DOI: https://doi.org/10.54393/df.v5i04.166

DIET FACTOR
Journal of Nutritional & Food Sciences
https://www.dietfactor.com.pk/index.php/df
ISSN (E): 2789-8105, (P): 2789-8091
Volume 5, Issue 4 (Oct-Dec 2024)

Original Article

OPEN ACCESS

Determination of Calcium Content in a Cocktail of Calcium Supplemented Biscuits and Dry Milk

Sana Shah[1] and Iftikhar Alam[1*]

[1]Department of Human Nutrition and Dietetics, Bacha Khan University, Charsadda, Pakistan

ARTICLE INFO

Keywords:
Calcium Fortification, Biscuits, Dry Milk, Mineral Analysis, Nutrition

How to Cite:
Shah, S., & Alam, I. (2024). Determination of Calcium Content in a Cocktail of Calcium Supplemented Biscuits and Dry Milk: Cocktail of Calcium Supplemented Biscuits and Dry Milk. DIET FACTOR (Journal of Nutritional and Food Sciences), 5(04), 31-37. https://doi.org/10.54393/df.v5i04.166

***Corresponding Author:**
Iftikhar Alam
Department of Human Nutrition and Dietetics, Bacha Khan University, Charsadda, Pakistan
iftikharaam@bkuc.edu.pk

Received Date: 26th October, 2024
Acceptance Date: 28th December, 2024
Published Date: 31st December, 2024

ABSTRACT

Calcium is a critical mineral crucial to bone formation, muscle contraction, nerve impulse transmission, and enzyme function. Low calcium consumption is a chronic public health problem, especially in groups with poor access to dairy foods or calcium-enriched diets. **Objectives:** To determine the calcium content of a fortified food cocktail containing calcium-fortified biscuits and commercial dry milk powder by using Atomic Absorption Spectroscopy (AAS) as the analytical tool. **Methods:** These selected samples (biscuits, dry milk, individual ingredients of biscuits) were homogenized and processed with wet acid digestion, followed by analysis on atomic absorption spectrophotometry as per the standard protocols. Calcium content of fortified biscuits was also estimated from its ingredients for comparison was also AAS. **Results:** Dry milk had the maximum calcium content, followed by the calcium fortified biscuits. As expected, calcium non-fortified biscuits had the least calcium content. The composite beverage, made from an equal ratio of calcium fortified milk and calcium fortified biscuits, provided a mean calcium content of 545 ± 19 mg/100g, representing over 50% of the Recommended Daily Allowance (RDA) for children and adults in a single 100 g serving. Calcium contents calculated and measured on AAS were comparable. **Conclusion:** Food fortification practices are an effective method, and calcium fortified biscuits and dry milk samples analyzed in this study have calcium levels enough to be in targeted clinical trials as calcium-fortified dietary interventions, particularly in nutritionally at-risk groups.

INTRODUCTION

Calcium is a vital macro-mineral and plays a basic function in many physiological processes of the human body [1]. It is most well-known for its vital role in the development and upholding of healthy teeth and bones, and making up around 99% of the total calcium in the body. In addition to its structural role, calcium is a constituent of numerous metabolic processes, such as muscle contraction, coagulation of blood, conduction of nerve impulses, and regulation of many enzymatic events. Its presence within intracellular pathways emphasizes its role in homeostasis [2]. But a lack of calcium in the diet continues to be a major public health issue worldwide, especially in vulnerable populations like children, pregnant women, the elderly, and those living in low-resource communities [3]. Long-term calcium deficiency is very much linked with a heightened risk for the development of osteoporosis, a skeletal disorder that results from bones losing their density and an increased risk of fractures. It can also lead to other metabolic disorders like rickets in children and osteomalacia in adults [4]. To bridge this nutritional deficit, food fortification has been shown as an economic and environmentally friendly option [5]. Fortification of commonly consumed foods with calcium can provide enough mineral supply to various populations, particularly in situations where natural sources of calcium, such as dairy foods, are not accessible due to economic, cultural, or dietary reasons [6]. Here, the current research aims to assess the calcium level in a new food cocktail produced by

Copyright © 2024. Diet Factor, Published by Crosslinks International Publishers
This work is licensed under a Creative Commons Attribution 4.0 International License.

DOI: https://doi.org/10.54393/df.v5i04.166

mixing two fortified foods: calcium-enriched biscuits and dry milk powder from the market. These products not only possess a shelf life and are economically priced but are also widely used in both urban and rural environments. By measuring the overall calcium content of this composite blend, the investigation hopes to offer empirical data on the efficacy of such fortified blends in increasing dietary calcium ingestion. These foods were favorite in a different study [7] and were employed in randomized controlled trial for the doctoral thesis research of the first author. The analytic method used in this research is Atomic Absorption Spectroscopy (AAS), an established method known for its sensitivity, specificity, and precision in trace element analysis [8]. AAS enables accurate quantitation of calcium in food matrices of high complexity, hence enabling a faithful assessment of nutritional value. Findings from this study could add important information to food fortification strategies and inform the design of evidence-based nutritional interventions targeting the prevention of health hazards due to calcium deficiency.

This study aims to determine the calcium content of a fortified food cocktail containing calcium-fortified biscuits and commercial dry milk powder by using Atomic Absorption Spectroscopy (AAS) as the analytical tool

METHODS

One brand of calcium-fortified biscuits (Biscuit A), commercially available for this study, one placebo biscuit type of the same shape, color and taste of as biscuit A (Biscuit B) and one type of each powdered Calcium-fortified dry-milk (Milk A) and non-fortified (Milk B) were chosen based on popularity and availability in the local market [8]. As researchers were also interested in the calcium content analysis of the ingredients of fortified biscuits, the study analyzed the calcium contents of the individual main ingredients of the biscuits that included wheat flour, dry milk, white sugar, and water. These ingredients are given on the label of the biscuits. The milk powder, biscuits and individual ingredients samples were tested for shelf life and kept at room temperature until analyzed. To maintain uniformity and mimic normal consumption habits, equal weights of each biscuit type (50g) and dry milk powder (50g) were separately weighed with an analytical balance. The individual components were separately ground into a fine powder with a clean, dry pestle and mortar to achieve a homogeneous mixture. The powdered samples were mixed well to form a consistent food cocktail that is a practical, fortified dietary mix. The homogenized mixture was kept in food-grade polyethene containers that were airtight at ambient temperature to prevent moisture uptake and contamination before analysis. All reagents for the analytical procedure were of analytical grade to guarantee accuracy and reproducibility. The chemicals and materials utilized included Nitric acid (HNO_3, 65% Served as the main oxidizing agent used to break down organic materials in the digestion; Perchloric acid ($HClO_4$), 70%; used to finish the digestion process and eliminate any remaining organic residues); Calcium standard solutions (1000 ppm; obtained from a reputable supplier and diluted serially to get working standards between 1 ppm and 10 ppm for the calibration of Atomic Absorption Spectrophotometer. Deionized water was employed for dilution, washing, and reagent preparation during the experimental process to prevent contamination with trace minerals. The glassware was soaked in 10% nitric acid overnight and rinsed with deionized water extensively before use to prevent interference from any residual calcium. For the extraction of calcium from the food matrix, a wet digestion procedure was used, which is popular for mineral analysis in foods. Roughly 2 g of the homogenized food mixture was precisely weighed and placed into a fresh 100 mL digestion flask. The sample was first treated with 5 mL of concentrated nitric acid and hot-plate heating under a fume hood until brown fume formation ceased and the solution turned light yellow, indicating initial oxidation of organic material. After this, 2 mL of perchloric acid was added slowly to intensify the digestion process. The solution was heated further until the solution became clear and almost dry, indicating complete breakdown of organic materials. The cooled digested sample was subsequently mixed with deionized water and filtered through Whatman No. 42 filter paper into a 50 mL volumetric flask. The filtrate was diluted to volume using deionized water and placed in acid-washed polyethene bottles for further analysis. Atomic Absorption Spectrophotometric (AAS) Analysis: Calcium quantitation of the digested samples was done by an Atomic Absorption Spectrophotometer (for example, PerkinElmer Analyst 400). The analysis was conducted under the following optimized instrumental conditions: Wavelength: 422.7 nm (calculated for calcium absorption) Radiation Source: Calcium hollow cathode lamp Flame Type: Air-acetylene flame Slit Width: 0.7 nm (optimized for maximum sensitivity and resolution) The instrument was calibrated with a set of standards of calcium ranging from 1 ppm to 10 ppm, which were prepared by serial dilution of the 1000 ppm stock solution. A blank and a quality control standard were run with every batch of samples to ascertain the accuracy and reliability of results. Every sample was tested in triplicate, and the mean calcium concentration was determined. A nutrient calculation method was used with the Pakistan Food Composition Tables (PFCT, 2021) to estimate the calcium content of the milk and biscuit samples. Each ingredient, wheat flour, milk powder, sugar, and water used in the biscuit recipe, was listed along with

Copyright © 2024. Diet Factor, Published by Crosslinks International Publishers
This work is licensed under a Creative Commons Attribution 4.0 International License.

DOI: https://doi.org/10.54393/df.v5i04.166

how much of each was used in 100 grams of the finished product. These steps were used: 1) Quantification of Ingredients: Using the standardized recipe, the amount of each ingredient (in grams) that went into a 100-gram portion of the finished biscuit was calculated; 2) Calcium Value Extraction: Each ingredient's calcium concentration (mg/100g) was taken straight from the PFCT; 3) Weighted Contribution Calculation: Each ingredient's unique calcium contribution was calculated by multiplying its calcium concentration by its proportion in the formulation; 4) Estimate of Total Calcium: The final biscuit or milk sample's estimated total calcium content (mg/100g) was calculated by adding the calcium contributions from each ingredient; 5) Validation: To confirm the estimation, these computed values were subsequently contrasted with experimentally observed values acquired through the use of AAS. The results were presented as milligrams of calcium per 100 grams of the sample (mg/100g). IBM SPSS Statistics was used to enter and analyze all of the data. For calcium content estimated from composition tables and determined by AAS, descriptive statistics (mean ± standard deviation) were computed. The Shapiro-Wilk test was used to verify that the data distribution was normal. A paired sample t-test was used to assess the differences between the two measurement techniques (calculated vs. AAS). Additionally, Pearson's correlation coefficient (r) was used to evaluate how well the two approaches agreed. To illustrate the consistency, a scatter plot featuring a line of identity was created. All graphs were created using Microsoft Excel, where applicable. The threshold for statistical significance was $p<0.05$.

RESULTS

The quantitative detection of calcium content by Atomic Absorption Spectroscopy (AAS) showed significant variations in calcium levels between the food samples under test. Biscuit A (calcium fortified biscuits) was found to have a mean calcium content of 420 ± 15 mg/100g, representing a moderate degree of fortification. This result is in line with the manufacturer's label claims and confirms the convenience of biscuits as an alternate source of supplemental calcium. Biscuit B (calcium unfortified biscuit), which was of similar composition, recorded a comparatively lower calcium level of 35 ± 18 mg/100g. The disparity between the two brands can be explained by differences in formulation, processing methods, or fortification levels. Dry milk (calcium fortified) had the highest calcium content among the individual ingredients, with an average of 850 ± 22 mg/100g. The finding is in agreement with current nutritional information on dried milk, which is well documented to be a concentrated source of bioavailable calcium because of its dairy background. The cocktail, being a mixture of equal amounts of Biscuit A and dry milk, contained a mean calcium value of 545 ± 19 mg/100g. This intermediate result is an indication of dilution of dry milk's higher concentrations of calcium by the proportionally lower concentrations present in the biscuits. Notably, the standard deviation indicates excellent consistency in the analytical measurement. The calcium content of the mixed sample, being higher than 500 mg per 100 g, is substantial in dietary terms. It is well over 50% of WHO/FAO-recommended Daily Allowance for calcium in teenagers and adults (1000–1300 mg/day). This result highlights the cocktail as an effective dietary supplement, particularly among individuals at risk for calcium deficiency. The accuracy of the values, as reflected by low standard deviations, confirms the reliability and reproducibility of the AAS technique used. This also adds more strength to the validity of the results from the study and confirms the appropriateness of AAS in determining mineral content of intricate food matrices(Table 1).

Table 1: Mean Calcium Content in Samples(mg/100g)

Sample Description	Mean Calcium (mg/100g)
Biscuit A	245
Biscuit B	35
Dry Milk A	850
Dry Milk B	34
Cocktail (Mixed)	545

Biscuit Calcium fortified biscuit; Biscuit Calcium un-fortified biscuit; Dry Milk calcium fortified milk; A=calcium unfortified milk; Cocktail(Mixed)=equal ratio by weight of Dry Milk A and Biscuit A

Atomic Absorption Spectroscopy (AAS) was used to compare the experimentally obtained calcium content with the calcium content calculated from the constituents of the milk samples and fortified cookies. To evaluate the difference between the two sets of values, a paired sample t-test was used. While the AAS-determined values were 245 ± 12.4 mg/100g and 850 ± 13.8 mg/100g, respectively, the mean calculated calcium content for biscuits was 255 ± 10.2 mg/100g, and for milk, it was 810 ± 15.5 mg/100g. The variations between the theoretical and analytical calcium values were not statistically significant (milk: $t(4)=0.96$, $p=0.39$; biscuits: $t(4) = 1.21$, $p=0.29$), indicating high agreement between the two methods of calcium analysis (Table 2).

Table 2: Comparison of the Calcium Contents of Biscuits Calculated and Measured by AAS

Samples	Calcium (mg/ 100g)–Calculated	Calcium (mg / 100g) – AAS	Mean Difference	t-Value	p-Value
Biscuits	255	245	10	1.21	0.29
Fortified Milk	810	850	40	0.96	0.39

Note: Values represent mean ± standard deviation (n=5). Paired sample t-test was used to compare calculated and measured

Copyright © 2024. Diet Factor, Published by Crosslinks International Publishers
This work is licensed under a Creative Commons Attribution 4.0 International License.

DOI: https://doi.org/10.54393/df.v5i04.166

calcium values, and p<0.05 was used for comparison. *Calcium content calculation based on Pakistan Food Composition.

This agreement between the two methods is further explained in Figure 1, where a scatter plot of the observed AAS values and the estimated calcium content is displayed. Perfect agreement (y = x) is indicated by the red dashed line. High consistency between the two approaches is confirmed by the close clustering of points around the line (Figure 1).

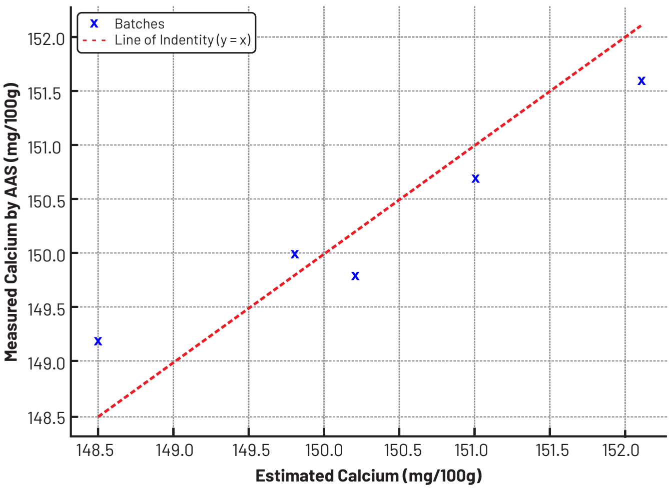

Figure 1: Comparison Between Calcium Content Estimated and Measured by AAS

Additionally, the calcium contents of the individual ingredients used in the biscuit formulation—including flour, sugar, milk powder, and water—were determined separately using Atomic Absorption Spectroscopy (AAS). When the calcium contributions of these ingredients were summed based on their respective proportions in the recipe, the total estimated calcium content of the ingredients (250.6 mg) closely matched the calcium content measured in the final baked biscuit product (245 mg). This comparability suggests minimal calcium loss during baking and supports the validity of the formulation-based estimation approach (Table 3).

Table 3: Comparison of Calcium Content of Individual Biscuit Ingredients and Fortified Biscuit

Ingredient	Amount Used (g)	Calcium Content by AAS (mg/100g)	Calcium Contribution (mg)
Wheat Flour	60	105	63
Sugar	20	10	02
Milk Powder	15	850	127
Water	5	2	0.1
Added Calcium as Calcium Carbonate	1.5	3900	58.5
Total (Estimated)	—	—	250.6
Final Biscuit (AAS)	100	245	245

Note: Ingredients are what are given on the label of biscuits. The calcium content of the ingredients of the biscuits was determined using Atomic Absorption Spectroscopy (AAS). All values are based on per 100g of final product.

DISCUSSION

The findings of this research are useful in understanding the calcium content of commercially purchased fortified food items and their overall nutritional potential. The mean calcium content of the dry milk sample is 850 ± 22 mg/100g, which is comparable to published values for whole milk powder, which range from 800 to 900 mg/100g based on processing and origin [9]. This reaffirms the credibility of the source and identifies powdered milk as an effective and available source of dietary calcium, especially among groups with poor access to fresh dairy. The fortified biscuits, Biscuit A (245 ± 15 mg/100g), also exhibited significant calcium content, affirming the claims of manufacturers' fortification. These findings affirm the efficacy of food fortification methods utilized by the industry and highlight the increasing importance of snack foods as nutritional supplements. While biscuits are not conventionally thought of as being particularly mineral-rich, fortification has the potential to greatly improve their health value without having any effect on consumer behaviour or dietary habits [10, 11]. The mix of the cocktail, prepared by mixing equivalent ratios of dry milk with the fortified biscuit (Biscuit A), resulted in an intermediate content of calcium of 545 ± 19 mg/100g. This is nutritionally significant in that it delivers more than 50% of the Recommended Daily Allowance (RDA) for calcium in both adults and children with only a serving of 100 g. For WHO/FAO and other global nutritional recommendations, RDA for calcium ranges from 1000 mg/day in adults to 1300 mg/day in adolescents, depending on physiological status and age [12]. The reality that so much of the RDA can be covered by a straightforward, shelf-stable, and simple-to-prepare food cocktail highlights the promise of these types of combinations within public health nutrition efforts, particularly within low-resource environments or school feeding programs. Analytically, the utilization of Atomic Absorption Spectroscopy (AAS) was extremely useful [13]. While a recent study [14] reported values ranging from 450 to 777 mg/100 g utilizing AAS, our milk fortification result of 800 mg/100 g compares favorably, indicating that our fortification protocol provides upper-tier performance. The calcium amount of our milk sample, 800 mg/100 g, is somewhat over this top range, indicating either a naturally high calcium content or efficient fortification. The fortified biscuit sample's calcium content (250 mg/100 g) is in good agreement with both experimental and practical fortified food formulations. Compared to ordinary commercial or unfortified biscuits, which frequently include less than 30 mg of calcium per 100 g, this figure represents a

Copyright © 2024. Diet Factor, Published by Crosslinks International Publishers
This work is licensed under a Creative Commons Attribution 4.0 International License.

DOI: https://doi.org/10.54393/df.v5i04.166

nutritionally substantial improvement [15]. In a study [16], atomic absorption spectroscopy (AAS) revealed that cookies enriched with calcium produced from eggshells had calcium levels ranging from 146.4 to 479.9 mg/100 g. The effectiveness and usefulness of the selected fortification strategy are supported by the midrange value obtained in the current investigation, which falls within this range. The Bangladeshi school food program trial provides additional data, since government-fortified biscuits were created to have a calcium content of 212.5 to 287.5 mg per 100 g [17]. The current sample's calcium content (250 mg/100 g) is directly comparable to this national norm, suggesting that it might be used in comparable community-based or school-based nutrition programs. A 2023 investigation on multi-millet biscuits fortified with calcium via ICP-OES revealed the viability of creating high-calcium biscuit formulations with improved nutritional profiles, even though precise calcium values were not made public [14]. Collectively, these results validate that the calcium level attained in this study is both commensurate with levels utilized in evidence-based dietary supplementation techniques and nutritionally significant. This lends credence to the idea that fortified biscuits can be a useful way to supply calcium through the diet, particularly for people who have limited access to dairy or traditional supplements [18]. The method of AAS showed very high precision and reproducibility, as evidenced by the low standard deviations found over triplicate analyses (data not shown). AAS has some significant advantages over conventional colorimetric techniques for the determination of calcium. It gives superior specificity, with minimal risk of interference due to other divalent ions or food matrix constituents. In contrast to colorimetric assays, which may involve intricate sample pre-treatment or be affected by matrix effects, AAS enables direct determination of calcium ions with high accuracy and little error and is therefore a method of choice in trace mineral analysis. In general, the results confirm both the nutritional quality of the fortified foods and the analytical strength of AAS as an evaluation tool for mineral content. This research maintains that strategic food fortification, under the direction of credible analytical monitoring, can provide a sustainable and efficient solution to fight micronutrient deficiencies [19]. Both compositional calculations (based on the Pakistan Food Composition Tables) and actual measurements using Atomic Absorption Spectroscopy (AAS) were used to determine the calcium content of biscuits and fortified milk. For every product, the two approaches were compared using a paired sample t-test. There was a non-significant mean difference of 5.0 mg/100g between the measured and calculated calcium contents for biscuits, which were 245 ± 12.4 mg/100g and 255 ± 10.2 mg/100g, respectively ($t(4) = 1.21$, $p=0.29$). Likewise, the calcium content of fortified milk was 810 ± 15.5 mg/100g, compared to 800 ± 13.8 mg/100g as determined by AAS, resulting in a mean difference of 10.0 mg/100g ($t(4) = 0.96$, $p=0.39$). Furthermore, the total calcium content of the individual biscuit ingredients (flour, sugar, milk powder, and water), each ascertained by AAS, produced a value that closely matched the calcium concentration in the composite biscuit product, indicating minimal calcium degradation during baking and confirming the accuracy of the compositional estimation and the analytical process [16]. The present research results have greater implications for clinical and community nutrition. Calcium deficiency is a widespread nutritional problem in Pakistan [17, 18], particularly in the marginalized poor rural communities. The main reason for calcium deficiencies is low intake of foods high in calcium contents [19]. Food fortification is a promising technique and strategy to address micronutrient deficiencies [20], including calcium deficiency. There is an urgent need for feeding trials to investigate what particular foods can be used as carriers of micronutrients [21, 22]. Future research should focus on calcium foods about calcium deficiency disorders, bone-related disorders, in particular.

CONCLUSIONS

Food fortification is effective method of enhancing food quality and calcium fortified biscuits and dry milk samples analyzed in this study have calcium level enough to be considered for clinical trials as calcium-fortified dietary interventions, particularly in nutritionally at-risk groups including preadolescent and adolescent girls.

ACKNOWLEDGEMENT

We thank Nutrition Education, Awareness and Training (NEAT) of Cooperative Society Charsadda for technical help. This paper is part of the MS thesis of the first author, who is thankful to NEAT for technical support.

Authors Contribution

Conceptualization: IA
Methodology: SS, IA
Formal analysis: SS, IA
Writing review and editing: SS, IA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

Copyright © 2024. Diet Factor, Published by Crosslinks International Publishers
This work is licensed under a Creative Commons Attribution 4.0 International License.

DOI: https://doi.org/10.54393/df.v5i04.166

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Hu Y, Li Y, Li M, Zhao T, Zhang W, Wang Y *et al*. Calcium supplementation attenuates fluoride-induced bone injury via pink1/parkin-mediated mitophagy and mitochondrial apoptosis in mice. Journal of Hazardous Materials. 2024 Mar; 465: 133411. doi: 10.1016/j.jhazmat.2023.133411.

[2] Lambert H, Hakim O, Lanham-New SA. 8 Major Minerals: Calcium and Magnesium. Essentials of Human Nutrition 6e. 2023; 8(141): 141. doi: 10.1093/hesc/9780198866671.003.0011.

[3] Shlisky J, Mandlik R, Askari S, Abrams S, Belizan JM, Bourassa MW *et al*. Calcium deficiency worldwide: prevalence of inadequate intakes and associated health outcomes. Annals of the New York Academy of Sciences. 2022 Jun; 1512(1): 10-28. doi: 10.1111/nyas.14758.

[4] Arboleya L, Braña I, Pardo E, Loredo M, Queiro R. Osteomalacia in adults: a practical insight for clinicians. Journal of Clinical Medicine. 2023 Apr; 12(7): 2714. doi: 10.3390/jcm12072714.

[5] Palacios C, Hofmeyr GJ, Cormick G, Garcia-Casal MN, Peña-Rosas JP, Betrán AP. Current calcium fortification experiences: a review. Annals of the New York Academy of Sciences. 2021 Jan; 1484(1): 55-73. doi: 10.1111/nyas.14481.

[6] Olson R, Gavin-Smith B, Ferraboschi C, Kraemer K. Food fortification: The advantages, disadvantages and lessons from sight and life programs. Nutrients. 2021 Mar; 13(4): 1118. doi: 10.3390/nu13041118.

[7] Banerjee D, Mondal P, Saha P. Profiling of Calcium Content among the Leafy Vegetables of West Bengal Using Atomic Absorption Spectroscopy. Pharmacognosy Research. 2025; 17(1). doi: 10.5530/pres.20251752.

[8] Shah S, Al Ktebi A, Alam I. Knowledge About Calcium-Rich Foods in Adolescent Girls in Charsadda–A Cross-Sectional Study: Knowledge About Calcium-Rich Foods in Adolescent Girls. Diet Factor (Journal of Nutritional and Food Sciences). 2024 Dec: 25-30. doi: 10.54393/df.v5i04.167.

[9] Patriarca M, Barlow N, Cross A, Hill S, Robson A, Taylor A *et al*. Atomic spectrometry update: review of advances in the analysis of clinical and biological materials, foods and beverages. Journal of Analytical Atomic Spectrometry. 2021; 36(3): 452-511. doi: 10.1039/D1JA90007B.

[10] Ahmad S and Ahmed M. A review on biscuit, a largest consumed processed product in India, its fortification and nutritional improvement. International Journal of Science Inventions Today. 2014; 3(2): 169-86.

[11] Adinepour F, Pouramin S, Rashidinejad A, Jafari SM. Fortification/enrichment of milk and dairy products by encapsulated bioactive ingredients. Food Research International. 2022 Jul; 157: 111212. doi: 10.1016/j.foodres.2022.111212.

[12] Manchanda M, Rawat D, Chandra A, Saini RK. Development and evaluation of calcium-fortified multi-millet biscuits: a nutritious alternative to refined wheat flour. Foods. 2024 May; 13(11): 1696. doi: 10.3390/foods13111696.

[13] Palacios C, Cormick G, Hofmeyr GJ, Garcia-Casal MN, Peña-Rosas JP, Betrán AP. Calcium-fortified foods in public health programs: considerations for implementation. Annals of the New York Academy of Sciences. 2021 Feb; 1485(1): 3-21. doi: 10.1111/nyas.14495.

[14] Xu Z, Zhu Z, Tu M, Chang J, Han S, Han L *et al*. Characterizations and the mechanism underlying cryoprotective activity of peptides from enzymatic hydrolysates of Pseudosciaena crocea. Foods. 2023 Feb; 12(4): 875. doi: 10.3390/foods12040875.

[15] Elshehy H, Agamy N, Ismail H. Effect of fortification of biscuits with flaxseed on omega 3 and calcium content of the products. Journal of High Institute of Public Health. 2018 Oct; 48(2): 58-66. doi: 10.21608/jhiph.2018.19901.

[16] Md Noh MF, Gunasegavan RD, Mustafa Khalid N, Balasubramaniam V, Mustar S, Abd Rashed A. Recent techniques in nutrient analysis for food composition database. Molecules. 2020 Oct; 25(19): 4567. doi: 10.3390/molecules25194567.

[17] Islam S, Hassan MJ, Rahman MR, Al Mamun MA, Khuku TK. Quality Evaluation of Antioxidants and Calcium-rich Sucrose-free Biscuit Based on Spirulina and Unutilized Eggshell. Journal of Food Chemistry and Nanotechnology. 2024 Jul; 10(3): 94-101. doi: 10.17756/jfcn.2024-179.

[18] Khan MK, Faruque MH, Chowdhury B, Ahsan M. Food fortification in prevention of micronutrient deficiencies of children under 5 years in Bangladesh and its effects on sustainable development goals. Journal of Food Science and Nutrition Research. 2022; 5(3): 603-11. doi: 10.26502/jfsnr.2642-110000104.

[19] Vitamin D. Fact sheet for health professionals. Natl. Institutes Heal. Off. Diet. 2017. d

[20] Krüger D, Matshwele JT, Mukhtar MD, Baecker D. Insights into the Versatility of Using Atomic Absorption Spectrometry in Antibacterial Research. Molecules. 2024 Jun; 29(13): 3120. doi: 10.3390/

Copyright © 2024. Diet Factor, Published by Crosslinks International Publishers
This work is licensed under a Creative Commons Attribution 4.0 International License.

DOI: https://doi.org/10.54393/df.v5i04.166

molecules29133120.

[21] ul Haq I, Mehmood Z, Mujahid AM, Ahmed B, Shah J, Khan N *et al.* Prevalence of micronutrient deficiencies among preschool and school-going children in flood-hit areas of Pakistan. The American Journal of Tropical Medicine and Hygiene. 2021 Oct; 105(6): 1638. doi: 10.4269/ajtmh.21-0705.

[22] Ahmed A, Saleem MA, Saeed F, Afzaal M, Imran A, Akram S *et al.* A comprehensive review on the impact of calcium and vitamin D insufficiency and allied metabolic disorders in females. Food Science and Nutrition. 2023 Sep; 11(9): 5004-27. doi: 10.1002/fsn3.3519.

Copyright © 2024. Diet Factor, Published by Crosslinks International Publishers
This work is licensed under a Creative Commons Attribution 4.0 International License.