, which could enhance calcium absorption in humans (Hassan et al., 2015). The impact of cookies was most significantly observed in calcium levels. The incorporation of eggshell powder resulted in a substantial rise in the calcium and iron concentrations in the enriched butter cake (Salem et al., 2012).

Sensory evaluation of cookies

The impact of supplementing cookies with eggshell and shiitake mushroom powder on their sensory properties was examined. A panel of semi-trained judges assessed the cookies based on organoleptic attributes.

Figure 1. Formulated cookies samples.

The average scores for color, flavor, taste, texture, and overall acceptability of the control cookies without fortification were 9.0, 9.2, 9.5, 9.5, and 9.5, respectively. The findings also indicated that there were no statistically significant differences noted between the control cookies and those enhanced with 3%, 5%, 7%, and 9% eggshell powder or 3%, 4%, 5%, and 6% shiitake mushroom powder regarding color. The addition of 9% eggshell to the cookies led to significant differences ($P<0.05$) in taste and appearance. The sensory evaluation further revealed that the crispiness, flavor, and taste profile remained similar up to the 6% level of the same calcium source. Conversely, with the addition of higher amounts of calcium sources, a more pronounced fishy odor was detected due to the increased levels of calcium (Swiatkiewicz et al., 2015). This was corroborated by sensory tests showing that the firmness of the eggshell increased as the level of supplementation rose. The acceptability of the fortified eggshell calcium powder appeared to be stable up to 6% eggshell fortification. Brun et al. (2013) discovered that the optimal method for utilizing chicken eggshell as a calcium dietary supplement is to grind it into a powder to be added to bread, pizza, or spaghetti, as this results in minimal alterations to texture and no changes in flavor.

CONCLUSION

It can be concluded that eggshell powder serves as a suitable and economical source of calcium for human dietary needs and can be prepared easily at home. The addition of eggshell powder to wheat flour did not significantly alter the flavor and texture of cookies at levels up to 6%.

Acknowledgement: The authors are sincerely thankful to the Pokhara Bigyan Tatha Prabidhi Campus for chemical and equipment support.

Conflicts of interest: The authors declare no conflict of interest

REFERENCES

Adebayo, O., Olatidoye, Ogundipe, Balogun, O.O. and Aro F O (2010) Quality Characteristics of Cookies Produced from Composite Flours of Wheat And Mushroom. J. of Sci. and Multidisciplinary Res. II. Available at: www.cenresin.org.

Aditya,S., Stephen J. and Radhakrishnan, M. (2021). Utilization of eggshell waste in calcium-fortified foods and other industrial, applications: A review. Trends in Food Science and Technology, 115: 422–432

Afzal, F., Mueen-ud-Din, G., Nadeem, M., Murtaza, M.A. and Mahmood, S. (2020) Effect of eggshell powder fortification on the physicochemical and organoleptic characteristics of muffins. Pesqui Agropecu Bras, 9, 1488–1496

Animashaun, O. H., Bamgbose, A., & Olorode, O. O. (2016). CHARACTERISTICS OF COOKIES PRODUCED FROM WHEAT AND EGG SHELL FLOUR. *Journal of Sciences and Multidisciplinary Research*, *8*(1).

Bilal AW, Bodha RH, Wani AH. Nutritional and medicinal importance of mushrooms. J Med Plants Res 2010; 4:2598-604

Brun, L.R., M. Lupo, D.A. Delorenzi, V.E. Di Loreto and A. Rigalli, 2013. Chicken eggshell as suitable calcium source at home. International Journal Food Science and Nutrition, 64(6): 740-3

Daengprok, W., W. Garnjanagoonchorna and Y. Mine, 2002. Fermented pork sausage fortified with commercial or hen eggshell calcium lactate. Meat Sci., 62: 199-204

Ermiş, E., Tuğla, B. K., & Külsoy, B. (2020). Effects of adding eggshell powder and olive seed powder to biscuit formulation on some quality properties. *Türk Tarım-Gıda Bilim ve Teknoloji dergisi*.

FAO, The State of Food and Agriculture 2019. Moving Forward on Food Loss and Waste Reduction (FAO, Rome, 2019).

Hassan, N. M. (2015). Chicken eggshell powder as dietary calcium source in biscuits. World Journal of Dairy & Food Science, 10(2): 199-206.

Hassan, N. M. (2015). Chicken eggshell powder as dietary calcium source in biscuits. *World J. Dairy Food Sci*, *10*(2), 199-206.

Khan, F., Ameer, K., Qaiser, M., Pasha, I., Mahmood, Q., Anjum, F., Riaz, A and Amir, R. (2021). Development and analysis of bread fortified with calcium extracted from chicken eggshells of Pakistani market. Food Sci. Technol, Campinas, 41(1): 14-20

Krystyjan M, Gumul D, Ziobro R, Korus A. 2015. The fortification of biscuits with bee pollen and its effect on physicochemical and antioxidant properties in biscuits. LWT Food Science and Technology 63: 640–646. doi:10.1016/ j.lwt.2015.03.075

Kshetri, s (2017) Preparation and quality evaluation of multigrain cookies. , 1–73

M. Waheed, M.S. Butt, A. Shehzad, N.M. Adzahan, M.A. Shabbir, H.A. Rasul Suleria et al., Eggshell calcium: a cheap alternative to expensive supplements. Trends Food Sci. Technol. 91, 219–230 (2019)

Maria EV, Talía HP and Octavio PL. Edible Mushrooms: Improving Human Health and Promoting Quality Life. International Journal of Microbiology Volume 2015; Article Clinical ID 376387. 3.

Oliveira, D. A., Benelli, P. and Amante, E. R. (2013). A literature review on adding value to solid residues: Egg shells. Journal of Cleaner Production, 46: 42–47

S. Ray, A. Kumar Barman, P. Kumar Roy, B. Kumar Singh, Chicken eggshell powder as dietary calcium source in chocolate cakes. Pharma Innov. 6(9), 1–4 (2017)

Salem, I.S., A.S.M. Ammar and R.A. Habiba, 2012. Effect of Eggshell Powder Addition as a Source of Calcium Fortification on Butter Cake Quality. Journal of Agricultural and Veterinary Sciences, 5(2): 109-118

Silva F de O, Miranda TG, Justo T, Frasão B da S, Conte-Junior CA, Monteiro M, Perrone D. 2018. Soybean meal and fermented soybean meal as functional ingredients for the production of low-carb, high-protein, high-fiber and high isoflavones biscuits. LWT - Food Science and Technology 90: 224–231. doi:10.1016/j.lwt.2017.12.035

Swiatkiewicz, S., Arczewska-Wosek, A., Krawczyk, J., Puchala, M. and Józefiak, D. (2015). Effects on performance and eggshell quality of particle size of calcium sources in laying hen with different Ca concentration. Journal of Archive Animal Breed, 58: 301-307

USDA (2019) FoodData Central. Agricultural Research Service. Available at: https://fdc.nal.usda.gov/fdc-app.html#/food-details/168436/nutrients [Accessed December 21, 2022]

Van Toan, N., & Thu, L. N. M. (2018). Preparation and improved quality production of flour and the made biscuits from shitake mushroom (Lentinus edodes). *Clinical Journal of Nutrition and Dietetics*, *1*(1), 1-9.

Van Toan, N., & Thu, L. N. M. (2018). Preparation and improved quality production of flour and the made biscuits from shitake mushroom (Lentinus edodes). *Clinical Journal of Nutrition and Dietetics*, *1*(1), 1-9.

Wilderjans, E., Luyts, A., Brijs, K. and Delcour, J.A. (2013) Ingredient functionality in batter type cake making. Trends Food Sci Technol, 30, 6–15.

Zulkeflee, W., Chompoorat, P., & Siva, R. (2020). Physicochemical and sensory properties of bread added with chicken eggshell powder. *Malaysian Journal of Analytical Sciences*, *24*(6), 873-881.

THIS PAGE INTENTIONALLY LEFT BLANK