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Eggshells Calcium Extraction and the Application in Food Fortifications

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Eggshells are the hard, outer covering of eggs. It is known that eggshells are discarded as waste materials, although they contain a significant amount of calcium. The study was aimed to extract and quantify calcium from the eggshells and fortification on the biscuit and yogurt products. The extraction of calcium was done using calcium chloride with HCL solution at different propositions (1:1, 1:5, 1:10, 1:15, and 1:20). After extraction, the sample was dried at 50 °C temperature for 3 hours to obtain dry calcium chloride. Calcium was fortified at a concentration of 100 ppm, 1000 ppm, and 2000 ppm in both the biscuits and yogurt, respectively. The calcium-fortified samples were analyzed for sensory properties and chemical composition. The ash content of calcium-fortified yogurt (0.47) was slightly higher than normal yogurt (0.44), while the other chemical components remains similar to the control. For the sensory evaluation result, the biscuit with 2000 ppm calcium-fortified biscuit and 1000 ppm calcium-fortified yogurt was found to be highly acceptable among the calcium-fortified samples. The extraction of calcium chloride from eggshells was obtained the highest for eggshells on HCl ratio 1:20 (w/v) where calcium chloride was found 32.92%, 26.95%, and 23.63% for duck, layer chicken, and local chicken eggshells, respectively. The extraction rate of calcium chloride of duck eggshells was higher than the local and layer chicken's eggshells. Therefore, it may be opined that the fortified products (2000 ppm Ca) contained a considerably higher amount of calcium content than the control sample.

Keywords: Extraction, Eggshells, Calcium, Fortification, Products.

1. INTRODUCTION

Eggshell is an excellent source of dietary calcium for health and was approved to be an effective source for increasing bone mineral density (BMD) and reduce the pain of the elder population with osteoporosis [1]. Eggshell matrix protein (1%) attached with calcium carbonate ($CaCO_3$) can enhance calcium transportation in the body through intestinal epithelial cell $CaCO_2$ [2]. An average-sized egg (55–60 g) consists of 10% shell and membrane [3]. Calcium is considered a vital nutrient required for maintaining many important biological activities such as nerve cells, muscle cells, mitosis, chronic diseases, and coagulation of blood [4, 5]. The dietary reference intake (DRIs) of calcium is 800–1300 mg/day based on the age of the consumer [6]. Usually, milk and dairy products are considered as the best natural sources of calcium from which approximately 75% to 89% are being ingested from aforesaid sources [7]. The calcium obtained from dairy sources possesses high bioavailability and can be attained at a relatively lower cost by considering the nutritional value [8]. Considering this issue, food industries are continuously working on developing an alternative source for calcium and develop and introduce new calcium-fortified products so that the consumers can get the opportunity to meet their daily calcium requirements [8]. Fortification is the process of adding up micronutrients such as essential vitamins to any food items to supply the level of this micronutrient/element [9]. The fortification of certain nutrients to various food products has been practiced

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worldwide such as vitamin D, vitamin B2, and B3 for more than 80 years to prevent people from different types of diseases [10, 11]. Researchers have demonstrated that the calcium fortification to various food items could be treated as an economical way to achieve additional calcium as well as ensuring the total requirement of calcium [12, 13]. Eggshell can be utilized to treat industrial waste effectively and efficiently [14].

Considering the importance of calcium fortification, the current research has been carried out for the extraction and characterization of calcium from eggshell wastes. Consequently, the different proportion of extracted calcium was fortified into model food system (yogurt and biscuit) to formulate the best acceptable product in terms of nutritional and sensory evaluations.

2. MATERIALS AND METHODS

2.1. Sample Collection and Reagents

The duck, layer, and local chicken eggshells were collected from the residential area of Bangladesh Agricultural University, Mymensingh. The necessary ingredients such as sugar, shortening agent, milk powder, baking powder, salt, eggs, etc. were collected from the local market. While reagents and chemicals were first grades.

2.2. Extraction Process of Calcium Chloride ($CaCl_2$) from Eggshells

2.2.1. Preparation of Membrane-Free Eggshells Powder

Eggshells were rinsed thoroughly with distilled water to eliminate adhered filth and extraneous particles. Shell membranes were separated from eggshells manually. Membrane-free shells were sterilized in water at 100 °C for 20 min to kill the pathogenic microorganism [15]. Eggshells were crushed to small pieces then dried in a cabinet dryer at 60 °C for 6 hr. The dried eggshells were ground by a grinder and sieved through a 30 mm mesh. The fine eggshell powder was packed in high-density polyethylene (HDPE) bag and stored at the ambient condition to using further.

2.2.2. Extraction of Calcium Chloride from Eggshells

The eggshell powder was mixed with different amounts of HCl solution with a ratio of powder to HCl solution of 1:1, 1:5, 1:10, 1:15, and 1:20 (w/v) [16]. Mixtures were transferred on a hot plate with a magnetic stirrer and heated with continuous stirring at 50 °C for 3 hr. The mixtures were cooled to room temperature and the eggshells, calcium chloride was packed in HDPE bags for further analyses.

2.3. Chemical Analysis

Chemical analysis of eggshells such as moisture, carbohydrate, crude fiber, ash, and protein contents was carried out according to the methods described by Refs. [17–19]. Minerals such as calcium, magnesium, arsenic lead, and zinc contents were analyzed [20–23] with slight modifications.

Table I. Formulation of biscuit.

Ingredients	Treatment			
	B_1	B_2	B_3	B_4
Wheat flour (g)	60	60	60	60
Fat (g)	16	16	16	16
Sugar (g)	20	20	20	20
Calcium chloride (extracted from duck eggshells with 10 ml HCl) (ppm)	0	100	1000	2000
Salt (g)	0.50	0.50	0.50	0.50
Baking powder (g)	1.25	1.25	1.25	1.25
Milk powder (g)	2.25	2.25	2.25	2.25
Egg	(1/2) no	(1/2) no	(1/2) no	(1/2) no
Vanilla essence	2 drops	2 drops	2 drops	2 drops

2.4. Formulation and Preparation of Fortified Biscuits

The biscuits were formulated as shown in Table I by the following method described by Das et al. [24]. The dough was rolled to obtain a thin uniform sheet of 3 mm thickness. The biscuits were prepared from the thin sheets using a round biscuit cutter of 3.6 cm diameter. The prepared biscuits were baked at 180 °C for 15 min in the baking oven. The biscuits were cooled, packed, and stored at ambient temperature for further analysis.

2.5. Formulation and Preparation of Fortified Yogurt

The ingredients of the yogurt are shown in Table II were used by the following method described by Abdelazez et al. [5]. Processed yogurt was analyzed for moisture, fat, protein, pH, acidity, and ash contents.

2.6. Sensory Evaluations

Twenty judges evaluated the sensory properties of the fortified biscuits and yogurt products for different formulations according to the method described by Vivek et al. [25] for color, flavor, texture taste, and overall acceptability with the help of the 9-point hedonic rating test.

Table II. Formulation of yogurt.

Ingredients	Treatment			
	Y_1	Y_2	Y_3	Y_4
Milk (ml)	100	100	100	100
Sugar (g)	12	12	12	12
Culture (g)	4	4	4	4
Calcium chloride (extracted from duck eggshells with 10 ml HCl) (ppm)	0	100	1000	2000

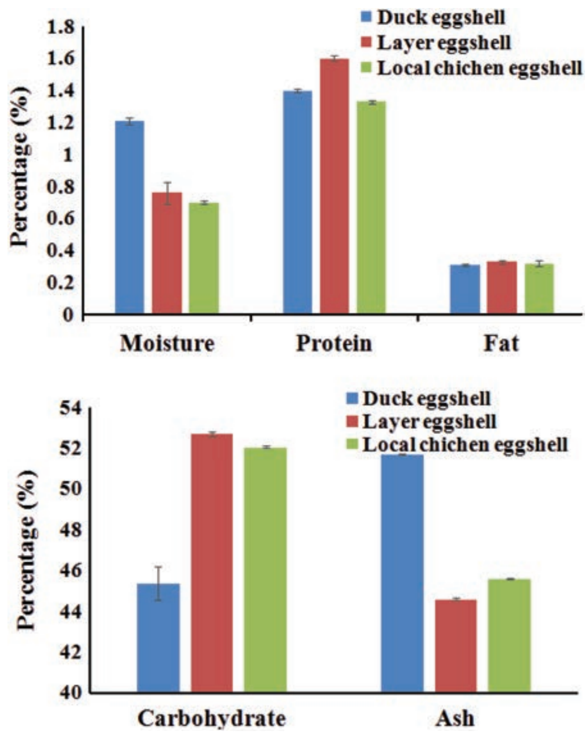

Fig. 1. Composition of eggshells.

3. RESULTS AND DISCUSSION

3.1. Chemical Composition of Eggshells

The eggshells of duck, layer chicken, and the local chicken were analyzed for moisture, protein, fat, ash, and carbohydrates contents, while the result is presented in Figure 1. The duck eggshells contained the highest moisture content followed by layer eggshells while the lowest content was for local eggshells. This analysis also showed that duck eggshells contained the highest ash content, while the layer eggshells had the lowest ash and carbohydrate contents. The chemical compositions of different eggshells were consistent with the results depicted by Al-awwal and Ali [26], where chicken eggshell contained $0.5 \pm 0.03\%$ moisture, $43.5 \pm 0.0316\%$ ash, $1.35 \pm 0.4\%$ protein, $0.30 \pm 11\%$, and fat, $51.65 \pm 0.44\%$ carbohydrate by wet basis. The minor differences observed in these compositions can be due to different species or even food habits.

3.2. Mineral Composition of Eggshells

The eggshells were analyzed for mineral composition. The analyzed results are shown in Figure 2. The analysis showed that duck eggshells contained 52.8 mg calcium, 3.68 mg magnesium, 1.4 mg zinc, 0.18 mg lead, and 0.15 mg arsenic. Mineral matters of layer eggshells were 42.9 mg calcium, 3.49 mg magnesium, 3.2 mg zinc, 0.44 mg lead, and 0.32 mg arsenic, whether the local eggshell contained 39.9 mg calcium, 3.86 mg magnesium, 2.7 mg zinc, 0.17 mg lead, and 0.13 mg arsenic, respectively. The observed mineral values for the current study were very close to Al-awwal and Ali results [26]. In conclusion, the analysis showed that duck eggshells represented the highest calcium content (52.8 mg), while the lowest content was for local eggshells (39.9 mg). Local eggshells had the highest magnesium content (3.86 mg) than duck eggshells and layer eggshells. The analysis also showed that layer eggshells contained the highest zinc (3.2 mg), lead (0.44 mg), and arsenic content (0.32 mg).

3.3. Extraction Rate of Calcium Chloride from Eggshell

The yield of calcium chloride from eggshells according to the different ratios of HCl has been presented in Figure 3. The highest amount of calcium chloride was found from the duck eggshells when the same amount of HCl was used for extraction. Similarly, local eggshells provided the highest amount of calcium chloride than layer eggshells while the same amount of HCl was used for extraction. The highest yield was obtained from eggshells to HCl ratio 1:20 (w/v) for the duck, local, and layer eggshell were 32.92%, 26.95%, and 23.63%, respectively. Extraction amount with 10 ml HCl was the best option in the point of cost-effectiveness. No significant changes were detected by applying 15 ml and 20 ml HCl application.

3.4. Composition of Calcium-Fortified Biscuit Product

The compositions of calcium-fortified biscuits are given in Table III. The examined compositions were similar to those reported by Foda et al. [27], where moisture, protein, fat, ash, and total carbohydrate contents in the biscuit were found in the range of 2.24–6.97%, 6.90–10.80%,

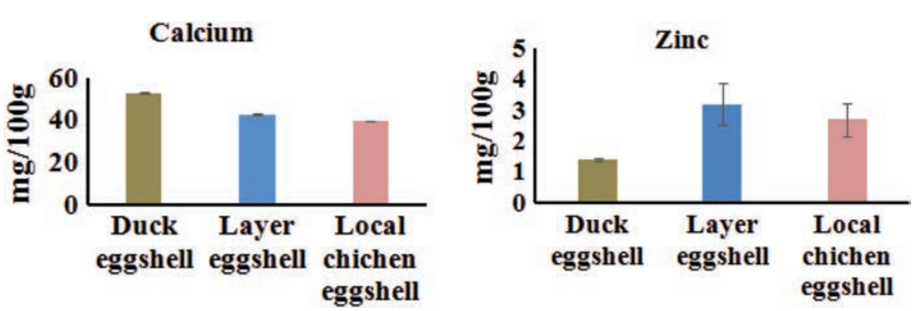

Fig. 2. Continued.

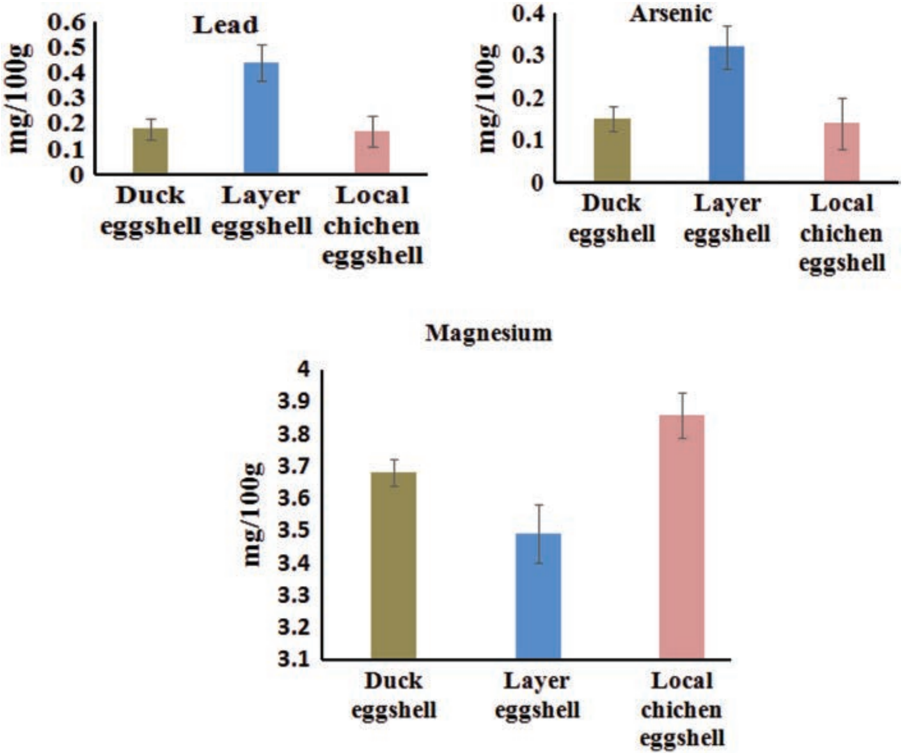

Fig. 2. Mineral compositions of eggshells.

5.66–26.67%, 0.49–1.90%, and 64.31–83.45%, respectively. The moisture content of calcium-fortified biscuits was slightly higher than biscuits with no calcium fortification. Similarly ash content of calcium-fortified biscuits is slightly higher than control biscuits. It is seen that the maximum amount of Ca was found in the sample fortified with 2000 ppm Ca (4206 ppm) followed by 1000 ppm and 100 ppm Ca, while the lowest Ca was found in the control sample (2337 ppm).

3.5. Composition of Calcium-Fortified Yogurt Product

The compositions of yogurt was given in Table IV. It was revealed that the chemical compositions of calcium-fortified yogurt were similar to the regular whole milk yogurt. There are no significant differences between them ($P < 0.05$). The acidity of whole milk yogurt was slightly higher (1.1) than calcium-fortified yogurt (0.95–0.90). On the other hand, pH of calcium fortifies yogurt slightly higher than the normal whole milk yogurt. Calcium content in the fortified yogurt was the highest for 2000 ppm Ca fortified yogurt (3071 ppm) whereas the lowest was found in the control sample (1225 ppm).

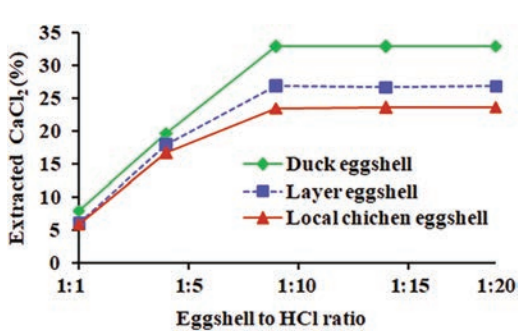

Fig. 3. Extraction of calcium chloride from eggshells.

3.6. Sensory Properties of Calcium-Fortified Biscuits and Yogurt Products

A panel of twenty people evaluated calcium-fortified biscuits for color, flavor, texture, overall acceptability, as shown in Figure 4. From the obtained mean scores for color, it is seen that the biscuits fortified with 2000 ppm Ca (B_4) secured the highest score and followed by B_3 and B_2. Concerning flavor, B_4 secured the highest score and was equally acceptable as samples B_3, B_2, and B_1, and all of the samples were ranked as like moderately, while for texture, sample B_4 (2000 ppm Ca) secured the highest score. Thus for texture evaluation, there were no significant differences among different levels of calcium fortification at a 5% level of significance. For overall acceptability, it was observed that sample B_4 (2000 ppm Ca) obtained the highest score and significantly differed from another level of fortification at a 5% level of significance. From the sensory evaluation, it was observed that samples B_4 and B_1 secured the highest rank and lowest rank, respectively. It may be concluded that sample B_4 (2000 ppm Ca fortification) was best among the calcium-fortified biscuit samples.

Sensory evaluation conducted with the developed yogurt samples for their color, flavor, texture, taste, and overall acceptability values and mean scores are represented in

Table III. Chemical analysis of calcium fortified biscuit.

Samples	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Carbohydrate (%)	Calcium (ppm)
B_1 (Control)	4.5 ± 0.20^{c}	17.25 ± 0.32^{a}	10.20 ± 0.56^{a}	1.53 ± 0.1^{b}	66.26 ± 1.18^{b}	2337 ± 44^{d}
B_2 (100 ppm Ca)	5.24 ± 0.25^{b}	17.24 ± 0.3^{a}	10.22 ± 0.27^{a}	1.53 ± 0.13^{b}	65.77 ± 0.95^{c}	2422 ± 26^{c}
B_3 (1000 ppm Ca)	5.05 ± 0.27^{bc}	17.26 ± 0.43^{a}	10.19 ± 0.53^{a}	1.54 ± 0.09^{ab}	66.96 ± 1.32^{a}	3289 ± 44^{b}
B_4 (2000 ppm Ca)	5.81 ± 0.23^{a}	17.25 ± 0.46^{a}	10.19 ± 0.39^{a}	1.57 ± 0.15^{a}	65.18 ± 1.23^{d}	4206 ± 36^{a}

Notes: Average ± S.D., $n = 5$, Samples having the same superscript do not differ at 5% level of significance (α).

Table IV. Chemical analysis of calcium fortified yogurt.

Sample	Moisture (%)	Ash (%)	pH	Acidity (%)	Fat (%)	Protein (%)	Calcium (ppm)
Y_1 (Control)	70.1 ± 0.3^{a}	0.44 ± 0.01^{c}	4.38 ± 0.0^{b}	1.1 ± 0.03^{a}	5.8 ± 0.14^{a}	4.29 ± 0.1^{a}	1225 ± 15^{d}
Y_2 (100 ppm ca)	69.93 ± 0.2^{ab}	0.44 ± 0.02^{c}	4.39 ± 0.0^{b}	0.95 ± 0.03^{b}	5.79 ± 0.1^{a}	4.29 ± 0.1^{a}	1312 ± 23^{c}
Y_3 (1000 ppm Ca)	69.87 ± 0.3^{a}	0.45 ± 0.02^{b}	4.42 ± 0.0^{ab}	0.95 ± 0.01^{b}	5.79 ± 0.4^{a}	4.27 ± 0.12^{a}	2176 ± 44^{b}
Y_4 (2000 ppm Ca)	68.8 ± 0.32^{b}	0.47 ± 0.01^{a}	4.44 ± 0.0^{a}	0.90 ± 0.03^{c}	5.8 ± 0.01^{a}	4.28 ± 0.09^{a}	3071 ± 29^{a}

Notes: Average ± S.D., $n = 5$, Samples having the same superscript do not differ at 5% level of significance (α).

Figure 5. The sample Y_3 (1000 ppm Ca) scored the highest score and was significantly different from others at a 5% level of significance. In the case of flavor preference, Y_4 (2000 ppm Ca) secured the highest score, followed by Y_3, Y_2, and Y_1, respectively. Hence, it could be remarked that the sample with 1000 ppm Ca (Y_3) fortification was the best among the yogurt sample fortified at different Ca levels.

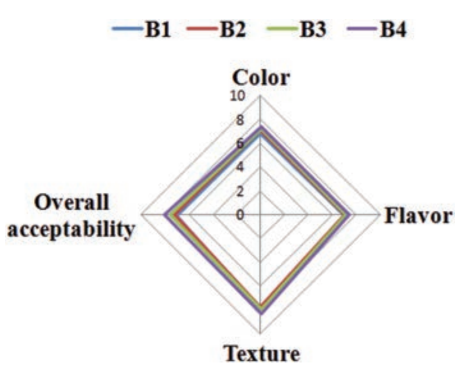

Fig. 4. Sensory scores of calcium fortified biscuit product.

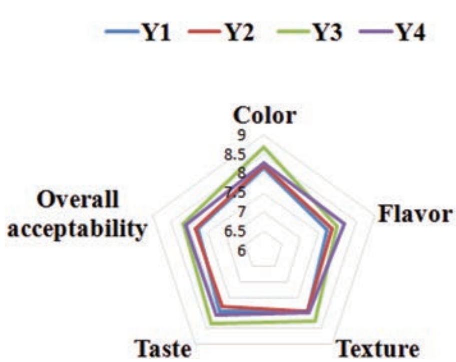

Fig. 5. Sensory scores of calcium fortified yogurt product.

4. CONCLUSION

Calcium was extracted from three different eggshells and was used in the preparation of biscuits and yogurt products. Findings evinced that the calcium content of duck eggshells was the topmost than layer eggshells and local eggshells, while extraction with 20 ml HCl resulted in the highest extraction rate. Duck eggshells had more moisture and ash than layer and local chicken eggshells whereas protein, fat, and carbohydrate content were highest for layer eggshells. The proximate composition of calcium-fortified biscuits and yogurt was not differing in the different percentages of calcium fortification. Sensory evaluation data showed that fortified biscuits and yogurt were more acceptable than non-fortified products. Biscuit fortified with 2000 ppm calcium was found to be the best among the calcium-fortified biscuits in the sensory evaluation. On the other hand, yogurt with 1000 ppm calcium fortification was found to be the best among the sensory evaluation. Further research is recommended for the extraction of calcium as calcium ions which can be used in milk products and other food products.

Ethical Compliance

There are no researches conducted on animals or humans.

Conflicts of Interest

There are no conflicts to declare.

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References

1. Daengprok, W., Garnjanagoonchorn, W., Naivikul, O., Pornsinlpatip, P., Issigonis, K. and Mine, Y., **2003**. Chicken eggshell matrix proteins enhance calcium transport in the human intestinal epithelial cells, Caco-2. *Journal of Agricultural and Food Chemistry*, *51*(20), pp.6056–6061.
2. Miller, G.D., Jarvis, J.K. and McBean, L.D., **2001**. The importance of meeting calcium needs with foods. *Journal of the American College of Nutrition*, *20*(2), pp.168S–185S.
3. Nicklas, T.A., **2003**. Calcium intake trends and health consequences from childhood through adulthood. *Journal of the American College of Nutrition*, *22*(5), pp.340–356.
4. Sami, R.A., Khojah, E.Y., Elgarni, E.A. and Benajiba, N., **2017**. Evaluation of nutritional status for some sensitive sets and its relationship to natural antioxidants. *Journal of King Abdulaziz University-Medical Sciences*, *24*, pp.1–9.
5. Abdelazez, A., Muhammad, Z., Zhang, Q.X., Zhu, Z.T., Abdelmotaal, H., Sami, R. and Meng, X.C., **2017**. Production of a functional frozen yogurt fortified with bifidobacterium spp. *BioMed. Research International*, *2017*(6438528), pp.1–10.
6. Benajiba, N. and Eldib, R.S., **2018**. Exploring attitudes related to sweetened soft drinks consumption among adults in Saudi Arabia. *Nutrition and Food Science*, *48*, pp.433–441.
7. Sami, R., Bushnaq, T., Radhi, K., Benajiba, N. and Helal, M., **2020**. Prevalence of thinness cases and dietary diversity among learners of various education stages in taif region, Saudi Arabia. *African Journal of Food, Agriculture, Nutrition and Development*, *20*, pp.17081–17094.
8. Abdelazez, A., Abdelmotaal, H., Evivie, S.E., Melak, S., Jia, F.F., Khoso, M.H., Zhu, Z.T., Zhang, L.J., Sami, R. and Meng, X.C., **2018**. Screening potential probiotic characteristics of lactobacillus brevis strains in vitro and intervention effect on type I diabetes in vivo. *BioMed. Research International*, *2018*(7356173), pp.1–20.
9. Khojah, E.Y. and Sami, R., **2016**. Fatty acids composition and oxidative stability of peanut and sesame oils with the sensory evaluation of mayonnaise prepared by different oils. *Assiut Journal of Agricultural Sciences*, *47*(6–2), pp.460–472.
10. Sami, R., Li, Y., Qi, B., Wang, S., Zhang, Q., Han, F., Ma, Y., Jing, J. and Jiang, L., **2014**. HPLC analysis of water-soluble vitamins (B2, B3, B6, B12, and C) and Fat-soluble vitamins (E, K, D, A, and β-carotene) of okra (*Abelmoschus esculentus*). *Journal of Chemistry*, *2014*, DOI: 10.1155/2014/831357.
11. Abdelazez, A., Abdelmotaal, H., Zhu, Z.T., Fang-Fang, J., Sami, R., Zhang, L.J., Al-Tawaha, A.R. and Meng, X.C., **2018**. Potential benefits of lactobacillus plantarum as probiotic and its advantages in human health and industrial applications: A review. *Advances in Environmental Biology, 12*, pp.16–27.
12. Rokayya, S., Garsa, A., Eman, E. and Helal, M., **2021**. Saudi community care awareness food facts, nutrients, immune system and covid-19 prevention in Taif city among different age categories. *The African Journal of Food, Agriculture, Nutrition and Development*, *21*, pp.17213–17233.
13. De Angelis, G., Medeghini, L., Conte, A.M. and Mignardi, S., **2017**. Recycling of eggshell waste into low-cost adsorbent for Ni removal from wastewater. *Journal of Cleaner Production*, *164*, pp.1497–1506.
14. Katha, P.S., Ahmed, Z., Alam, R., Saha, B., Acharjee, A. and Rahman, M.S., **2021**. Efficiency analysis of eggshell and tea waste as low cost adsorbents for Cr removal from wastewater sample. *South African Journal of Chemical Engineering*, *37*, pp.186–195.
15. Than, M.M., Lawanprasert, P. and Jateleela, S., **2012**. Utilization of eggshell powder as excipient in fast and sustained release Acetaminophen tablets. *Mahidol University Journal of Pharmaceutical Sciences*, *39*(3–4), pp.32–38.
16. Garnjanagoonchorn, W. and Changpuak, A., **2007**. Preparation and partial characterization of eggshell calcium chloride. *International Journal of Food Properties*, *10*(3), pp.497–503.
17. Bishai, D. and Nalubola, R., **2002**. The history of food fortification in the United States: Its relevance for current fortification efforts in developing countries. *Economic Development and Cultural Change*, *51*(1), pp.37–53.
18. West, C.E., Eilander, A. and van Lieshout, M., **2002**. Consequences of revised estimates of carotenoid bioefficacy for dietary control of vitamin a deficiency in developing countries. *The Journal of Nutrition*, *132*(9), pp.2920S–2926S.
19. Keller, J.L., Lanou, A.J. and Dbarnard, N.E.A.L., **2002**. The consumer cost of calcium from food and supplements. *Journal of the American Dietetic Association*, *102*(11), pp.1669–1671.
20. Sami, R., Alshehry, G., Ma, Y., Abdelazez, A. and Benajiba, N., **2019**. Evaluation of some specific components existences in okra (*Abelmoschus esculentus L.* (Moench)) cultivated from different areas. *Journal of Food and Nutrition Research*, *2019*(7), pp.155–161.
21. Frisbie, S.H., Mitchell, E.J., Yusuf, A.Z., Siddiq, M.Y., Sanchez, R.E., Ortega, R., Maynard, D.M. and Sarkar, B., **2005**. The development and use of an innovative laboratory method for measuring arsenic in drinking water from western Bangladesh. *Environmental Health Perspectives*, *113*(9), pp.1196–1204.
22. Sami, R., Elhakem, A., Alharbi, M., Benajiba, N., Almatrafi, M. and Helal, M., **2021**. Nutritional values of onion bulbs with some essential structural parameters for packaging process. *Applied Sciences*, *11*(2317), pp.1–11.
23. Korn, M.D.G.A., Ferreira, A.C., Teixeira, L.S.G. and Costa, A.C.S., **1999**. Spectrophotometric determination of zinc using 7-(4-nitrophenylazo)-8-hydroxyquinoline-5-sulfonic acid. *Journal of the Brazilian Chemical Society*, *10*, pp.46–50.
24. Das, P.C., Rana, M.S., Saifullah, M. and Islam, M.N., **2018**. Development of composite biscuits supplementing with potato or corn flour. *Fundamental and Applied Agriculture*, *3*(2), pp.453–459.
25. Vivek, K., Singh, S.S., Sasikumar, R. and Sami, R., **2021**. Consumer preference study on combined ultrasound and sodium hypochlorite treated freshcut kiwifruits coated with chitosan using the fuzzy logic approach. Sensory study of chitosan coated fresh-cut kiwi fruits. *Journal of Microbiology, Biotechnology and Food Sciences*, *11*(1), pp.1–5.
26. Al-awwal, N.Y. and Ali, U.L., **2015**. Proximate analyses of different samples of egg shells obtained from sokoto market in Nigeria. *International Journal of Science and Research*, *4*(3), pp.564–566.
27. Foda, Y.H., Allam, M.H., Mahmod, R.M. and Elshatanovi, G.A., **1984**. Quality of biscuits supplemented with low fat soya flour. *Annals of Agricultural Science, Egypt*, *29*(1), pp.327–335.

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