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DIET FACTOR
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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)

# The Use of Preservatives in Food: A Shift Toward Natural Alternatives

OPEN ACCESS

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## ARTICLE INFO

***How to Cite:***

Imran Sajid, M. (2024). The Use of Preservatives in Food: A Shift Toward Natural Alternatives. DIET FACTOR (Journal of Nutritional and Food Sciences), 5(04), 01 https://doi.org/10.54393/df.v5i04.154

Preservatives are defined as anything that elongates the shelf life of food. It helps to prevent the spoilage of food by microorganisms and enhance its flavor, taste, and color. It is estimated that a total of 2,00,000 tons of food preservatives are used annually [1]. Salt, honey, and sugar are the natural preservatives used to preserve food for centuries. With the increase in food demand, many chemicals have been prepared to preserve food more effectively and efficiently.

Artificial preservatives have increased greatly during the last decade because of modernization, the food industry, and media advertisement [2]. The artificial food preservatives can provide us with prompt effects but in the long run, it could act as a poison if constantly ingested. When converted into nitrous acid inside, these artificial preservatives can be toxic: nitrates and nitrites. These preservatives can cause cancer in various organs. Benzoates which are used as antimicrobial agents cause asthma, allergies, and rashes.

Sorbates, another class of antimicrobial agents, have a lower rate of conversion into harmful substances within the body. There are some reports claiming it causes skin conditions such as urticaria and dermatitis [3]. As a result of this, there is an increased public awareness of the harmful effects of artificial preservatives on health, leading the public to demand safer alternatives.

This demand led many researchers to find alternative natural preservatives that can give the same antimicrobial and antioxidant efficacy. The widely studied natural preservatives alternative to artificial preservatives are polyphenols. Polyphenols are the secondary metabolites produced by plants in response to infection, stress conditions, and UV exposure.

Polyphenols have shown promising effects and no side effects on human health have been observed. It has been noticed that polyphenols have low stability, and their antioxidant activity is not efficient if they are directly used in food products. Various environmental factors such as heat, pH, electrolyte concentration, and oxidants affect their stability. Encapsulation has emerged as an effective solution to improve polyphenols' stability and controlled release in food products. It not only improves the stability and antioxidant character, but it is also seen that sensory characteristics (aroma, flavor, and food acceptability), shelf life, and antioxidant activity of polyphenols have shown better scores after encapsulation.

In conclusion, while artificial preservatives have been widely used for their effectiveness, growing concerns over their health implications have led to increased interest in natural alternatives like polyphenols. Encapsulation technology shows promise in addressing the challenges of polyphenol instability, offering a safer and more sustainable option for food preservation.

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DOI: https://doi.org/10.54393/df.v5i04.143

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)

Review Article

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# Effectiveness of Fenugreek Seeds against Polycystic Ovarian Syndrome

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## ARTICLE INFO

***Keywords:***

Fenugreek Seeds, Polycystic Ovary Syndrome, Insulin Resistance, Phytotherapy, *Trigonella foenum-graecum*

***How to Cite:***

Zafar, U., Amjad, U. A., Ghaffar, B., Fatima, A., Habiba, U.- e, Malik, R., Mustafa, S., Sattar, N., Abbas, T., & Ashraf, R. (2024). Effectiveness of Fenugreek Seeds against Polycystic Ovarian Syndrome. DIET FACTOR (Journal of Nutritional and Food Sciences), 5(04), 02-06. https://doi.org/10.54393/df.v5i04.143

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Received Date: 13th August, 2024
Acceptance Date: 11th December, 2024
Published Date: 31st December, 2024

## ABSTRACT

Polycystic Ovarian Syndrome (PCOS) is one of the most common hormonal issues which predominantly affects women among reproductive years and can lead to irregular menstrual cycles, infertility, miscarriages, and excessive facial hairs. Infertility, obesity, lipid metabolism disorders, and insulin resistance are at the root of Polycystic Ovarian Syndrome. It is diagnosed with ultrasound and blood hormonal tests. Phytomedicines have been used in ancient times and recent research has approved potential benefits to advance disease treatment. Asia, Europe, and the Mediterranean region are home to fenugreek. Fenugreek may regulate estrogen and testosterone levels, which is effective against Polycystic Ovarian Syndrome. In women with polycystic ovary syndrome, fenugreek improved body weight, number of ovarian cysts, ovary size, irregular hair growth, and monthly regularity. Sonographic results and menstrual cycle are improved in polycystic Ovarian Syndrome women receiving adjuvant therapy to the fenugreek seed extract. The effectiveness of a standardized *Trigonella foenum- graecum* extract (Furocyst®) as a phytotherapeutic agent for the efficient management of Polycystic Ovarian Syndrome has been reported in literature [1]. Fenugreek seeds act as functional food that can give various health related benefits beyond basic nutrition. This review aimed to highlight the therapeutic effects of fenugreek seeds in the management of Polycystic Ovarian Syndrome.

## INTRODUCTION

Polycystic ovarian syndrome (PCOS), a frequent and complex endocrine condition, affecting roughly 7% women of reproductive age [2]. A thorough physical examination and a detailed medical history, including family history, are the first steps in evaluating a girl who exhibits symptoms and indicators of PCOS. Appropriate laboratory testing is then performed [3]. The latest recommendations state that this initial laboratory panel should include erythrocyte sedimentation rate, complete blood count, comprehensive metabolic profile, serum LH and FHS, and pregnancy test [4]. Prolactin, total testosterone, androstenedione, SHBG, thyroid function, dehydroepiandrosterone sulfate (DHEAS), and the concentration of 17-hydroxyprogesterone may also be included. Additionally, lipid values, fasting glucose, and glycated hemoglobin (HbA1c) are usually asked [5]. Additionally, prior research revealed that fenugreek had neuroprotective, antidepressant, and antianxiety qualities in addition to improving cognitive abilities and Parkinson's symptoms. According to a recent study, fenugreek and its extract have positive benefits on hormonally associated conditions such male impotence and galactagogue in nursing mothers. Along with other vital nutrients required for healthy growth and development, fenugreek is regarded as

**DOI:** https://doi.org/10.54393/df.v5i04.143

a rich source of dietary fiber. Research has also confirmed fenugreek's physiological and nutraceutical qualities, supporting its prospective use in the creation of various medicinal and functional food items [6]. Many traditional cuisines have made extensive use of fenugreek as a flavor enhancer. Furthermore, fenugreek is a valuable ingredient for the food and pharmaceutical industries due to its anticarcinogenic, antidiabetic, antioxidant, hypo cholesterolemic, anti-lithogenic, antibacterial, and immunological qualities [7].

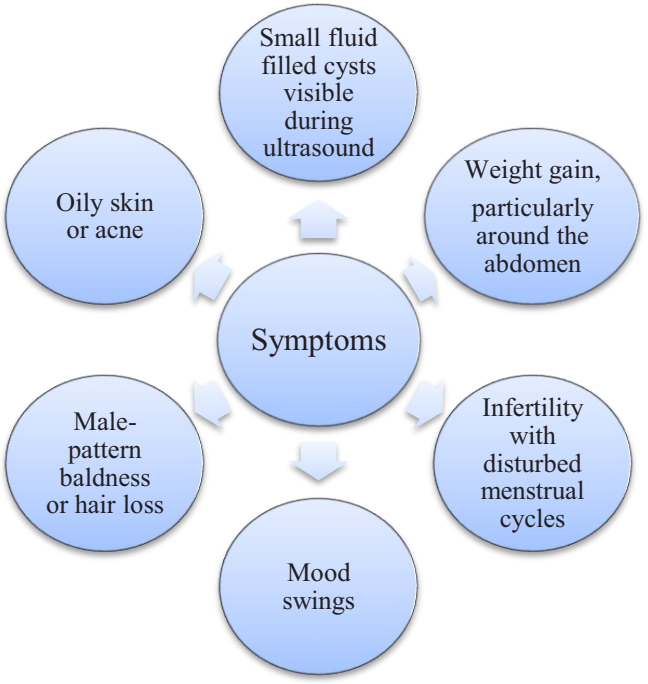


**Figure 1:** Symptoms of Polyovarian Cystic Syndrome

Natural plant components known as phytochemicals have a variety of medicinal uses. For ages, traditional remedies have made use of these chemicals' advantageous qualities, underscoring their potential as therapeutic candidates [8, 9]. A tiny, fragrant herb known as fenugreek (*Trigonella foenum-graecum*) belongs to the legume family (*Fabaceae*). Originating in the Eastern Mediterranean region and the Indian subcontinent, it is regarded as one of the first medicinal herbs and is now highly valued as food, spice, condiment, and traditional medicine (*Trigonella foenum-graecum, or fenugreek*) is a tiny, aromatic herb with seeds that belongs to the Fabaceae family of legumes. The Greek word trigonous is derived from the source of this plant's name. Because of the triangular shape of the leaflets, which implies a triangle, and is meant by the phrase *Foenum-graecum* is Greek hay or Greek grass. This versatile crop is now expanded globally on a commercial basis as a result of growing demand and specific uses in the value chains for food, wellness, and pharmaceuticals. For example, the market for fenugreek seed extract is anticipated to expand at a compound annual growth rate of 10% between 2024 and 2032 due to rising awareness of the extracts possible health advantages [10].

**Table 1:** Nutritional Composition of Fenugreek Seeds

| Nutrients | 100g of Fenugreek Seeds | References |
|---|---|---|
| Dietary Fiber | 24.6g | [10] |
| Carbohydrates | 58.4g | [10] |
| Protein | 23g | [10] |
| Fat | 6.4g | [10] |
| Ash | 3.4g | [10] |
| Moisture | 8.8g | [10] |
| Crude | 23g | [10] |

Tigogenin, Diosgenin, Trigonelline, the steroidal sapogenins B, C, D, E, and G, as well as apigenin, kaempferol, luteolin, atroside, and yamagenin Graecunin (Saponins of Spirostanol) Gitogenin, also known as dihydroxy-sapogenins Triterpenoids a complicated carbohydrate called galactomannan Isoleucine. The important amino acid 4-hydroxylaseAlkaloids: fenugreekine glycosides that hydrolyze to produce steroidal sapogenins (diosgenin, yamogenin, tigogenin, and neo tigogenin); sitosterol and cholesterol; vitamins A, B1, C, and nicotinic acid. Amino acids and protein: According to a study that was done, the amino acid profile of fenugreek seeds is made up of globulins, albumins, prolamins and glutelins. The substantial presence of amino acids in fenugreek seeds, including arginine, threonine, glutamic acid, aspartic acid, and leucine, led to this conclusion. This is because the seeds have relatively low levels of histidine and methionine, but high levels of lysine, an important amino acid. Fenugreek seeds have an average protein concentration of 23%, with a range of 13% to 39%. This is similar to the high protein values of other cultivated legumes, like the mung bean, cowpea, and field pea. It was discovered that it varied from 25% to 39% in five distinct NDM kinds, primarily depending on the fenugreek variety [11]. It lowers the blood cholesterol levels by preventing its absorption via intestines. Flavonoids which are excellent antioxidants are also present among fenugreek which plays the defensive role against free radical. Fenugreek seeds are antioxidant in nature which improves the immune system and helps to fight against chronic diseases. It is known that fenugreek seeds contain alkaloids, such as choline and trigonelline. Because it increases insulin sensitivity, Tripoline has been demonstrated to have antidiabetic effects in relation to blood sugar regulation. Bio active compounds of fenugreek seeds Galactomannan and volatile compounds: A primary polysaccharide in fenugreek, and a component of the cell wall. It has the ability to lower plasma glucose level and has a preventative impact on diabetes [12].

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**Table 2:** Bioactive Component of Fenugreek Seeds

| Classes | Bioactive Components | Role in PCOS | References |
|---|---|---|---|
| Saponins | Trigofoenosides A-G, graecunins, Neogitogenin | In women with PCOS, increased pregnancy, regular menstrual cycle and reduced size of cyst. | [13] |
| Flavanoids | Orientin, Calycosin, Luteolin, Vitexin, Isovitexin, Irilone, Tricine, and Apeginin. | Antioxidant qualities and anti-inflammation in rats with PCOS | [14] |
| Fatty Acids in Lipids | Oleic, palmitic, stearic, linoleic, and A-linolenic acids: Cycloartenol, campesterol, Bsitosterol, Glycerides of triacyls. | Anti-inflammatory actions improve hormonal and metabolic abnormalities by reducing chronic inflammation in PCOS. | [15] |
| Alkaloid | Carpaine, Gentienne, Choline, and Trigonelline | Enhance insulin sensitivity and glucose metabolism to help treat insulin resistance, a major problem in PCOS. | [16] |
| Fiber | Soluble and Insoluble | Promote satiety, enhance gut health, and stabilize blood sugar levels to help manage weight. can aid in lowering cholesterol levels, which are frequently raised in PCOS. | [17] |

It is also found to affect the feeding behaviour in human resulting in reduced fat consumption [18]. Some studies reported the role of fenugreek in stimulating hair growth [19]. Fenfuro, a fenugreek seed extract enriched with furostanolic saponins is found to play a vital role in the management of type 2 diabetes without causing any potential side effects [20]. Yamogenin controls the expression of genes linked to fatty liver tissue, hence reducing lipid accumulation in Hepatocytes' generation of acid. Subsequent research has shown that fenugreek's fiber content dramatically suppresses hunger, especially in obese experimental subjects. Research has demonstrated that fenugreek dietary supplements are effective at facilitating temporary weight loss. Fenugreek powder was given to a group of obese rats for 14 weeks, during which time significant changes in body composition, dimensions, and nutritional parameters was observed [21]. Effectiveness of fenugreek seeds against PCOS: The yearly fenugreek seeds (*Trigonella foenum-graecum L.*) is cultivated as a traditional spice crop in Asia, it plays a role in PCOS. There are ten to twenty aromatic golden seeds in its crust. Fenugreek seeds possesses anti-diabetic and anti-insulin resistance properties in women with PCOS. An open label, one arm, non-randomized post- marketing monitoring study with 50 premenopausal women (aged 18 to 45, BMI of 23.88 4.72 kg/m2, PCOS diagnosis) examined the effectiveness of fenugreek (*Trigonella foenum-graecum*) seeds extract increased in furostanolic saponins (*Furocyst*). Individuals showed a significant reduction in ovarian volume at trial's end compared to baseline measurements. Whereas the left ovary's volume dropped by 17.82%, the right ovary's reduced by 28.25% [22]. According to the study when rats with PCOS were treated with metformin and fenugreek seeds, their estrous cycles returned to normal. Ovarian morphological alterations include the emergence of polycystic ovaries with few corpora lutea in the group with PCOS vs the group without the condition [23]. A normal estrus cycle was shown by the decreased number of cysts and varying numbers of corporal lutea in the fenugreek-treated group. Rats with PCOS who received metformin treatment displayed a dense layer of granulosa cells in their corpus luteum and ovarian cortex. Numerous hormonal and metabolic abnormalities, including hyperglycemia, insulin resistance, and consequent hyperinsulinemia, are linked to PCOS. In animal models of PCOS produced by letrozole, numerous investigations have documented the induction of hyperglycemia Following points illustrate the effectiveness of fenugreek seeds against PCOS. 1. Increased Sensitivity to Insulin: The impact of fenugreek supplementation on insulin resistance in women with PCOS was examined in a 2023 randomized, double-blind, placebo-controlled study that was published in the Journal of the Science of Food and Agriculture. In comparison to the placebo group, the study revealed that fenugreek dramatically decreased insulin resistance and enhanced blood sugar management, indicating that it may be useful in treating the metabolic components of PCOS [24]. Controlling Menstrual Cycles: The use of fenugreek in conjunction with other herbal remedies to control menstrual periods in women with PCOS was investigated in a research published in Phytotherapy Research (2022). Significant improvements in menstrual cycle regularity and a decrease in symptoms such delayed ovulation was observed in the study. It is thought that fenugreek's capacity to affect hormone control is responsible for these benefits [25]. Effects that prevent Androgenesis: In 2021, a clinical study that was published in The Journal of Clinical Endocrinology & Metabolism examined how fenugreek supplementation affected the levels of androgen and hirsutism in women with PCOS. Fenugreek may help lessen hyperandrogenism in women with PCOS, according to the data, which revealed a significant decrease in both blood testosterone levels and the degree of hirsutism [26]. Antioxidant and Anti-Inflammatory Properties: Fenugreek's anti-inflammatory and antioxidant qualities, as well as its potential application in the treatment of metabolic diseases like PCOS, were examined in a 2020 study published in Molecules. According to the review's findings, fenugreek seeds may help lower inflammatory indicators, which could enhance PCOS-afflicted women's general metabolic health [27]. Managing Weight: The

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impact of fenugreek supplementation on body weight in overweight women with PCOS was investigated in a 2019 study published in The Journal of Nutrition. The findings showed a little decrease in body weight and body fat, most likely as a result of fenugreek's capacity to boost fullness and reduce caloric consumption [28].

## CONCLUSIONS

This review compares different studies to highlight the effectiveness of fenugreek seeds against PCOS. From all the various aspects it can be concluded that use of Fenugreek seeds can better manage hormonal imbalance and PCOS. However further studies should be conducted to prove clinical efficacy as it can offer an economical approach to treat PCOS with no or less adverse effects as compared to allopathic medicines. It can open new avenues for nutraceuticals especially for patients with unfavorable economic conditions.

## Authors Contribution

Conceptualization: UZ
Methodology: UAA, BG, UEH, RM, NS, TA, RA
Formal analysis: SM
Writing, review and editing: AF

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

## Conflicts of Interest

All the authors declare no conflict of interest.

## Source of Funding

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

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**DOI:** https://doi.org/10.54393/df.v5i04.143

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**DOI:** https://doi.org/10.54393/df.v5i04.148

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

# Development and Evaluation of Cookies Made with Different Ratios of Red Beans and Chia Seeds

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## ARTICLE INFO

***Keywords:***

Chia Seeds, Red Kidney Beans, Nutritious Snacking, Sensory Evaluation

***How to Cite:***

Saleem, I., Dar, S. N., Nadeem, A., Ijaz, M., Batool, F., Awan, I. S., & Kanwal, H. (2024). the Development and Evaluation of Cookies Made with Different Ratios of Red Beans and Chia Seeds: Cookies Made with Different Ratios of Red Beans and Chia Seeds. DIET FACTOR (Journal of Nutritional and Food Sciences), 5(04), 07-13. https://doi.org/10.54393/df.v5i04.148

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Received date: 5th November, 2024
Acceptance date: 26th December, 2024
Published date: 31st December, 2024

## ABSTRACT

Chia seeds and red kidney beans are rich in fiber and protein content respectively and also help in the prevention of heart disease and maintaining blood glucose levels. **Objectives:** To alternate heavy and fatty snacking with nutritious snacking so it can be consumed by all individuals and provide nutritious benefits. **Methods:** The process was done by making flour of chia seeds and red kidney beans by washing and soaking them in water for 7-8 hours and after sun drying grinding them into the powder form. Then cookies were baked by using different ratios of chia seeds and red kidney beans. After their preparation, physicochemical properties, sensory evaluation and proximate analysis were also done to examine the properties of 3 control groups of cookies. **Results:** The sample cookie B with a ratio (60:40) has overall acceptability for appearance, texture, smell and taste. The Cookie Sample B (60:40) has the highest protein (43.30%) and fiber (24.40). The dry matter and ash% of cookie sample C (70:30) are the highest at (55.37%) and (8.86%). The fat of cookie C (70:30) is the highest (8.86) among all the three samples. The cookie sample B (60:40) has the lowest fat content of about (22.45%). **Conclusions:** It was concluded that cookie sample B (60:40) has overall better nutritional qualities as compared to the other two samples and all the ingredients used in making the recipe of cookies are easily available in local markets.

## INTRODUCTION

The baking industry is experiencing significant growth, recognized as a vital sector in food processing, with bread and cookies representing approximately 80% of all bakery products. Cookies are particularly popular due to their convenience, affordability, and extended shelf life, which is attributed to their low moisture content, minimizing the risk of microbial spoilage. As consumer preferences shift toward health-promoting functional foods, there is an increasing demand for products that not only satisfy hunger but also offer nutritional benefits, reducing the reliance on pharmaceuticals [1]. Chia seed flour has emerged as a popular ingredient in various baked goods, enhancing their nutritional profiles. Research indicates that fortifying muffins with chia seeds can increase their protein content by 15% [2]. Cookies enriched with chia seeds are noted for their high fiber, omega-3 fatty acids, and antioxidant content, making them a nutritious alternative to traditional cookies. Nutritional analysis of these cookies demonstrates favourable results in terms of protein, fat, carbohydrates, moisture, and ash content, confirming the feasibility of incorporating nutrient-dense ingredients into commercially available products [3]. Chia seeds, which can be found in various colors brown, white, and black—are not only versatile but also packed with essential nutrients such as calcium, iron, and dietary fiber. Their rich antioxidant profile contributes to reducing

**DOI:** https://doi.org/10.54393/df.v5i04.148

chronic inflammation and improving overall health. Furthermore, chia seeds can aid in weight management by promoting feelings of fullness and supporting healthy blood sugar levels, making them a valuable addition to weight-reducing beverages [4]. The addition of chia seeds is also beneficial in puddings and breads, enhancing their nutritional attributes and overall quality [5]. While the health benefits of chia seeds are significant, it is essential to consume them in moderation. Excessive intake can lead to gastrointestinal issues, including bloating and diarrhea, especially when combined with certain medications for hypertension and diabetes [6]. This caution is vital for manufacturers as they seek to incorporate chia seeds into their product lines, given their nutritional profile. In addition to chia seeds, the fortification of baked goods with red kidney beans is gaining attention. Rich in fiber and protein, kidney beans serve as an affordable plant-based protein source, often referred to as "poor man's meat." They offer significant health benefits, including improved blood sugar control due to their slow-digesting carbohydrates, which are particularly beneficial for individuals with type 2 diabetes. Red kidney beans also provide essential vitamins and minerals, such as folate, potassium, and iron, along with bioactive compounds that promote health and weight management. Despite their numerous advantages, raw kidney beans can be toxic if not properly cooked, emphasizing the importance of safe food preparation methods [7]. Studies indicate that fortifying cookies with red kidney beans enhances their nutritional content and antioxidant activity while maintaining acceptable sensory qualities [8]. Furthermore, cookies fortified with these legumes exhibit favourable quality parameters, such as spread factor and diameter, while retaining a soft texture and acceptable shelf life of up to 90 days [9]. In conclusion, the integration of nutrient-rich ingredients like chia seeds and red kidney beans into baked goods not only enhances their nutritional value but also meets the growing consumer demand for healthier snack options.

This study aims to develop a recipe to make snacking nutritious and beneficial and to find innovative ways to fortify snacks to improve their nutritional value.

## METHODS

This was an experimental study. Development and evaluation of cookies were made with different ratios of red kidney beans and chia seeds. The first group was 50:50, in which half proportion of white flour and the other half proportion of kidney beans and chia seeds flour. The second group was 60:40, in which 60% is flour and 40% is kidney beans and chia seeds flour. The third group was 70:30, in which 70% flour and only 30% is kidney beans and chia seeds flour [10]. A standard recipe for 12 servings of cookies was applied. This recipe is for making 12 servings of the cookies with 50:50 ratios in which half portion is purpose flour and half is chia seed and red kidney beans flour. The recipe can be adjusted according to other ratios 60:40 and 70: 30. 12 cookies from each group were made and then compared (Table 1).

**Table 1:** Recipe for 12 Servings of Cookies for 50:50 Ratio

| Ingredients | Quantity |
|---|---|
| Brown Sugar | 100g |
| White Sugar | 50g |
| Melted Butter | 1 Cup |
| Egg | 1 Medium (Room Temperature) |
| Vanilla Extract | 1 Tea-Spoon |
| Salt | ½ Tea-Spoon |
| Milk | 1¼ Cup |
| Chia Seed Powder | 1 tablespoon (9 Grams) |
| Chia Seeds | 1/2 Tea-Spoon |
| Red Kidney Beans Powder | 9 Table Spoons (70 Grams) |
| All-Purpose Flour | 10 Table Spoons (78 Grams) |
| Baking Powder | 1 Tea-Spoon |

The red kidney beans and chia seeds were sifted and washed to remove surface crumbs, weed seeds, and other foreign matter. Raw red beans were soaked overnight and dried in the sun for 7-8 hours. After that, the beans were ground in a grinder to obtain a powdery consistency. The flour was then placed in a zip-lock bag and stored at room temperature. Whereas, chia seeds were soaked for 30 minutes to expand, after which the seeds were dried in normal air for approximately 3-4 hours. The seeds were then ground to a powder in a grinder and stored at room temperature in zip lock bags for preparation of cookies: Sugar, salt and butter were combined in a large bowl and mixed until a smooth texture formed. Eggs and vanilla extract were added and mixed until light lumps formed. Red kidney beans, chia seeds, all-purpose flour, and baking powder were mixed with a spatula. Slowly milk was added until semi-soft dough was formed. Half a teaspoon of chia seeds was folded and the dough was chilled for at least 30 minutes. After that, the oven was preheated at 350°F (180°C). Using an ice cream scoop the batter onto a parchment-lined baking sheet, leaving at least 10cm between the cookies and at least 5cm around the edge of the mold to spread the cookies evenly. Baked for 12-15 minutes or until edges were lightly browned [11]. The physicochemical properties of the cookies were calculated. It includes the weight of the cookies, Thickness of the cookies, diameter of the cookies and spread factor of the dough. The weight of cookies is measured by weight scale in gram units. So, for measuring the weight of cookies first of all we will put the scale readings on zero value. Then, place the China dish on it for weighing and note down the weight. After this, press the zero buttons and again place the dish on the weight scale by putting the cookies' sample in it and then note down the readings of weight. We

**DOI:** https://doi.org/10.54393/df.v5i04.148

measured the thickness of cookies in centimetres by using a Vernier caliper. It has two main scales that are named as zero scale and the Vernier scale. The zeroes of these scales concurred with each other when their jaws were going to meet each other. For measuring the thickness of cookies, first of all, by using a magnifying glass, we will ensure that the zeroes of Vernier and main scale are ideally concurring with each other. Then, we will write down the number of divisions of these scales to avoid errors. By slightly loosening the screw, we will release the moveable jaw. Without any pressure; slightly move it to grasp the cookies between the jaws of the Vernier caliper. Then, perpendicularly align it to the thickness of the cookies. Now at this point, we will slightly tighten the screw and point out the position of the zeroes of the Vernier scale against the zero scale. Which normally will not concur with the divisions of the main scale. After this, write down the readings of the Vernier scale and main scale divisions' coincidence; from left to right. Then, jot down the values that are obtained from the least count of Vernier caliper and add them to the readings of the main scale. Now, in a tabular column, we will record the observations of proper reading (If required, then apply zero correction). Now, find the arithmetic mean of the thickness of cookies. Diameter is also measured by using a Vernier caliper, for measuring the diameter of cookies, first of all, by using a magnifying glass, we will ensure that the zeroes of Vernier and the main scale are ideally concurring each other. Then, we will write down the number of divisions of these scales to avoid error [12]. By slightly loosening the screw, we will release the moveable jaw. Without any pressure; slightly move it to grasp the cookies between the jaws of the Vernier caliper. Then, perpendicularly align it to the diameter of the cookies. Now at this point, we will slightly tighten the screw and point out the position of the zeroes of the Vernier scale against the zero scale. Which normally will not concur with the divisions of the main scale. After this, write down the readings of the Vernier scale and main scale divisions' coincidence; from left to right. Then, jot down the values that are obtained from the least count of Vernier caliper and add them to the readings of the main scale. Now, in a tabular column, we will record the observations of proper reading (If required, then apply zero correction). Now, find the arithmetic mean of the diameter of cookies. The spread factor is measured by dividing the values of diameter by the thickness of the cookies. The spread factor is defined as the characteristics or acceptability of cookies. If cookies have the highest spread factor ratio, then they will be considered more desirable or profitable. Spread factor=diameter of cookies/thickness of cookies. Sensory analysis was accomplished by the 15 members of the expert panel from the School of Health Sciences. The sensory characteristics like appearance, taste, smell and texture were analysed of A, B, and C samples of cookies and graded according to the Hedonic scale [13]. The hedonic scale is used to analyze food acceptability or food preferences; to like extremely, like very much, neither like nor dislike or dislike extremely [14]. The main nutritional components of these cookies; protein, fat, moisture or ash content are estimated by proximate analysis. Proximate analysis was held at labs of the University of Veterinary and Animal Sciences [15]. The raw material used defines the dry matter content of any substance [16]. For the estimation of mineral or ash content, first of all, we weighed an empty china dish; with having flat bottom. Then we put the sample in this dish and measured the weight of the sample whose values would be equal to W1. Place it in an oven at 105°C for at least 4 hours. After the desired temperature; remove the China dish let it cool in a desiccator and again weigh it. Again, place the dish in an oven for 4 hours and weigh it. We will repeat this process until constant reading is obtained. Then, after drying we will estimate the sample of cookies along with a dish that is equal to w2. Moisture%=(weight of fresh sample-weight of the sample after drying × 100)/weight of the sample. The moisture content of these cookies was estimated by charring process; by placing the crucible in a muffle furnace at 650°C for 4 hours until white ash is obtained. Then execute the crucible after ashing that contains weight gain and ash. Ash% is calculated by: Ash%=(weight of ash+ crucible weight gain - the weight of crucible × 100)/weight of fresh sample. Protein content is measured by the Kjeldahl method, which consists of three steps; digestion, distillation and titration [17]. In this method, firstly we grab a dry and clean digestion flask then take one to five food samples of 5 grams of digestion mixture. Then put 30ml of concentrated solution of H2SO4 in a digestion flask. Now, put the flask in a fume hood and increase the temperature for 2 to 3 hours until the digestion mixture turns into copper sulphate or sea light green colour and acts as a catalyst. After this, let the flask cool down and place it with distilled water, in a 250ml volumetric flask. Now grab the diluted sample of 10ml and pour 10ml of 40% sodium hydroxide into it. Then transfer the flask to the tube of the distillation apparatus through the fitting tube. Place the beaker in the distillation apparatus and add 10 ml of boric acid solution of 4% with 2 to 3 drops of indicator in a separate beaker. During this process, the colour of the boric acid solution will turn from pink to yellow-golden. The volume of acid used for titration would be calculated by noting down the titrate distillate against H2SO4 or 0.1 NH4Cl till the light pink colour is obtained. Hence, firstly we determined the total nitrogen content of cookies by using this equation: Nitrogen%= (volume of H2SO4 used - normality of H2SO4 – 0.0014*volume of dilution*100)/weight of sample × 10. Then, use the value of N% to find crude protein by this formula:Crude protein= $\%N^2$*6.25.Lipid content is estimated by the Soxhlet extraction method. In which; a TLC plate is mainly used to

**DOI:** https://doi.org/10.54393/df.v5i04.148

estimate different types of lipids in cookies. In this procedure, first of all, make a thimble by using filter paper and weighing (W1) it. In thimble paper, the accurate weight represents the dry sample (5g). Now for extraction of lipids, the thimble has to be placed in the Soxhlet apparatus. Then by using petroleum ether, extract fat from food for at least 8 to 12 cycles (Siphon off). Turn the heat off after complete extraction and remove the sample by using filter paper. At 70-100°C, evaporate petroleum ether till constant weight is achieved. After this, let it cool to room temperature. Then weigh again by using filter paper and samples after extraction. Fat content is also calculated by using this equation: Lipid%= (weight of thimble with sample before fat extraction – the weight of thimble fat – free sample after extraction × 100)/weight of the sample. Total fiber content is estimated by adding 95% ethanol solution to precipitate fiber then this solution is filtered and fiber is collected, dried and weighed. In the process of determining fiber content in food about 2g of food sample is required which should be dry, representative and defatted and placed in a digestion flask then add 200ml boiling 1.25% sulphuric acid in it. In the next step, attach the flask with the condenser and boil it at least for 30 minutes. By using muslin cloth or filter paper, filter the content of the flask in a fluted funnel. Then wash the remaining residue by using distilled water until washings are free from acid. Then repeat the process by transferring it to the digestion flask add 1.25 sodium hydroxide (200ml and connect the flask to the condenser. Boil it for half an hour. Then filter this hot material by using a muslin cloth wash it completely with boiling water and collect the remaining (residue) in a China dish. After washing the acid, alkali and dry sample at 70°C in the oven to constant weight. Cool it at room temperature and weigh it (W1). Then place it in a weight crucible(W2). Place the ignited sample in a muffle furnace at 625◦C for about 4 hours and then weigh again (W3). At last, record weight loss and calculate the percentage of crude fiber. Moreover, it is also calculated by this equation: Fiber%= (weight of crucible with sample before ashing – the weight of crucible with ash after drying × 100)/weight of the sample. The three samples of cookies A, B, and C were compared according to the sensory properties by using one-way ANOVA on SPSS. It is used to check whether there is a significant difference between all the cookie groups. Multiple comparison tests (Post Hoc test) will be performed for each of the possible groups within cookies if there are significant differences between all groups.

## RESULTS

The three samples were developed from the recipe mentioned in the methodology portion. Each sample has the defined ratios of both chia seeds and red kidney bean flour as mentioned above. According to the physicochemical properties, cookie C with a ratio (of 70:30) has the highest weight of about 62g. In the same ratio cookie C has the largest diameter of about 6.7cm and the greatest thickness which is 6.2cm. The spread factor of cookie C with ratio (70:30) is also the largest (Table 2).

**Table 2:** Physicochemical Properties of the 3 Different Variations of Cookie Dough

| Variables | Dough A 50:50 | Dough B 60:40 | Dough C 70:30 |
|---|---|---|---|
| Weight (Grams) | 20g | 37g | 62g |
| Diameter | 5.8cm | 6.2cm | 6.7cm |
| Thickness | 5.2cm | 5.9cm | 6.2cm |
| Spread Factor | 1.115 | 1.0505 | 1.0806 |

By using a hedonic scale, the sensory attributes of the cookies were analyzed. It includes appearance. Taste, smell and texture. The results of the sensory evaluation were described through pie charts given below of 3 ratios of cookies. The figures below show that in appearance. The cookie sample A (50:50) and cookie sample B (60:40) are almost similar and liked extremely by almost 26.67% of the panel. 40% of the panel liked extremely the taste of cookie sample B (60:40). The smell of cookie B (60:40) and cookie sample C (70:30) was almost similar and each was liked by 20% of the expert panel. The Texture of cookie sample B (60:40) was highly acceptable by the panel. Hence the bar chart given shows the overall acceptability of the cookie sample B (60:40). It shows that the sample Cookie B has overall acceptability for appearance, texture, smell and taste (Figure 1).

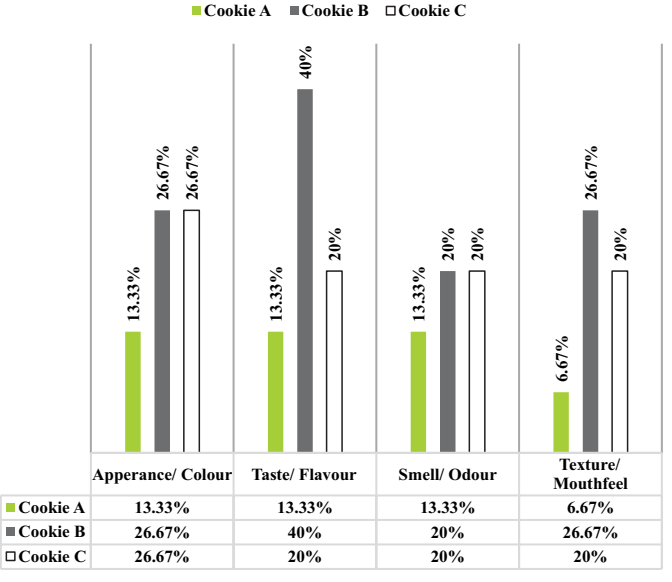


**Figure 1:** 3 Ratios of Cookies Sensory Analysis

After the graphical representation of the sensory attributes of cookies, the statistical analysis was performed. On SPSS, One-Way ANOVA shows p-value (0.007) is less than α (0.05) which means that there is a significant difference between all the cookie groups A, B, C (Table 3).

**DOI:** https://doi.org/10.54393/df.v5i04.148

**Table 3:** One-Way ANOVA

| No of People | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 12.667 | 2 | 6.333 | 9.120 | 0.007 |
| Within Groups | 6.250 | 9 | 0.694 | - | - |
| Total | 18.917 | 11 | - | - | - |

To check if there is a significant difference between groups A-B, A-C and B-C, a multiple comparison test (LSD) was performed. The p-value (0.002) for group A is less than α (0.05) which shows that there is a significant difference between cookies A and B. The p-value (0.031) for cookie A-C is also less than α (0.05) which shows that there is significant difference between cookie A and C. The p-value (0.124) for cookie B-C is greater than α (0.05) which shows that there is no difference between cookie B and C. So, the overall results of the statistical analysis show that Cookie B is extremely liked for all the sensory attributes as compared to Cookie A and Cookie C (Table 4).

**Table 4:** Post Hoc Test

| Cookie Type (I) | Cookie Type (J) | Mean Differences (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| A | B | -2.50000* | 0.58926 | 0.002 | -3.8330 | -1.1670 |
| | C | -1.50000* | 0.58926 | 0.031 | -2.8330 | -0.1670 |
| B | A | 2.50000* | 0.58926 | 0.002 | 1.1670 | 3.8330 |
| | C | 1.00000* | 0.58926 | 0.124 | -0.3330 | 2.3330 |
| C | A | 1.50000* | 0.58926 | 0.031 | 1.1670 | 2.8330 |
| | B | -1.00000* | 0.58926 | 0.124 | -2.3330 | 0.3330 |

Dependent Variables: no of people LSD

The cookie sample C (70:30) has the highest mean (7.93 ± 0.96) for appearance. The mean of cookie sample B (60:40) is the highest (7.7 ± 1.22) for taste attribute. The cookie sample C has the greatest mean (7.66 ± 1.11) for the attribute smell and (7.53 ± 1.18) for texture (Table 5).

**Table 5:** Statistical Analysis

| Samples | Appearance | Taste | Smell | Texture |
|---|---|---|---|---|
| Cookie A 50:50 | 7.53 ± 0.92 | 7.60 ± 0.91 | 7.60 ± 0.91 | 7.33 ± 0.98 |
| Cookie B 60:40 | 7.80 ± 0.86 | 7.73 ± 1.22 | 7.56 ± 1.16 | 7.40 ± 1.28 |
| Cookie C 70:30 | 7.93 ± 0.96 | 7.53 ± 1.30 | 7.77 ± 1.11 | 7.53 ± 1.28 |
| Total | 7.76 ± 0.91 | 7.62 ± 1.13 | 7.58 ± 1.01 | 7.42 ± 1.10 |

After the physicochemical and sensory properties of the three cookie samples proximate analysis was done to analyze the nutritional content of the cookies. Proximate analysis includes dry matter%, crude protein%, crude fiber%, fat%, ash % and NFE% [18]. Cookie sample B (60:40) has the highest protein (43.30%) and fiber (24.40%) content as compared to cookie samples A and C. The Dry matter and ash of cookie sample C (70:30) are the highest at (55.37%) and (8.86%). The fat of cookie C (70:30) is the highest (8.86) among all the three samples. The cookie sample B (60:40) has the lowest fat content of about (22.45%) (Table 6).

**Table 6:** Proximate Analysis

| Type of Tests | Sample A | Sample B | Sample C |
|---|---|---|---|
| Dry Matter (%) | 37.68 | 45.15 | 55.37 |
| Crude Protein (%) | 40.70 | 43.30 | 39.60 |
| Crude Fiber (%) | 22.85 | 24.40 | 19.35 |
| Fat (%) | 23.05 | 22.45 | 29.0 |
| Ash (%) | 7.50 | 7.16 | 8.86 |
| NFE (%) | 5.90 | 2.69 | 3.19 |

## DISCUSSION

Thus, the results show that cookie sample B (60:40) has overall better nutritional and sensory qualities as compared to the other two samples. This research was based on the development and evaluation of cookies made with red kidney beans and chia seed flour. Three different ratios were taken as 70:30, 50:50 and 60:40 [19]. According to past research, cookies were developed either using three ratios of only chia seed flour or three ratios of only red kidney bean flour. However, this research includes the use of both red kidney bean flour and chia seed flour in one cookie with three different ratios. This research proves that cookies made with 60:40 ratios have shown more appropriate results for nutrition. Nutritional evaluation was done by proximate analysis which is the standard official method and applied internationally [20]. According to previous research on cookies made with chia seeds flour, it was stated that the cookies with the lowest ratios that is 15% and 10% out of all the controls were acceptable both nutritionally and in texture, flavor etc. And researches on cookies, made with red kidney beans flour, it was stated that the cookies with 15% and 10% ratios were acceptable. But in this research, the cookies with Ratios of 40% of red kidney beans and chia seeds flour were acceptable both nutritionally and in texture, flavor etc. According to previous research, during sensory analysis, cookies made with 20% ratios of red kidney beans were not acceptable due to their texture and color appearance. Cookies of sample C, with the lowest ratios of red kidney beans or chia seeds flour, are 15% 15%and 10%were acceptable during sensory analysis. But in this research, the taste of all three samples; A, B, and C, were acceptable based on taste and odor; during proximate analysis and sensory analysis. But

**DOI:** https://doi.org/10.54393/df.v5i04.148

based on the texture, color, appearance and as well as taste and smell of cookies, sample B, with 60:40 ratios of red kidney beans and chia seeds flour was more acceptable. In the past research, the physical properties of dough with different ratios of 0%, 5%, 10%, and 15% were also measured. In which, the thickness of cookies was increased while the spread factor of cookies was increased according to the above ratios. But in this research, the thickness, and diameter of the 60:40 ratios were less than 50:50 ratios of cookies. On the other hand, the spread factor having a 60:40 ratios are more than the 50:50 ratios of cookies made with red kidney beans and chia seeds flour. A past study also showed that 90% wheat flour with 10% red kidney bean flour has the overall acceptability [21]. A study showed that the addition of 20% chia seeds in cookies has overall acceptability [22]. Moreover, according to former studies, cookies with ratios of 15% of red kidney beans have lower fiber content and higher fat content. But, in this research, cookies made with 60:40 ratios of red kidney beans and chia seeds flour have higher protein and fiber content as well as lower fat content. So, this research proves that the sample B of these cookies have more nutrient content and is acceptable as healthy snacking. Besides that, all the ingredients used in making the recipe of cookies are easily available in local markets. And these cookies are protein and fiber-rich due to red kidney beans and chia seeds flour. Moreover, there is still a need to research all the physicochemical properties, analysis of moisture content, shelf life and stability of the cookies.

## CONCLUSIONS

It was concluded that nutritious cookies were developed using chia seeds and red kidney beans flour as chia seeds are high in fiber, which can help to lower blood pressure whereas red kidney serve as a rich source of plant-based protein and helpful in maintaining blood glucose levels and promote colon health as well. In combination both ingredients also play a role in disease prevention. Its evaluation includes physiochemical properties, sensory analysis and proximate analysis were completed on the three samples that were extracted from the stock sample. Under the evaluation sensory analysis and proximate analysis were observed. Based on sensory analysis which was done by using a hedonic scale group B was mostly liked as it is high in protein and low in fat content according to proximate analysis and group C was also acceptable. Further, there is still a need for such studies to be conducted to make snacking healthier.

## Authors Contribution

Conceptualization: IS
Methodology: IS, SND, AN, MI, FB, ISA, HK
Formal analysis: IS, SND
Writing review and editing: IS
All authors have read and agreed to the published version of the manuscript.

## Conflicts of Interest

All the authors declare no conflict of interest.

## Source of Funding

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

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**DOI:** https://doi.org/10.54393/df.v5i04.135

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 the Temperature and Time Required for Formation of Safe Levels of Acrylamide in Bakery Products

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## ARTICLE INFO

***Keywords:***

Acrylamide, Temperature, Time, Bakery Products

***How to Cite:***

Qadeer, S., Basharat, S., Khan, R. B., Ishtiaq, S., Hasan, A., Zahur, M., & Ajmal, A. (2024). Determination of the Temperature and Time Required for Formation of Safe Levels of Acrylamide in Bakery Products: Temperature and Time for Formation of Acrylamide in Bakery Products. DIET FACTOR (Journal of Nutritional and Food Sciences), 5(04), 14–18. https://doi.org/10.54393/df.v5i04.135

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Received date: 11th May, 2024
Acceptance date: 16th December, 2024
Published date: 31st December, 2024

## ABSTRACT

Acrylamide as a toxic and carcinogenic substance is produced naturally during high-temperature methods used in baking. **Objectives:** To evaluate the temperature and time required for the formation of safe levels of acrylamide in bakery products. **Methods:** All developed bakery products were evaluated for their chemical elements i.e. fiber, moisture, ash, protein, crude, and crude fat were calculated according to their relevant methods. Bakery product ingredients were procured from the local market of Lahore. Furthermore, developed products were quantified for acrylamide concentration using high-performance liquid chromatography (HPLC) technique. Three samples were prepared Control group (T0), Treatment plan 1 (T1), Treatment plan 2 (T2). From each group, 10g of sample was procured for analysis. **Results:** Pizza treated at T0 (220°C, 15min), T1 (210°C, 20 mints), and T2 (230°C, 10 min) had the following concentration of acrylamide (15.66 ± 3.05, ND, 32.33 ± 2.08 µ/kg), T0 (18°C, 20 mints), T1 (175°C, 25 mints), T2 (195C, 15 mints), showed acrylamide as (66.66 ± 2.51, 42.66 ± 3.05, 90 ± 1.73 µ/kg) and Cake T0 (160-15 mints), T1 (150-20 mints), T2 (170C, 10mins) acrylamide quantified (15.66 ± 2.51, ND, 34.33 ± 2.08µ/kg) and Biscuit (66.66 ± 2.51, 42.66.33 ± 3.05, 90 ± 1.73µ/kg). **Conclusions:** It was concluded that treating T1 (210°C for 20 mints -ND), To (175°C for 25 mints - 48.33 µ/kg), and T3 (150°C for 20 mints-ND) can reduce and mitigate the formation of acrylamide following. Low temperature and high time could serve as an efficient strategy to reduce acrylamide and optimize the process.

## INTRODUCTION

Acrylamide is formed during the cooking of plant-based foods when the free amino acid asparagine reacts with reducing sugars such as glucose and fructose in the Maillard reaction [1]. While the Maillard reaction contributes to desirable flavor, colour, and aroma changes in cooked foods, it also produces processing contaminants such as acrylamide [2]. It is known as a processing contaminant. Acrylamide has been classified as potentially carcinogenic to humans by the International Agency for Research on Cancer (IARC) [3]. Acrylamide is a metabolite glycidamide and causes genetic damage when binds with DNA. Various heated products contain acrylamide such as carbohydrate-rich foods [4]. Exposure to high acrylamide levels is said to cause damage to the nervous system. After speedy confirmation of these observations, several activities targeting the origin and extent of exposure of acrylamide in food, its potential risk to human health, and mitigation of acrylamide in foods were initiated [5]. Presently the two methods for the detection of acrylamide are mass spectrometric and non-mass spectrometric-based techniques [6]. The majority of the basic stable industries of food manufacturing focus production of bread and bakery products. The majority of bakery products, bread, and biscuit pizza in the countries that are developed are manufactured through special automated production [7]. Foods that are prepared and eaten in homes are only 60 percent, mostly due to two working parents and individuals who are single parents, have less time to cook

**DOI:** https://doi.org/10.54393/df.v5i04.135

[8]. Biscuits are considered a famous food product that is consumed by a massive range of populations because of their variety, taste, big shelf life, and comparatively low cost making them economical [9]. Fernandes et al., reported a mass spectrometry method for the detection and the estimation of the quantity of acrylamide in particular food structures of biscuits. The established analytical process revealed 11.8 µg kg−1 and 3.55 as the limit of quantification (LOQ) and limit of detection (LOD). Data analysis was carried out to examine the comparison of the levels of Acrylamide with numerous manufacture specifications, like cooking time and temperature, the color, and water content in various biscuits [10]. A study was designed by Esposito et al., which was carried out to analyze acrylamide in the Italian market targeting bread and sweets. A sample of 200 were assessed and the level of acrylamide formation in bread ranged between 31 to 454 µg/kg and in sweets, it ranged between 204 to 400 µg/kg. No neurotoxic health effect was shown in the data [11]. In a study described by Zilic et al., an acrylamide concentration was detected by baking biscuits at two different time durations at 180 °C for 7, 10, and 13 min. Acrylamide was observed at different baking durations revealing a range of 72.3 - 861.7µg/kg after a baking time of 13 minutes. The data showed no link between the amount of acrylamide in biscuits and free asparagine present in flour. Moreover, rye flour, hulled oat, and durum wheat contain the highest amounts of 859.8,603.2 and 530.3 mg/kg, which produced the highest amount of acrylamide in biscuits after a baking time of 13 minutes. A low amount of acrylamide was observed in biscuits made from refined wheat flour and red maize whole meal flour as they contain a low amount of asparagine as well [12]. A study was investigated by Andacic et al., in 2020. The purpose of this examination was to compare and find out the amount of acrylamide in different baked products. The method used for acrylamide detection was the LC-MS/MS method. The second goal of the study was to assess the average exposure of adults to acrylamide through the bakery food category. A total of 100 samples were assessed, and content ranging below the limit of quantification (LOQ) to 237µg/kg was reported. The dietary bakery food category means exposure of acrylamide estimated as 0.16 µg/kg b.w. per day. For food safety in regards to reducing the level of acrylamide, European regulation has contributed a lot [13].

This study aims to develop acrylamide-free bakery products and to evaluate the quality of acrylamide bakery products

## METHODS

The experimental study design was carried out in Food Science and Technology (FST) Lab no.102, University Institute of Food Science and Technology (UIFST), Faculty of Allied Health Sciences (FAHS), The University of Lahore. The duration of research after synopsis approval was 9 months. In this study, oil and other ingredients were procured from the local market of Lahore and then packed in sealed bags and sealed jars to avoid any further contamination until further analysis at the laboratory facility of the University Institute of Food Science and Technology (UIFST) at the University of Lahore. A jury comprised of 10 men and women was selected from The University of Lahore, Lahore. The judges had knowledge of testing terms and they were requested to analyze the multiple samples of cookies, pizza, and cake for taste, crispiness/firmness, color, crust, internal texture, flavor, color, and general acceptability applying a 9-point Hedonicscalewhere9wasequivalent to like extremely and 1 meant dislike extremely [14]. All three developed products (P1, P2, P3) were analyzed by HPLC Acrylamide method. Acrylamide was analyzed by following a method adapted from Gokmen [16]. Bakery products like Pizza, biscuits, and cake were developed. Three samples were prepared Control group (T0) bakery products were baked at a standardized temperature and time, Treatment Plan 1 (T1) was baked at a low temperature with a long time duration, Treatment Plan 2 (T2) was baked at a high temperature and a short duration of time. Bakery products (pizza P1, biscuit P2, and cake P3) were evaluated for their chemical elements i.e. fiber, moisture, ash, protein, crude, and crude fat were calculated according to their relevant methods. Other micronutrients like minerals have been analyzed through various methods. For each separate food type sample (10g) analysis, extraction, and quantification were performed (Table 1).

**Table 1:** Development of Bakery Products Like Pizza, Biscuits, and Cake

| Treatment Plan | P1 Pizza | P2 Biscuits | P3 Cake |
|---|---|---|---|
| T0 | 220°C for 15 mints | 185°C for 20mints | 160°C for 15 mints |
| T1 | 210°C for 20 mints | 175°C for 25mints | 150°C for 20 mints |
| T2 | 230°C for 10 mints | 195°C for 15 mints | 170°C for 10 mints |

All three developed products were analyzed by the HPLC Acrylamide method. Acrylamide was analyzed by following a method adapted from Gokmen (2005) [15]. Analysis of the food extracts was performed by high-performance liquid chromatography meeting the requirements. Finely, ground (10g) all three samples were prepared. For each separate food type sample (10 g) analysis and extraction were performed. Firstly, all samples were homogenized. 100 ml hexane was added to the sample and was fixed with a flask on a wrist shaker for 20 minutes. Decant hexane and sample were dried on a hot plate. 100 ml acetone and 500 ml distilled water added to the sample and was shaken for 10 minutes. The flask with the sample was placed in a water bath at 40°C for 20 minutes. The sample was placed on a

**DOI:** https://doi.org/10.54393/df.v5i04.135

hot plate and acetone was evaporated. After the evaporation of acetone 5ml distilled water was added to the residue and filtered from a 0.25-micron syringe filter before the examination. LC-MS assessments were done by an HPLC method containing a binary pump, an auto-sampler, and a thermally controlled column oven, connected to the MS detector furnished with diverse interface employing the following interface variables: drying gas temperature of 350°C, drying gas (N2,20psig) flow of 5L/min, corona current of 5µA, the capillary voltage of 2000V, and nebulizer pressure of 20psig. Onan Atlant is T3 column (150×4.6mm, 3 µm) a critical division was executed by using the isocratic blend of 10 mM formic acid at a flow rate of 0.3 mL/min at 25°C. The LC solvent was administered to the MS arrangement for about 10 to 16 minutes employing MSD software. For the estimation of the quantity of acrylamide in the sample, the observed ions were m/z 72 and 55. Acrylamide was quantified for each sample.

## RESULTS

All three products (P1, P2, and P3) were quantified for acrylamide content. ND: (Not-detected)(Table 2).

**Table 2:** Acrylamide Concentration in Pizza

| Pizza | T0 | T1 | T2 |
|---|---|---|---|
| AAµ/kg | 15.66 ± 3.05 | ND | 32.33 ± 2.08 |

Acrylamide concentration in pizza was analyzed (Figure 2).

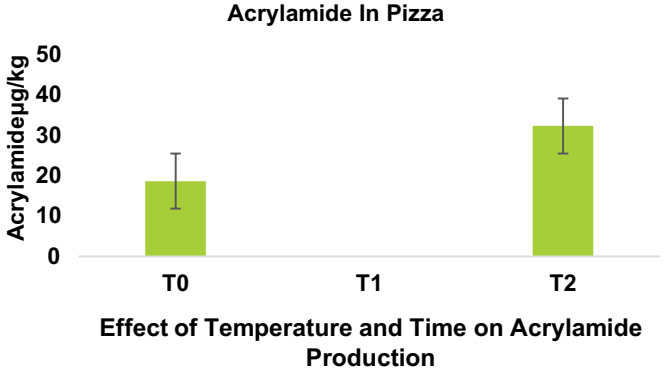


**Figure 1:** Acrylamide Concentration in Biscuit

Values represent the means of three replicate determinations and Mean + SD values with different superscripts within the same column are significantly different (p≤0.05)(Table 3).

**Table 3:** Acrylamide Concentration in Biscuit

| Biscuit | T0 | T1 | T2 |
|---|---|---|---|
| AAµ/kg | 66.66 ± 2.51 | 42.66.33 ± 3.05 | 90 ± 1.73 |

Acrylamide concentration in biscuits was analyzed (Figure 3).

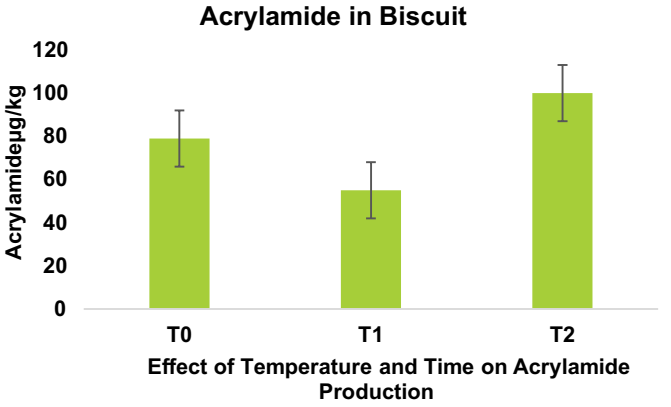


**Figure 2:** Acrylamide Concentration in Biscuit

Values represent means of three replicate determinations and Mean + SD values with different superscripts within the same column are significantly different (p≤0.05)(Table 4).

**Table 3:** Acrylamide Concentration in Cake

| Cake | T0 | T1 | T2 |
|---|---|---|---|
| AAµ/kg | 15.66 ± 2.51 | ND | 34.33 ± 2.08 |

Acrylamide concentration in cake was analyzed (Figure 4).

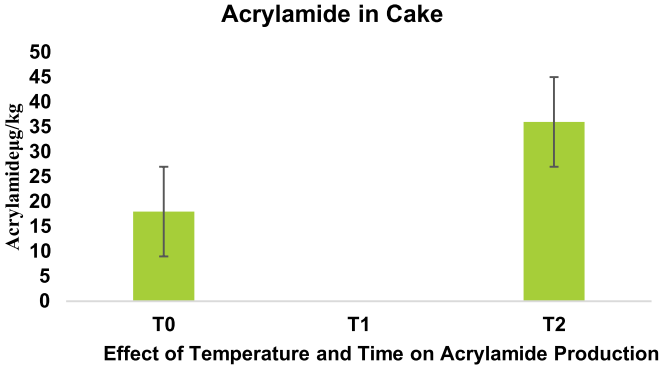


**Figure 3:** Acrylamide Concentration in Cake

## DISCUSSION

An experimental study was conducted to develop and evaluate acrylamide-free selected bakery products. Acrylamide is a toxic substance produced upon heating food products containing reducing sugar and asparagine. Acrylamide does not occur naturally and is formed only during heating treatment, thus known as a processing contaminant. Previously various studies have been conducted to quantify acrylamide amounts in many food products. The study revealed acrylamide T0 (15 µ/kg), and T2 (32.33 µ/kg) whereas in Treatment Plan T1 amount of acrylamide was detected. A study performed by Michalak *et al.*, investigated the amount of acrylamide in pizza and his findings were our result of 33µ/kg [16]. Another study performed previously by Crawford *et al.*, showed the same average value of acrylamide in pizza [17]. The value of acrylamide in T1 was not detectable supported by the findings of Eerola *et al.*, [18]. Detection of acrylamide using

**DOI:** https://doi.org/10.54393/df.v5i04.135

different strategies helps control measures to stop its formation as it is toxic to human health. In the control group, T0 acrylamide detected was (66.66 µ/kg), T1 (42.66 µ/kg) acrylamide content was slightly low compared to because of low temperature, whereas T2 showed the highest level of acrylamide (90 µ/kg) due to high temperature(°C). Similar findings were performed by Michalak *et al.*, [16]. Another study was similar to our results, performed by Hai *et al.*, [19]. Acrylamide content in control group T0 (15.66µ/kg) was low an amount due to appropriate baking temperature and time, whereas the content of acrylamide was almost double (34.66 µ/kg) as compared to T0 as it was treated at high temperature, moreover in Treatment plan T1, no amount of acrylamide was detected, as it was prepared data low temperature (°C). These concentrations are found in a cake and are within the range of the lowest concentration found in a cake in accordance to a study performed by Hai *et al.*, [19]. Davoodi, *et al.*, reported similar findings by using high-performance liquid chromatography for the detection of acrylamide in cake [20].

## CONCLUSIONS

It was conducted that bakery items are consumed globally. The present study targeted three bakery products pizza, biscuit and cake for the evaluation of acrylamide which is a toxic compound formed during processing. The study of baking products at different temperatures and times constitutes an approach to understanding the mechanisms of acrylamide formation during the baking process. Considering the data obtained in our study, treating pizza (210°C for 20 mints -ND), biscuit (175°C for 25 mints - 48.33 µ/kg) and cake (150°C for 20mints-ND) can reduce and mitigate the formation of acrylamide following. Acrylamide formation can be prevented by altering temperature and time. Low temperature and high time could serve as an efficient strategy to reduce acrylamide and optimize the process. Proper attention should be paid to the food processing methods to attain safe food. Food preferences that are alarming to public health must be avoided.

## Authors Contribution

Conceptualization: SQ
Methodology: SQ, SB
Formal analysis: AH, MZ, AA
Writing review and editing: RBK, SI

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

## Conflicts of Interest

All the authors declare no conflict of interest.

## Source of Funding

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

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**DOI:** https://doi.org/10.54393/df.v5i04.135

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**DOI:** https://doi.org/10.54393/df.v5i04.159

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

# Nutritional and Vitamin D Status of University Students in Khyber Pakhtunkhwa, Pakistan: A Cross-Sectional Study

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## ARTICLE INFO

***Keywords:***

Vitamin D Deficiency, Dietary Habits, Inflammatory Markers, Nutritional Status

***How to Cite:***

Rahman, H. U., & Alam, I. (2024). Nutritional and Vitamin D Status of University Students in Khyber Pakhtunkhwa, Pakistan: A Cross-Sectional Study: Nutritional and Vitamin D Status in Students. DIET FACTOR (Journal of Nutritional and Food Sciences), 5(04), 19-24. https://doi.org/10.54393/df.v5i04.159

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Received Date: 9th November, 2024
Acceptance Date:26th December, 2024
Published Date: 31st December, 2024

## ABSTRACT

Data on Vitamin D level and nutritional status of Pakistani university students are limited. **Objective:** To find dietary habits of the university students with special focus on vitamin D levels. **Methods:** This descriptive cross-sectional study included a total of 400 students (272/128 male/female) 18-26 years old students from Bacha Khan University Charsadda were included in this descriptive cross-sectional study. Socio-demographic, selected anthropometrics, dietary food intake, blood chemistry (lipid profile and CRP), and vitamin D status were measured. **Results:** Moderate energy deficiencies, particularly males (73% of RDA), and protein was meeting requirements in females but marginally short in males. Calcium (65% RDA), vitamin A (41% female, 30% male), and vitamin D (20% RDA) intake were significantly low. Iron intake was marginal in females (60%) but over met in males (135%). Low milk/dairy, egg, fish, and fruit/vegetable consumption reflected low micronutrient variety. Regular consumption of fast foods (3.4 times/week) and limited consumption of fish (0.5 servings/week) also indicated a shift toward nutrient-scarce dietary habits. Mean serum 25(OH)D level of 26.1± 12.6 ng/mL with A high number (32% ;95% CI: 13.5–27.3) of respondents were vitamin D deficient; 42% (95% CI: 35.3–52.8) were vitamin D insufficient; and only 26% (95% CI: 20.1 – 32.2) of the participants had normal vitamin D. In addition, high levels of inflammatory and lipid markers were observed. **Conclusion:** In summary, university students exhibited poor vitamin D status with high levels of CRP and blood lipid profile such that interventions are warranted.

## INTRODUCTION

University students are a unique and pivotal population, experiencing a life phase of increasing autonomy, academic pressure, and formation of lifelong behaviors [1-3]. This phase, typically synonymous with late adolescence and young adulthood, is marked by dramatic changes in habits of daily life, such as changes in food habits, sleep habits, exercise habits, and psychosocial activities. These changes in lifestyle have the ability to impact highly the level of nutrition among students and expose them to numerous short- and long-term health issues [2-5]. The campus life tends to encourage eating habits of poor quality, greater dependency on fast or convenience foods, and lower intake of highly nutritious meals with good balance [3]. At the same time, sleep deprivation, school stress of high intensity, and physical inactivity usually enabled by prolonged screen time and absence of organized physical activity contribute to nutritional imbalance and negative health consequences [4-9]. A high body of evidence shows that these elements of lifestyle are associated with higher prevalence of micronutrient inadequacies, overweight, and obesity, dyslipidemia, and inflammation among university students, thus at higher risk of having Non-Communicable Diseases (NCDs) in the long run [1, 9]. On this basis, it is crucial to determine the nutritional and biochemical status among university students. These results not only inform on the current health status of this group, but also inform the development of timely interventions for promoting

DOI: https://doi.org/10.54393/df.v5i04.159

healthier behaviors. The main objective of the present study was to evaluate vitamin D status of of the university students in Khyber Pakhtunkhwa, Pakistan. Particular emphasis is placed on gender-specific variation in body weight, body mass index (BMI), and significant biochemical parameters such as serum 25-hydroxyvitamin D [25(OH)D], lipid profiles total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides and high-sensitivity C-reactive protein (hs-CRP) as a marker of inflammation. The findings of this research are anticipated to identify common nutritional deficits and biochemical disturbances among university students, putting into perspective early markers of cardiometabolic risk.

Such information may be used as a platform for evidence-based health promotion strategies and campus wellness programs to optimize the overall health and academic achievement of students.

## METHODS

A descriptive cross-sectional study was conducted in Bacha Khan University Charsadda in the year 2021. Population was the students of Bacha Khan University. Cross-sectional design was employed since it provides data collection and analysis at a single time-point in time and hence suits to find out prevalence, pattern, or association within a defined population [10]. Students of university were included at 18-26 years age, both male and female and without chronic illnesses. Sample Size was calculated by Cochran's formula as discussed in the following: n = N / (1 + N e2) Where, n = Number of sample; N = Whole population and e = Error tolerance (level). Simple random sampling process was utilized. Complete list of enrolled students was obtained from director admission office. The sample as defined was selected at random from the list by using a random number generator. For anthropometrics, height and weight of the participants were recorded [11, 12]. Weight was measured on a calibrated balance with minimal clothing on (Model: 708; Seca, Hamburg, DE). Height was measured with a calibrated stadiometer (Model: 206; Seca, Hamburg, DE). Measurements and testing were carried out by highly trained dietitians at University clinics. BMI was calculated from the formula: weight (Kg)/height (m2).To analyze the body composition, the participants were requested to go to HND lab at BKUC. Body composition was examined on Bioelectric Impedance Analysis (BIA) following standard operating procedures designed by body composition core-lab. A 10 ml blood sample was obtained and analyzed for the following biochemical parameters: 25-OH vitamin D, C - reactive protein (CRP), blood lipid profile etc. As per standard protocol as supplied by the manufacturer. In short, CRP was measured on immunoturbidimetric (Tina-quant CRP detection method; Roche Diagnostics) on a Hitachi 717 analyzer with between-assay CV was 2.6% at 4.65 mg/L CRP. Serum 25-OH vitamin D was estimated by ELISA kits (MY Bio-Source, CATLOG # MBS2601819). 25-OH Vitamin D in all the samples was read at the same time with standards and the concentrations were calculated from external standards. Serum Triglycerides (TG), Total Cholesterol (TC), HDL, LDL was determined by automated chemistry analyzer (HumaStar 600- Germany). Cholesterol/HDL and LDL/HDL ratios were calculated by dividing total cholesterol and LDL respectively by HDL. Serum concentration of 25-OH D was measured by taking blood samples. A single blood sample from each subject was taken and examined in Thumbay hospital, Ajman-UAE laboratories. Outcomes were classified into four categories according to vitamin D concentration of 25-hydroxyvitamin: deficiency (<10 ng/dl), insufficiency (10–29 ng/dl), sufficiency (30–100 ng/dl), and likely intoxication (>150 ng/dl). The dietary recall data was analyzed by utilizing valid food composition software. Descriptive statistics were calculated for all variables: Means ± Standard Deviation (SD) for quantitative variables and frequencies and percentages for categorical variables. Normality was tested with the Shapiro-Wilk test and visual means (histograms, Q-Q plots). Variables satisfied parametric assumptions were tested with parametric tests; otherwise, their non-parametric equivalents were used. Two means comparisons (e.g. male vs female) were performed by student-t-test and a single mean comparison (e.g. mean nutrient intake vs RDA of that nutrient) was performed using one-sample t-test. The participants were grouped according to their hs-CRP, TC, LDL-C and HDL-C levels. Taking into account the hs-CRP (mg/L) levels, the participants were grouped in any of the three groups as follow: Low Risk (< 1.0); Moderate Risk (1.0 – 3.0); High Risk (>3.0); Based on their TC (mg/dL) levels, the participants were grouped in any of these groups; Optimal (<200); Border line high (200 – 239); High (≥ 240). Based on their HDL-C (mg/dL) data, the participants were categorized as following: No Risk (≥40 mg/dL); Moderate Risk (35–39 mg/dL); High Risk (<35 mg/dL). Similarly, based on their LDL-C (mg/dL) level, these cutoffs were considered for grouping the participants accordingly into any of the five groups: Optimal: <100; Near Optimum: 100-129; Borderline High: 130-159; High: 160–189; Very High: ≥190. These cutoffs were taken from well-established clinical guidelines, such as those from the National Cholesterol Education Program (NCEP ATP III) and American Heart Association (AHA) as reported elsewhere [13-17]. They were used to categorize individuals' cardiovascular (TC, LDL-C; HDL-C) and inflammation (hs-CRP) risks. These categories based on the LDL-C levels represent increasing risk for cardiovascular disease, especially when combined

DOI: https://doi.org/10.54393/df.v5i04.159

with high hs-CRP, low HDL-C, or elevated TC. Chi-Square Goodness-of-Fit Test was used to test whether the observed frequency distribution for a single categorical variable (e.g., Vitamin D levels or LDL-C categories) significantly differs from an expected distribution (such as a normal or theoretical distribution). Statistical significance was presumed for P<0.05. All the analyses were performed on SPSS Version 20.0.

## RESULTS

In the present study, most of the participating students were male (68%, N = 272). More than half of the students come from rural areas (64%, N = 256). The mean age of the students participating in the study was M = 21.21 ± 4.56. Detailed data are presented in table 1.

**Table 1:** Mean Anthropometrics and Nutritional Status by Gender

| Variables | Male Mean ± SD | Female Mean ± SD | Overall Mean ± SD |
|---|---|---|---|
| Weight (kg) | M = 75.63 ± 13.64 | M = 56.55 ± 7.66 | M = 66.2 ± 7.31 |
| BMI * | M = 24.43 ± 3.44 | M = 20.11 ± 3.33 | M = 22.24 ± 2.4 |
| Underweight (n = 40) | 10% (N = 27) | 10% (N = 13) | 10% (N=40) |
| Normal weight (n = 244) | 60% (N = 163) | 62% (N = 79) | 61% (N=244) |
| Overweight ( n=116) | 30% (N = 82) | 28% (N = 36) | 29% (N=116) |

Data were presented as mean ± SD or n (%). * According to Diagnostic and Statistical Manual of Mental Disorders (DSM)-V, a body mass index less than or equal to 17.5 kg/m2 is considered as a diagnostic criterion for anorexia nervosa.

### Dietary and Nutrients Intake

Table 2 showed the dietary intake of the students. The daily energy intake was 1834 ± 340 kcal, covering about 81% of the RDA for female and 73% of that for male University students, indicating a general state of moderate caloric deficit, particularly in male. Protein consumption was 52 ± 11 grams/day, covering 103% of the RDA for female and 83% for male students, indicating that protein requirements were sufficiently met in female students but that men might be at marginal risk of insufficient supply. Calcium consumption was significantly lower than recommended, with a mean consumption of 650 ± 180 mg/day, amounting to only 65% of the RDA, creating potential concerns about bone status and vitamin D metabolism. Iron consumption averaged 10.8 ± 3.2 mg/day, being equivalent to 60% of the female requirement but more than 135% in men, indicating gender differences in iron needs and potential dietary choices. The intake of Vitamin A was somewhat low (525 ± 110 µg RAE/day), covering only 41% of the RDA for female students and 30% for male. The intake of Vitamin D was particularly low, averaging 122 ± 60 IU/day, representing merely 20% of the suggested 600 IU/day, reflecting a very important deficiency matching general national concerns about hypovitaminosis D. In terms of food group intakes, milk and dairy intakes were 0.8 ± 0.5 servings/day, reaching only 41% of the minimum required intake, and egg consumption was 2.4 ± 1.7 per week, or 49% of the recommended intake. Fish intakes were very low (0.5 ± 0.8 servings/week), reaching only 21% of the recommendation, which could contribute to both low vitamin D and omega-3 fatty acid intakes. Individuals ate 2.3 ± 1.3 servings daily of fruits and vegetables, just 40% of the five recommended servings, reflecting inadequate micronutrient and fiber intake. Fast food, on the other hand, was eaten on a regular basis, with a mean of 3.4 ± 1.0 times weekly, reflecting a predisposition to convenience-dominant, nutrient-depleted diets. Sugar-sweetened beverage intake was 178 ± 78 ml/day, which fell within recommended parameters for the majority of the participants, although the cumulative effect on nutrient displacement deserves attention.

**Table 2:** Dietary and Nutrients Intake

| Food/Nutrient | Mean ± SD | Percentage of RDA Met* | p-Value |
|---|---|---|---|
| Energy (kcal) | 1834 ± 340 | ~81% (F), ~73% (M) | 0.358 |
| Protein (g) | 52 ± 11 | ~103% (F), ~83% (M) | 0.344 |
| Calcium (mg) | 650 ± 180 | 65% | 0.03 |
| Iron (mg) | 10.8 ± 3.2 | 60% (F), 65% (M) | 0.02; 0.02 |
| Vitamin A (µg RAE) | 525 ± 110 | 41% (F), 30% (M) | 0.003; 0.003 |
| Vitamin D (IU) | 122 ± 60 | 20% | 0.002 |
| Milk and Dairy (servings/day) | 0.8 ± 0.5 | 41% | 0.003 |
| Eggs (number/week) | 2.4 ± 1.7 | 49% | 0.004 |
| Fish (servings/week) | 0.5 ± 0.8 | 21% | 0.0003 |
| Fruits and Vegetables (servings/day) | 2.3 ± 1.3 | 40% | 0.003 |
| Fast Food (times/week) | 3.4 ± 1.0 | — | -* |
| Sugar-Sweetened Beverages (ml/day) | 178 ± 78 | Within limit | -* |

*there is no data on RDA available; P<0.05; All analysis were done using one-sample-t-test by comparing the mean of nutrient with its RDA; RDA values are based on WHO/FAO and NIH dietary guidelines for 18–25-year-olds: F=Female Students; M=Male Students

### Biochemical Status

The biochemical status of the students is shown in Table 3. Male and female students had the same vitamin D level (p>0.05). However, significant differences were observed in the concentration of other biochemicals between male and female students. The mean serum 25(OH) D level of the participants was 26.1± 12.6 ng/mL showing an overall insufficiency of vitamin D nutrition.

**Table 3:** Biochemical Status (n=50)

| Variables | Male Mean ± SD | Female Mean ± SD | Total Mean ± SD | p-Value |
|---|---|---|---|---|
| Serum 25(OH)D ng/mL | 26.5 ± 12.3 | 25.7 ± 13.1 | 27.1 ± 12.6 | 0.156 |
| CRP (mg/dL) | 7.4 ± 1.55 | 6.51 ± 2.11 | 6.62 ± 2.66 | 0.001 |
| TC (mL / dL) M ± DE | 178.8 ± 54.3 | 197.7 ± 46.3 | 191.3 ± 50.3 | 0.002 |
| HDL-C (mg / dL) M ± DE | 47.6 ± 21.7 | 47.2 ± 22.31 | 47.4 ± 21.7 | 0.004 |
| LDL-C (mg / dL) M ± DE | 141.4 ± 13.1 | 141.3 ± 13.4 | 141.4 ± 13.3 | 0.034 |
| Triglycerides (mg / dL) M ± DE | 132.7 ± 69.8 | 149.4 ± 61.8 | 140.1 ± 66.1 | 0.000 |

**DOI:** https://doi.org/10.54393/df.v5i04.159

### Distribution of Serum 25(OH) D Levels among Participants Students

The distribution of different serum 25(OH) D levels among the participants is presented in Table 4.A high number (32.% ;95% CI: 13.5–27.3) of respondents were vitamin D deficient; 42% (95% CI: 35.3–52.8) were vitamin D insufficient; and only 26% (95% CI: 20.1 – 32.2) of the participant had normal vitamin D. The $\chi^2$ (Chi-square) tests results show Significant results ($p < 0.05$) for HDL-C and LDL-C only, indicating that the observed frequencies in these categories deviate significantly from what would be expected by chance, reflecting a true underlying pattern (e.g., higher frequency in "high risk" LDL-C).

**Table 4:** Biomarkers Frequency (n=50)

| Variables | Frequency (%) | 95% CI* | $\chi^2$ (Chi-square) | p-Value |
|---|---|---|---|---|
| Vitamin D deficiency (<10 ng/dl**) | 16 (32) | 13.5-27.3 | 1.96 | 0.375 |
| Vitamin D insufficiency (10–29 ng/dl**) | 21 (42) | 35.3-52.8 | | |
| Normal (30–100 ng/dl**) | 13 (26) | 20.1-32.2 | | |
| **hs-CRP (mg/L)** | | | | |
| Low Risk (< 1.0) | 12 (24) | 19.2-28.9 | 3.04 | 0.219 |
| Moderate Risk (1.0 – 3.0) | 22 (44) | 32.4 -52.3 | | |
| High Risk (>3.0) | 16 (32) | 28.4 -37.2 | | |
| **TC (mg/dL)** | | | | |
| Optimal (<200) | 13 (26) | 20.-37.1 | 1.96 | 0.375 |
| Border line high (200 – 239) | 16 (32) | 28.7-38.2 | | |
| High (≥ 240) | 21 (42) | 32.9-49.2 | | |
| **HDL-C (mg/dL)** | | | | |
| No Risk (≥ 40) | 10 (20) | 15.9-27.3 | 7.0 | 0.03 |
| Moderate Risk (35 – 39) | 15 (30) | 26.2-38.2 | | |
| High Risk (< 35) | 25 (50) | 41.1-56.3 | | |
| **LDL-C (mg/dL)** | | | | |
| Optimal (< 100) | 10 (20) | 16.3-28.4 | 25.8 | 0.0003 |
| Near Optimum (100 – 129) | 2 (4) | 2.1-7.5 | | |
| Border Line High (130 – 159) | 10 (20) | 15.4- 26.3 | | |
| High (160 – 189) | 5 (10) | 6.5-18.7 | | |
| Very high (≥ 190) | 23 (46) | 35.9- 50.1 | | |

Ng/DL= Nanograms per decilitr; CI, confidence interval.

## DISCUSSION

The findings reveal 32% of students were Vitamin D deficient, a health condition also evidenced by other UAE and Iranian research [13, 14]. Confidence interval (CI: 13.5–27.3%) indicates a substantial number of this group might experience severe vitamin D deficiency. Moreover, 42% had low levels, indicating extensive deficiency in vitamin D but not being deficient. This continues to represent health risk, especially to immune and bone health [15, 16]. Vitamin D status in the current study was in the normal category in only 26% of the students. This indicates the prevailing trend of insufficiency in the population under study. These data indicate that most (74%) of the students had inadequate or deficient status of Vitamin D, indicating possible lack of sufficient exposure to sunlight, inappropriate dietary intake or both [16-18]. Elevated levels of hs-CRP, LDL and TC and decreased level of HDL are risk markers for several diseases [19-22]. Several regional studies have highlighted significant gaps in the nutritional knowledge and behaviors of university students in Khyber Pakhtunkhwa, Pakistan. Khan et al., reported that a large proportion of students in Peshawar had limited awareness regarding nutritional supplements, reflecting a broader deficiency in basic nutrition literacy among university populations [19]. Similarly, Haq et al., assessed knowledge, attitudes, and practices about vitamin D and found that many medical students in Swat lacked adequate understanding of its importance, sources, and deficiency consequences [20]. Zeb's study further explored eating attitudes and found that disordered eating behaviors were prevalent among university girls in Peshawar, closely linked to their nutritional status [21]. Moreover, Khan et al., evaluated the diet and physical activity habits of medical students and identified concerning patterns such as irregular meal timings, inadequate intake of nutrient-dense foods, and sedentary lifestyles, all of which contribute to poor nutritional status [22]. Lastly, Hussain et al., conducted a population-based preprint study and proposed region-specific reference ranges for vitamin D levels in Peshawar, stressing the need for localized standards to better interpret deficiency risks in this demographic [23]. The findings of the current study on hs-CRP are that 24% were with low risk, i.e., comparatively low systemic inflammation; 44% were with moderate risk and 32% with high risk, indicating the existence of underlying inflammation or chronic health stressors. More than half of the children (76%) had moderate to severe inflammation, which may have been triggered by infection, dietary imbalance, or psychosocial stress. Results for Total Cholesterol (TC) indicate that 26% were normal (<200 mg/dL); 32% were mildly elevated cholesterol (200–239 mg/dL) and 42% in high cholesterol levels (≥240 mg/dL). A staggering 74% of the children were borderline or high in total cholesterol, indicating nutrition imbalance, presumably high in saturated fats or processed foods. HDL-C 20% of the children had protective HDL levels (≥40 mg/dL), 30% were moderately at risk (35–39 mg/dL), and 50% were highly at cardiovascular risk with low HDL (less than 35 mg/dL). Poor HDL-C status was found in half of the children and is most likely a marker of increased cardiovascular risk as well as unhealthy lipid profiles due to inappropriate dietary intake and an inactive lifestyle. LDL-C 20% had normal (<100 mg/dL) LDL levels; 4% were in this category (100–129 mg/dL) and 20% were slightly high

DOI: https://doi.org/10.54393/df.v5i04.159

(130–159 mg/dL) and 10% were high (160–189 mg/dL) and 46% had very high (>190 mg/dL) LDL levels. Alarmingly, almost half (46%) of them had very high LDL-C, indicating an excess risk for future cardiovascular complications. Along with other cholesterol derangement, this is a picture of dismal dyslipidemia [22, 23]. Findings of the present study indicate very high malnutrition and cardiometabolic status of Pakistani university students. Large concerns are 1) very high prevalence of deficiency/insufficiency of vitamin D; 2) elevated prevalence of inflammatory markers (hs-CRP); 3) elevated prevalence of dyslipidemia (low HDL and high LDL). The data underscore the necessity of introducing nutritional intervention, better meal planning, physical exercise, and potentially supplementation to correct such deficiency states and avoid long-term health effects [16].

## CONCLUSIONS

The current research reports considerable nutritional and biochemical imbalances among Pakistani university students, and found that a high number were vitamin D deficient and/or had inadequate levels, suggesting that almost three-quarters (74%) of the participants were at risk based on less-than-ideal vitamin D status. This lack is of great concern to immune and bone health, and could be due to a lack of sunlight exposure, poor dietary consumption, or a combination of the two.

## 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: HUR, IA
Formal analysis: HUR, IA
Writing, review and editing: HUR, 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.

## Source of Funding

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

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**DOI:** https://doi.org/10.54393/df.v5i04.167

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

# Knowledge About Calcium-Rich Foods in Adolescent Girls in Charsadda – A Cross-Sectional Study

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## ARTICLE INFO

***Keywords:***

Pre-Adolescent, Calcium, Knowledge, Calcium-Rich Foods

***How to Cite:***

Shah, S., Ktebi, A. A., & Alam, I. (2024). 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), 5(04), 25-30. https://doi.org/10.54393/df.v5i04.167

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Received Date: 27th October, 2024
Acceptance Date: 24th December, 2024
Published Date: 31st December, 2024

## ABSTRACT

Calcium is a critical nutrient for adolescent bone development and overall health, yet intake often remains below recommended levels. **Objectives:** To identify and list calcium-containing foods available in Charsadda and to assess the knowledge level of adolescent girls about calcium foods and calcium nutrition. **Methods:** A cross-sectional study was conducted to list all foods and food products available in Charsadda. This study was conducted at two different time points in the same year, in the summer months and in the winter months of 2021. The purpose of the survey at two different time-points was to recognize and list all possible available calcium-rich foods in the locality throughout the year. This phase of the study was followed by cross-sectional surveys to find out pre-adolescent girls' knowledge about calcium-rich foods already identified in the first phase of the study. A multi-stage stratified sample (n=78) of pre-adolescent school-going girls completed the survey using a pre-tested questionnaire. **Results:** A high number (174) of calcium-rich foods and products were available at the local market and households of Charsadda city. Overall, pre-adolescent girls' knowledge about calcium-rich foods was poor. Only 60%, 45.3%, 46.7%, and 42.7% of the girls could identify, respectively, milk, cheese, yoghurt and lassi/malt-drink as calcium-rich foods. Most plant-based items were poorly recognized. **Conclusions:** Despite a high number of calcium-rich foods available in the local market of Charsadda, the knowledge level of school-going pre-adolescent girls was poor, warranting further community-based awareness and education programs on calcium nutrition.

## INTRODUCTION

Calcium is a critical micronutrient important for skeletal system development, especially during pre-adolescence, a time characterized by bone growth acceleration and hormonal development [1]. Proper calcium consumption during this window of development is critical in the attainment of peak bone mass and the prevention of osteoporosis, fractures, and other bone diseases in adulthood [2]. Global guidelines, like those of the World Health Organization (WHO) and the Institute of Medicine (IOM), recommend that children 9–12 years of age consume 1000 to 1300 mg of calcium each day to maximize growth and physiological functioning [3]. Nonetheless, there is evidence that calcium consumption in children living in South Asian nations, such as Pakistan, continues to be woefully inadequate, especially in rural and resource-poor populations [4]. This is further compounded by socio-economic limitations [5], restricted dietary variety [6], minimal exposure to dairy or fortified foods [7], and widespread ignorance about nutritional requirements [8, 9]. In Pakistan, where the dietary habits are predominantly cereal-based and calcium-containing foods are missing from typical diets, the risk of calcium deficiency is particularly high among young girls. Pre-adolescent female is a group of special concern because poor calcium consumption during this age can result in impaired bone structure, growth delay, and increased risk of persistent

**DOI:** https://doi.org/10.54393/df.v5i04.167

nutritional deficiency. Although the seriousness of this problem is considerable, little evidence supports the availability of calcium-rich foods in the semi-rural regions of Pakistan, especially KP, where socio-cultural and economic obstacles are unique. Information regarding the calcium sources in foods is critical to any nutrition policy development in addressing the common calcium deficiencies [9, 10]. The outcomes of this research are anticipated to guide specific nutrition interventions and assist with the design of culturally sensitive approaches to enhancing calcium consumption among school-age girls in comparable contexts.

This study aims to assess the knowledge level of pre-adolescent girls of Charsadda, an old traditional city of Khyber Pakhtunkhwa of Pakistan. Also to recognize and list all calcium-rich foods, as there is no such database which is necessary for diet planning and related research studies.

## METHODS

This descriptive cross-sectional study was completed in two phases. Phase 1 was a market survey to recognize and enlist calcium-rich foods and food products in the study area. Phase 2 was a general survey of the pre-adolescent girls to assess their knowledge about calcium-rich foods and other related information. All the surveys conducted were cross-sectional and descriptive. For identification and enlisting calcium-containing foods, the protocol previously reported [11], a form of ethnographic techniques to determine local food sources that are available and acceptable to vulnerable communities, was used without any qualitative content analysis or any coding method involved. Briefly, the survey was completed in the following standard 6 steps with minor modifications as reported previously [11]: 1) background market survey, installation of food system data tables form; 2) key-informant interviewing and development of family food lists, food system data tables; 3) preparation for structured interviews: examination of preliminary data from key-informant interviews; preparation and pretesting of structured interview schedule about diets, with particular focus on foodstuffs and foods with calcium; 4) preparation of structured interviews: analysis of key-informant information on cultural beliefs and practices regarding xerophthalmia; preparation and pre-testing of structured interviews on the signs and symptoms of nutritional blindness; 5) structured interviews with respondents; and 6) final market survey, data consolidation, and writing of reports. For this purpose, a cross-sectional market survey was conducted at two different time points of the same year in the city of Charsadda; once in summer (May-June, 2021) and a second time in the winter months (November-December, 2021). The purpose of these two market/household surveys in different seasons of the year was to get a comprehensive picture of the calcium-rich foods available across the year to the consumers in the local market of Chasadda city. Before the survey, an exhaustive list of all potential calcium-rich foods was made by a panel of 6 researchers having expertise in the field of nutrition and dietetics. They were requested to make lists of calcium-rich foods. These lists were prepared based on their knowledge about the food composition of Pakistani dishes as previously reported [12-14]. The independent lists prepared by these experts were later combined into a single consolidated calcium-rich foods list. For the data collection procedure, a meeting with a group of student researchers (n=4) was held in June 2022. The calcium-containing food list already prepared was shared with them. All foods on the list were explained with their local names as well as English and scientific names. A food manual with coloured illustrations was prepared. The students were advised to visit a diverse range of food outlets, including supermarkets, franchise convenience stores, local grocery stores, and fresh markets, to list both packaged and fresh calcium-rich foods available to school students. Online markets were excluded due to age-based purchasing restrictions. These student researchers prepared lists of foods rich in calcium content. A record form was used to collect data on packaged items, documenting the product name, calcium content, ingredients, and nutritional label information. The same survey by the same student researchers was repeated in the winter months of 2022. In each season, all the outlets were surveyed twice in one particular season, one week apart. In this way, each outlet was surveyed four times in total. The list of foods collected by the student researchers was presented to the local expert panel (n=5), with specialized expertise in food labels and food composition. A checklist of fresh high-calcium foods, developed from three references, guided the identification of unlabeled fresh items. All products meeting the definition of "high-calcium" were identified and listed. Calcium content was obtained from labels or estimated using reference data. Products were categorized into five groups: (1) meat and meat products, (2) vegetables and fruits, (3) dairy and dairy products, (4) legumes, nuts, and seeds, (5) Starch Foods, and (6) calcium supplements. A cross-sectional survey, conducted twice in 2022: Survey 1 in January-February, 2022 and Survey 2 in June-July, 2022. The purpose of conducting surveys at two different seasons of the year was to capture a comprehensive and representative pre-adolescent girls' level of knowledge about calcium-rich foods and products at a medium-sized lower secondary school in a semi-urban area of Charsadda City, representing similar schools in the area. The study targeted students from grades 5–9 (ages 9–14). Out of 120

**DOI:** https://doi.org/10.54393/df.v5i04.167

students enrolled, 79 students participated after obtaining guardian consent. Given the limitations of funds available, the survey could be conducted at a single school. However, the school is the largest in the city with students from diverse urban and rural demographic backgrounds, and it was assumed to be a fair representative of the sample of interest for the current study. For the sampling and sample size, the inclusion criteria were: 1) the subject must be normal without any medical diseases or disorders at the time of the survey; 2) must be from the local community, and 3) must have parental consent to participate. Using a 70% estimated proportion (based on a pilot), a 5% margin of error, and a 95% confidence level, the minimum sample size required was 65. A total of 85 students showed willingness to participate [15]. All of these 85 students were considered for participation, taking into consideration the possibility of dropout, incomplete questionnaire or absence. The final data could be completed on 78 students out of 85 students, as 7 students were absent on the day of data collection. Whereas the computed sample size was 65, 78 students completed data collection. This was more than the needed sample and was kept to enhance the statistical power of the study and ensure robust results. Oversampling more than the minimum needed is a standard approach in survey and observational research since it compensates for possible missing or unusable data and increases the external validity of results. Oversampling can also be an insurance against unforeseen exclusions during the cleaning of data or subgroup analysis. For the data collection tools, a self-administered questionnaire was developed and validated by 6 academic experts and clinical experts (University teachers and dietitians). The questionnaire had two parts: Part 1 included demographic data (sex, age, grade, weight, height, and daily allowance). Part 2 had 44 questions. Questions 1-36 were about the names of 12 high calcium-containing foods, and the students were to report 'yes' or 'no' in front of each food item. Questions 37-44 were questions about general knowledge regarding calcium nutrition. The market survey (ethnographical analysis) was the identification and listing of foods that have an appreciable amount of calcium (low-to-high calcium content). A food providing 5% of the DV or less was a low source, while a food that provides 10–19% of the DV was considered a good source, and a food that provides 20% of the DV or more was considered an excellent source of calcium. However, for simplicity, such further classification was not reported. Foods identified were put in different categories, including a) meat/meat products, b) vegetables and fruits, c) dairy products, d) legumes/nuts/seed products, e) starch foods and f) calcium supplements. For phase 2 of the study, descriptive statistics were used for demographics and anthropometric data. Data on Student Knowledge on Calcium-containing Foods and Calcium Awareness were analyzed and reported in numbers with percentages. Foods and products familiarity and consumption were summarized. Data were analyzed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA).

## RESULTS

The majority of these foods were from calcium supplements (45), followed by 'meat and meat products' and 'starchy foods' (both with 30 products each), 'legumes, nuts, and seed products' (25), 'vegetables (24), and dairy product group (20). A total of 174 calcium foods were identified as shown (Table 1).

**Table 1:** Calcium Containing Foods and Products in Charsadda City

| Categories of Food Items | Number of Food Items | Calcium per Serving (mg) | Examples of Foods |
|---|---|---|---|
| Meat and Meat Products | 30 | 150–500 | Egg, red meat, white meat, fish, chicken paye, paye, kabab, kufta, |
| Vegetables and Fruits | 24 | 150–400 | Cabbage, cauliflower, Collard Greens, Lettuce, Kale, Okra, Carrots, Rhubarb, tomatoes, Broccoli, barsandi, radish, banana, apple, |
| Dairy and Dairy Products | 20 | 150–800 | Milk, cheese, lassi, yoghurt, almond milk, Cream, double, whipped cream/full custard, ice-cream, vanilla pudding, pudding pancake, waffle, cheese cake, koya, barfee, krut |
| Legumes, Nuts, and Seed Products | 25 | 200–800 | Peas, lentils, almonds, white beans, red beans, French beans, soyabean, |
| Starchy Foods | 30 | 150–800 | Pasta, rice, parata, potatoes (boiled), white Bread, whole-meal (cereals), naan |
| Calcium Supplements | 45 | 600–100 | Calce, other commercial tablets, syrups, homeopathic products, |
| Total | 174 | – | – |

The mean age of the girls who participated in the survey on the assessment of the knowledge level of pre-adolescent girls was 12.4 years. Regarding awareness of common calcium-containing foods (Q1-4), milk (60%), cheese (45.3%), and yogurt (46.7%) were identified correctly by nearly half or more of the respondents, reflecting general familiarity with dairy products. However, malt drink/lassi was identified by fewer students (42.7%), possibly due to varied formulations or misconceptions about its nutrient value. Most plant-based items were poorly recognized as calcium sources. Spinach (14.7%), cabbage (6.7%), chia seeds, sesame seeds, flax seeds, broccoli, kale, and bok choy (all 0%) show a complete lack of awareness of leafy greens and seeds as good sources. Nuts and dried fruits like Almond (2.7%), Pistachios (4%), Figs (2.7%), Raisins (8%), and Apricots (5.3%) were also largely overlooked.

**DOI:** https://doi.org/10.54393/df.v5i04.167

Animal-based foods beyond dairy (meat, chicken, egg, fish). These were not well associated with calcium either (all under 5%), possibly due to the common link of calcium with only dairy. Regarding Calcium Knowledge Questions (Q37, Q39, Q41, Q43, Q44), showed alarmingly low awareness: Only 2.7% knew calcium's primary function in the body. Only 5.3% correctly identified milk as a calcium source when mixed with distractors. Just 2.7% knew that calcium isn't found only in dairy products. No one could explain whether white foods are calcium-rich or how many glasses of milk are needed daily (Table 2).

**Table 2:** Student Knowledge of Calcium-Containing Foods and Calcium Awareness

| Question No. | Question 1–12: Which of the Following Items Are Sources of High Calcium? | Number of Respondents That Answered Correctly (%) |
|---|---|---|
| 1 | Milk | 45 (60%) |
| 2 | Malt Drink / Lassi | 32 (42.7%) |
| 3 | Cheese | 34 (45.3%) |
| 4 | Yogurt | 35 (46.7%) |
| 5 | Raita | 21 (28.0%) |
| 6 | Spinach | 11 (14.7%) |
| 7 | Cabbage | 5 (6.7%) |
| 8 | Almond | 2 (2.7%) |
| 9 | Pistachios | 3 (4.0%) |
| 10 | Chia Seeds | 0 (0.0%) |
| 11 | Sesame Seeds | 0 (0.0%) |
| 12 | Flax Seeds | 0 (0.0%) |
| 13 | Lentils | 2 (2.7%) |
| 14 | Brocolli | 0 (0.0%) |
| 15 | Bok Choy | 0 (0.0%) |
| 16 | Kale | 0 (0.0%) |
| 17 | Turnip Greens | 2 (2.7%) |
| 18 | Mustard Greens | 3 (4.0%) |
| 19 | Okra | 7 (9.3%) |
| 20 | Podded Peas | 3 (4.0%) |
| 21 | Soybean Sprouts | 4 (5.3%) |
| 22 | Orange | 2 (2.7%) |
| 23 | Apple | 7 (9.3%) |
| 24 | Banana | 2 (2.7%) |
| 25 | Apricot | 4 (5.3%) |
| 26 | Currant (Dried Gooseberry) | 2 (2.7%) |
| 27 | Raisins (Dried Grapes) | 6 (8.0%) |
| 28 | Figs | 2 (2.7%) |
| 29 | Meat | 2 (2.7%) |
| 30 | Egg | 3 (4.0%) |
| 31 | Chicken | 2 (2.7%) |
| 32 | Fish (Fresh Water) | 2 (2.7%) |
| 33 | What Is Calcium Mainly Used for in the Body? | 2 (2.7%) |
| 34 | Which of the Following Is a Good Source of Calcium? A) Candy, B) Milk, C) Soda, D) Potato Chips. | 4 (5.3%) |
| 35 | Calcium Is Only Found in Dairy Products. (True/False) | 2 (2.7%) |
| 36 | How Many Glasses Of Milk Do We Need to Meet Our Daily Calcium Needs? | 2 (2.7%) |
| 37 | Do You Think White Color Foods Are Good Sources of Calcium? Why? | 0 (0.0%) |

The data suggests a critical need for structured nutrition education, especially to broaden understanding of non-dairy calcium sources, improve functional knowledge of calcium's role in health, and dispel myths and misconceptions (e.g., white food as a sign of calcium, dairy exclusivity).

## DISCUSSION

A total of 174 foods and food products also including supplements were available with low-to-high calcium contents in the local market of Charsadda city. This number is higher than what is reported, for example, in a study conducted in the Thailand, where 93 foods and food products with high calcium contents were reported previously [16]. This difference in the number of calcium foods between our study and that reported by [16] may be because this included only very high calcium foods in their list, while we included all foods and food products with low-to-high calcium content to maximize the spectrum of choice from a greater variety of foods for any intervention studies in future. In our analysis majority of the available high calcium foods and food products were from calcium supplements (45), followed by meat and meat products and starch foods (both with 30 products each), legumes, nuts, and seed products (25), vegetables and fruits (24), and dairy product group (20). Calcium in the form of supplements is emerging in calcium nutrition [16]. Meat and meat products, vegetables, and legumes are all plant-based sources of calcium [17]. Worrisome is the fact that the students exhibited poor knowledge regarding calcium nutrition (Table 2). As indicated, a poor awareness of calcium-containing food and calcium function among young respondents. Although more than half had correct recognition of dairy sources such as milk (60%), yoghurt (46.7%), and cheese (45.3%), which may be a reflection of superficial knowledge influenced by traditional marketing. Only fewer identified malt drink/lassi (42.7%) as a source of calcium, possibly because formulations are variable and confusing in advertisements. The most concerning result is the virtual lack of recognition for plant-based sources of calcium. Foods like spinach (14.7%) and cabbage (6.7%) were rarely recognized, and chia, sesame, flax seeds, and leafy greens such as kale and bokchoy (Chinese palak in Urdu) were not recognized at all. Nuts and dried fruits like almonds, figs, and raisins were also mostly unrecognized, all at levels below 8%. Non-dairy animal food items like eggs, chicken, and fish were also poorly recognized. Knowledge questions also revealed the gap: 2.7% only

**DOI:** https://doi.org/10.54393/df.v5i04.167

knew calcium's primary function in bone health, and only 5.3% correctly chose milk from distractors. Misconceptions such as pairing all white foods with calcium remain, and no one answered related items correctly. These results necessitate culturally appropriate nutrition education to expand knowledge of calcium sources, clarify its biological roles, and dispel misinformation. Our results on the number of students with good knowledge about the calcium contents of foods are comparable with some previous studies; for example, a study conducted in India reported that only <25% of students could identify calcium-rich food sources [18]. Results of our study show that although a majority of participants knew that milk and yoghurt are good sources of calcium, they lacked much knowledge about non-dairy foods containing calcium, such as leafy green vegetables, nuts, seeds, and fortified cereals. The same outcome was found in earlier research, where people mostly identified dairy as the primary source of calcium and had low knowledge about substitutes [19]. Misconceptions about the amount of calcium in soft drinks and ready-to-eat foods were also seen, showing poor nutritional education. Conversely, studies with targeted nutrition education interventions showed considerably enhanced knowledge of dairy and non-dairy sources of calcium among the students [18]. In the present research, only 2% of participants were able to identify correctly the main role of calcium in the body, that is, its essential role in constructing and upholding strong bones and teeth. This very low rate indicates a dismal lack of fundamental nutritional understanding. These are the same results obtained in earlier research, where adolescents mainly linked calcium to overall health or were not aware of its particular role in bone development [19]. Research with populations presented to nutrition education interventions indicated substantially higher knowledge, and appropriate answers amounted to up to 40% in certain instances [20]. These contrasts highlight the necessity of integrating focused nutritional knowledge to promote awareness regarding critical nutrients such as calcium among youth populations. In this present research, awareness of certain sources of calcium was found to be very low. A mere 5.3% of the participants were able to identify milk as a good source of calcium from the available options. Equally, only 2.7% correctly answered that calcium does not occur solely in dairy foods, reflecting a widespread misunderstanding about the sources of calcium. Further, when questioned about how many glasses of milk are required for daily calcium intake, and whether white foods are good sources of calcium, only 2.7% answered correctly. These results are similar to previous studies that point to low levels of awareness of calcium-containing foods outside of dairy, as well as confusion over daily calcium needs for young adults and teenagers. These findings highlight the imperative for timely overall and context-specific nutrition education to enhance the basics of knowledge about calcium and bone health.

## CONCLUSIONS

It was concluded that despite a high number of calcium-rich foods and food products available in the local market and households of Charsadda, the knowledge level of the school-going pre-adolescent girls was poor, warranting further community-based awareness and education programs on the sources of calcium foods and their importance in human health.

## 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: SS
Methodology: SS, AAK, IA
Formal analysis: SS, AAK, IA
Writing review and editing: SS, AAK, 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.

## Source of Funding

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

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**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

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## 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

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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

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

**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

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).

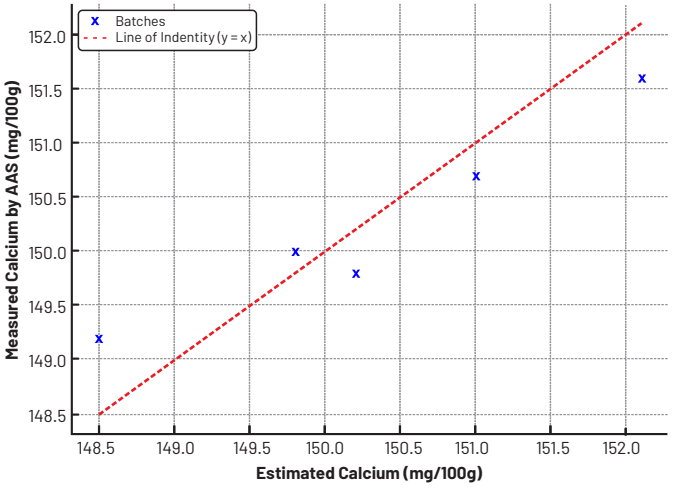


**Figure 1:** Comparison Between Calcium Content Estimated and Measured by AAS

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

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.

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.

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**DOI:** https://doi.org/10.54393/df.v5i04.151

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)

**Systematic Review**

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# Addressing Food Security and Nutritional Challenges: A Comprehensive Systematic Review of Health Administration Strategies for Vulnerable Communitiesin South Asia

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## ARTICLE INFO

***Keywords:***

Food Security, Malnutrition, Health Strategies, South Asia

***How to Cite:***

Aslam, A., & Zameer, Z. (2024). Addressing Food Security and Nutritional Challenges: A Comprehensive Systematic Review of Health Administration Strategies for Vulnerable Communities in South Asia: Health Strategies for Food Security. DIET FACTOR (Journal of Nutritional and Food Sciences), 5(04), 38-47. https://doi.org/10.54393/df.v5i04.151

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Received date: 5th November, 2024
Acceptance date: 24th December, 2024
Published date: 31st December, 2024

## ABSTRACT

South Asia having problems with food deficiency and malnutrition, which are made more problematic by ongoing poverty, fast increase in population, environmental loss, and unstable sociopolitical circumstances. This systematic approach analyzes how good is the health administration techniques that increased food security and nutritional benefits for the region's most disadvantaged inhabitants work. It evaluated a lot of health policies and initiatives that have been seen in South Asian nations, such as Bangladesh, Nepal, Bhutan, India, Pakistan, and Sri Lanka. Material from 2013 to 2023 was included in the review, which was occurred by employing a comprehensive search plan across seven electronic databases. Studies that were relevant to South Asian countries were given favoritism when selecting the studies, which focused on peer-reviewed publications that evaluated health policies and treatments related to food security and malnutrition. Thirteen studies in all were included following accurate screening program and a quality evaluation utilizing PRISMA guidelines. The study summarized research on women's empowerment plan, integrated resource management, agricultural interventions, and direct food assistance. It highlighted both effective strategies and areas in need of development, providing evidence-based suggestions for program optimization and policy improvements. The final objective was to develop practical insights that help improve food security, strengthen health administration procedures, and boost nutritional values for the most vulnerable communities in the region.

## INTRODUCTION

Food insecurity is referred to as absence of access to adequate quantities of safe and nutritious food needed for an active and healthy life. In case of South Asia, this is compounded by a double burden of malnutrition that comprise of undernutrition, hidden hunger and obesity among children Azmi FR *et al.*, in 2023 [1]. The South Asian region where more than a quarter of the comprehensive population resides has various issues hindering healthy diets such as food insecurity and malnutrition. Approximately 43.8% of the global population in poverty resides in South Asia where the hunger rate stands at 15.9 % in contrast with the global average of 9.3%. Moreover, it has been speculated that the population of the area could grow by 40% over the next 30 years Hibino G and Masui T in 2024 [2].The prevalence of high-rate of malnutrition in South Asia reflects a daunting image of the region entangled with public health related issues.Various indicators portray the status of nutritional deficiencies in the region.The stunting charges are high as 36.9% in Pakistan, and anemia in women impacting up to 53% in India. Around 17.3% of children are wasted every year in India Zhang X *et al.*, in 2022 [3]. Childhood obesity is common in many countries, with 5.2% in Bhutan Awasthi A *et al.*, in 2023 [4]. Figure 1 clearly shows that Pakistan and

DOI: https://doi.org/10.54393/df.v5i04.151

Afghanistan are the most vulnerable countries with maximum cases of stunting Zhang X *et al.*, in 2022 [3]. Stunting, anemia, and wasting are a complex combination of malnutrition indicators that highlight the urgent need for focused health administration measures to address these concerns Azmi FR *et al.*, in 2023 [1]. This is a need to enhance the nutritional outcomes for vulnerable communities in South Asian.

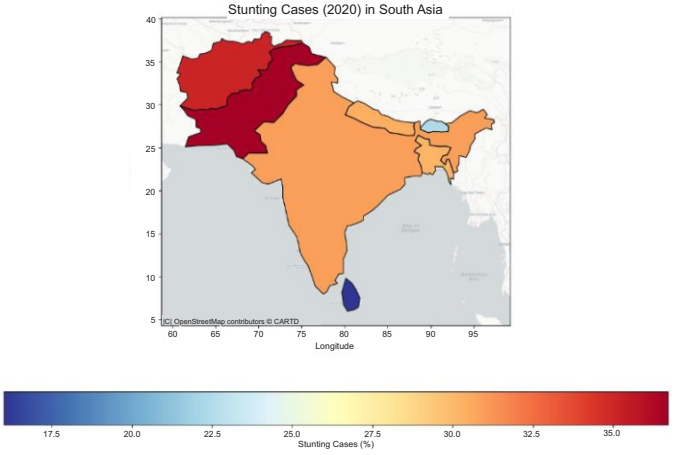



**Figure 1:** Heat map of South Asia reflecting country-wise status of stunting (%) Zhang X *et al.*, in 2023 [3]

The main drivers of food insecurity in South Asia include: poverty, environment degradation, social and economic inequalities, and high population growth rates. These confronts have been compounded by recent economic instabilities, political turmoil's and conflicts resulting in high food inflation and inadequate access of basic goods. Hence, the incidences of diseases related to malnutrition and other linked health complications have become rampant (World Bank, 2021). Consequently, this systematic review intends to show that improving food security and nutrition in the area is central to the question of sustainable development in the region. Considering recent surge in the population of South Asia, which is struggling with food insecurity and malnutrition, it becomes very important to come up with viable health administration policy.Therefore, it is the high time to assess the rate of malnutrition in the area and review current policies, strategies and interventions.Many studies need to be conducted and analyzed to assess the effectiveness of various health administration systems in addressing complications associated with nutrition and food insecurity in the region Mughal M and Fontan Sers C in 2020 [5]. Such as, several studies emphasize the importance of joining the interventions to promote local farming, enhance the communities' infrastructure, and supplement the food purchases with the food distribution Miller L and Malacarne J in 2023; Mishra P *et al.*, in 2023 [6, 7]. Such an analysis may provide insights into whether these strategies are effective or not, besides providing recommendations to practitioners and politicians. This systematic review will therefore assess the impact of health administration approaches in South Asia and their impact on food security and nutrition results.It will also relate different intervention strategies of the various regions in order to build those that are most effective in dealing with food insecurity and malnutrition. In order to achieve these purposes, this review presents an analysis of related literature and and evaluation of various approaches with the aim of banding the best practices that could be employed to enhance the effectiveness of existing health policies and programs. The aim here was to gain fresh understandings regarding what can be done to improve the design of interventions for food insecurity and malnutrition in the region and in turn, advance the health and quality of life of vulnerable populations in South Asia.

## METHODS

To assess different options for health administration concerning food security in South Asia, we performed a literature review in scientific databases. The procedures followed in conducting the literature search and analysis matched the standards of the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA) Moher D *et al.*, in 2009 [8].

### Information Sources

The databases that were used for the search to include seven of the most easily accessible open-access and free databases to conduct research between the years 2013 to 2023.The preliminary search was made using seven electronic databases. The databases searched PubMed Central (PMC), Google Scholar, the Directory of Open Access Journals (DOAJ), BioMed Central (BMC), the Social Science Research Network (SSRN), Health Systems Evidence, and the Lancet.

### Search Strategy

A thorough search of the published databases was conducted using an extensive set of keywords. Boolean operators were employed to combine these keywords and narrow down the search results (Table 1). According to Table 1, the primary search topics included 'South Asia', 'Food Security', and 'Health Policies'.

**Table 1:** Search Concepts and Key Terms

| Concept | Search Terms |
|---|---|
| South Asian Region | "South Asia" OR "Asia" OR "Pakistan" OR "India" OR "Afghanistan" OR "Bangladesh" OR "Nepal" OR "Bhutan" OR "Maldives" |
| Food Security | "Food security" OR "Food availability" OR "Nutritional Challenges" OR"Malnutrition" OR "Micronutrient Deficiencies" OR "Nutritional Deficiencies" OR "Dietary Intake" |
| Health Policies | "Health administration strategies" OR "public health policies" OR "health programs" OR "health management" |

DOI: https://doi.org/10.54393/df.v5i04.151

### Study Selection

The process for screening and selecting studies was based on the following inclusion criteria: 1) peer-reviewed, 2) available full text articles, 3) published in English, 4) studies focusing on vulnerable communities in South Asia, including countries India, Pakistan, Bangladesh, Sri Lanka, Nepal, Bhutan, and the Maldives, and 5) studies that evaluate health administration strategies or public health policies aimed at improving food security and nutrition, with relevant outcomes such as dietary intake, nutritional status, and food availability. Articles that did not meet the specified criteria were excluded. The stages of systematic mapping exercise are presented in Figure 2.

### Quality Assessment and Potential Bias

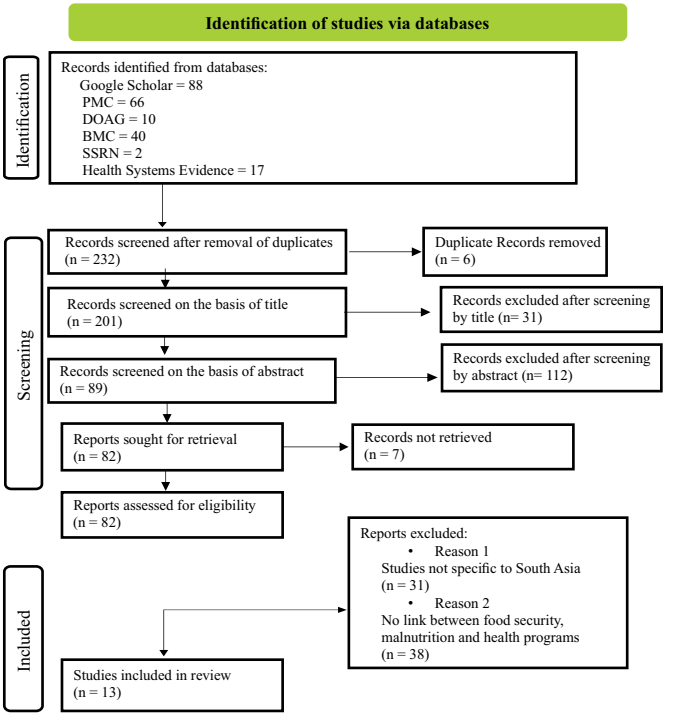
A strict quality estimate was used in compliance with PRISMA criteria to guarantee the validity of this systematic review. All the articles that have been considered in the review were reviewed for methodological quality and sources of bias according to the study type using appropriate and reliable instruments. A possible presence of biases was considered within the screened studies at various stages of selection. These measures were taken to reduce the potential sources of bias-including publication and selection bias-such as the detailed evaluations of the inclusion criteria and the search methods used to screen papers. Also, at each study, conflict of interest and funding for the purpose of determining was used that could bias the study results. Priority was given to studies on the basis of relevancy to the topic; focused on health policies aimed to cater food insecurity and malnutrition in South Asia.

### Data Charting

Details such as sample characteristics, intervention descriptions, and fidelity, outcomes, and effect sizes were carefully identified and documented from each of the included publication to conduct meta-synthesis systematically. The extracted data involved the name of the first author, the year of the publication, location of study, design, aim, key findings and conclusion about the role health policies regarding food security/malnutrition prevention in South Asia (Table 2). Based on the information published in the books and articles, all the related information was organized and presented in Table 2. This table provides a structured overview of the key aspects of each study, allowing for easy comparison and analysis of the evidence regarding the effectiveness of health policies and their impact on food security and nutritional outcomes in the region. This complex extraction method proved very useful because it allowed to grasp various outcomes reported by different studies, as well as the ways in which the given issue was addressed (Table 3).

### Study Selection

In order to ensure a transparent and rigorous procedure to select studies for inclusion in the selection process of systematic review, the guidelines of PRISMA were followed (Figure 2). Initially, a comprehensive search across seven databases yielded a total of 238 articles. After the removal of 6 duplicates, 201 articles were selected for further screening for relevant title and abstract. A total of 89 articles were selected, and 7 articles were excluded on the basis of unavailability of full text. Further 69 articles were excluded for non-relevance and lack of focus on vulnerable communities in South Asia. These selected studies were then evaluated for quality and bias analysis. In the end, 13 studies were screened to be eligible for inclusion. The selected articles offer insightful information about the efficacy of various health administration techniques.




## RESULTS

### Characteristics of Screened Studies

Each selected study was assessed based on their applicability to the region's most vulnerable population, and how effectively it added to the efficacy of different health interventions and policies. The table 2 provides a detailed summary of each study's focus, objectives, and conclusions, which gives a comprehensive overview of these diverse strategies applied in different countries and the overall region of South Asia. It further aids in comparing and contrasting the effectiveness of various health administration strategies, and identifying key factors contributing to their success or limitations.

DOI: https://doi.org/10.54393/df.v5i04.151

**Table 2:** Characteristics of the Studies included in the Review (n = 13)

| Lead Author | Study Focus (Country/ Region) | Publication Year | Objective of Study | Conclusions |
|---|---|---|---|---|
| Gillespie [9] | South Asia | 2019 | To analyze how agriculture and agri-food systems impact nutrition in South Asia, focusing on LANSA research and governance effects. | Combining nutrition and agriculture strategies with good governance is essential for sustainable development and effective malnutrition combat in agri-food systems. |
| Rasul [10] . | South Asia | 2016 | To assess South Asian challenges in meeting food, water, and energy needs, and evaluate policies' impact on resource sustainability. | Wheat production subsidies harm resources and increase water and energy demand, threatening SDG progress. Cross-sectoral cooperation and coherent policies are needed for sustainability. |
| Bird [11] | South Asia | 2019 | To evaluate peer-reviewed research from 2012-2017 on agricultural interventions' impact on nutrition in South Asia, focusing on key countries. | Nutritional habits and anemia have improved, but child growth data is mixed. LANSA's research aims to find practical solutions for South Asian nutrition. |
| Vir [12] | South Asia | 2016 | To examine how direct nutrition interventions and non-nutritional factors likegender inequality affect women's nutrition, birth outcomes , and child stunting in South Asia. | Combining nutrition interventions with women's empowerment—through economic,educational, and anti-abuse measures—can significantly reduce child stunting in South Asia. |
| Dewey [13] | South Asia | 2016 | To identify strategies that enhance nutrition for pregnant women and young children in South Asia, improving nutrient density in staple diets. | Dietary diversification and supplements can boost child growth, but infections and mixed results limit effectiveness; integrated, evaluated interventions are crucial. |
| Alam [18] | Pakistan | 2022 | To examine how financial stress and socio-economic deprivation during COVID-19 affected the nutritional status of young children in Bahawalpur. | COVID-19 lockdown worsened stunting and underweight in children, with increased risks linked to financial stress and socio-economic deprivation. |
| Leyvraz [21] | India | 2016 | To assess how well the fortified food product Bal Amrutham, provided by ICDS in Telangana, is used and identify effectiveness barriers. | Bal Amrutham is well-used and accepted, but effectiveness is limited by supply issues and intra-household sharing in Telangana. |
| Harris-Fry H [22] | South Asia | 2017 | To evaluate factors affecting stunting reduction in four Indian states from 2006-2016, focusing on maternal, household, and intervention variables. | Effective stunting reduction in India requires targeted maternal and household interventions, with a focus on comprehensive health and nutrition strategies. |
| Harding KL [23] | South Asia | 2018 | To examine factors influencing stunting reduction in four Indian states from 2006-2016, focusing on maternal, household, and intervention variables. | Stunting reduction in India depends on targeted maternal and household interventions, alongside effective health and nutrition strategies. |
| Pradyumna [24] | India | 2022 | To explore how national-level food systems policies in India address health concerns and the involvement of the health sector across various relevant policy domains. | This study aimed to explore how national-level food systems policies in India address health concerns and the involvement of the health sector across various relevant policy domains. |
| Wali [25] | South Asia | 2023 | To evaluate nutrition programs and policies in eight South Asian countries targeting child malnutrition and micronutrient deficiencies for 2025 goals. | South Asian countries struggle with implementing and evaluating nutrition policies, requiring better tracking and contextualization to improve effectiveness. |

**Diverse Regional Approaches to Address Food Security and Nutritional Concerns in South Asia**

Regional disparities in food security and nutritional outcomes in South Asia reveal significant variations with respect to challenges and interventions (Table 3). In India, food availability and access are the pivotal issues contributing to high stunting rates and anemia prevalence, with key programs such as the Integrated Child Development Services (ICDS) and the National Nutrition Mission addressing these challenges Gillespie, Van Den Bold, and Hodge 2019. In contrast, Bangladesh's

DOI: https://doi.org/10.54393/df.v5i04.151

National Food Policy and Social Safety Nets Program prioritizes enhancing nutritional diversity and quality in order to fight child malnutrition (Bird, Pradhan, Bhavani, and Dangour, 2019). With the help of programs like the Benazir Income Support Program and the Pakistan Integrated Nutrient Management Program, Pakistan is working to alleviate child stunting and underweight by focusing on the availability and affordability of food (Rasul, 2016). The School Feeding Program and the National Multi-Sectoral Nutrition Plan are two of Nepal's strategies for improving access to food security and reducing micronutrient deficiencies (Dewey, 2016). The significance of localized policies designed to address particular food security and nutritional demands is highlighted by these geographical changes.

**Table 3:** Regional Variation in Food Security and Nutritional Outcomes

| Region/ Country | Food Security Indicator | Nutritional Status Indicator | Key Programs/Policies |
|---|---|---|---|
| India | Food availability, access | Stunting rates, anemia prevalence | Integrated Child Development Services (ICDS), National Nutrition Mission |
| Bangladesh | Nutritional quality, diversity | Child malnutrition rates | National Food Policy, Social Safety Nets Program |
| Pakistan | Food availability, affordability | Child stunting, underweight | Benazir Income Support Program, Pakistan Integrated Nutrient Management Program |
| Nepal | Food security access | Micronutrient deficiencies | School Feeding Program, National Multi-Sectoral Nutrition Plan |

### Evaluation of Health Administration Strategies in South Asia and their Impact on Target Population

Significant differences in food security and nutritional results are shown by the interpretation of regional data across South Asia, emphasizing unique challenges and policy solutions in various nations. A comparative summary of the health administration approaches used to address these problems in different South Asian nations is given in Table 4. The effects of these tactics on malnutrition and food security are further illustrated in Table 5. While food security is determined by availability and access, stunting and anemia rates are important markers of nutritional status in India Gillespie S in 2019 [9]. Pakistan addresses child stunting and underweight issues while enhancing food security through measures of availability and cost, relying on the Pakistan Integrated Nutrient Management Program and the Benazir Income Support Program Rasul, in 2016 [10]. The Integrated Child Development Services (ICDS) and the National Nutrition Mission are noteworthy programs. Bangladesh prioritizes food diversity and nutritional quality, focusing on child malnutrition through programs like the Social Safety Nets Program and the National Food Policy Bird FA *et al*, in 2019; Gillespie S in 2019 [11, 9].

**Table 4:** Comparative Summary of Health Administration Strategies and their Effectiveness on Target Population

| Strategy | Region/ Country | Objective | Key Findings | Effectiveness | Target Population |
|---|---|---|---|---|---|
| Direct Food Assistance | South Asia | Improve food availability and accessibility | Mixed results; some improvements in dietary intake | Variable effectiveness | Vulnerable populations |
| Agricultural Interventions | India, Bangladesh | Enhance dietary diversity and quality | Improvements in dietary practices; mixed results in nutritional outcomes | Partially effective | Farmers, rural communities |
| Integrated Approaches | South Asia | Address interconnected issues of food, water, and energy | Improved resource management and sustainability | Promising, needs further integration | General population |
| Women's Empowerment | South Asia | Improve nutritional outcomes through women's education and economic opportunities | Positive impacts on nutrition and child stunting | Effective in specific contexts | Women and children |
| Fortified Foods | India, Pakistan | Increase micronutrient in take through fortified food programs | Increased micronutrient intake; reduction in anemia and stunting rates | Generally effective | Children |
| Mobile Health Interventions | India | Improve nutrition knowledge and practices through mobile health platforms | Enhanced nutrition knowledge; positive impacts on child nutrition practices | Effective in improving knowledge | Rural women |
| Social Safety Nets Programs | South Asia | Enhance food access and overall nutritional status among vulnerable populations | Improved food access; positive impact on nutritional status | Effective in improving food security | Vulnerable populations |
| Nutrition-Specific Interventions | South Asia | Target specific nutritional deficiencies through targeted programs | Significant reduction in stunting and anemia; improved dietary intake | Effective with proper implementation | Children and mothers |

Direct food assistance programs aim to improve food availability and accessibility, showing mixed results with variable effectiveness across the region Bird FA *et al.*, in 2019 [11]. In Bangladesh and India, agricultural interventions aimed at improving the diversity and quality of diets have shown mixed results in terms of overall nutritional outcomes Gillespie S *et al.*,

DOI: https://doi.org/10.54393/df.v5i04.151

in 2019; Vir, in 2016 [9, 12]. Although they need further integration to optimise advantages, integrated approaches to food, water, and energy challenges have demonstrated encouraging gains in resource management and sustainability Rasul G in 2016[10]. Although their effectiveness varies by local context and implementation, women's empowerment programs that emphasise economic possibilities and education have been successful in improving nutrition and lowering child stunting Dewey, in 2016 [13]. Child feeding practices have improved as a result of the Mobile Vaani initiative in India, which has increased rural women's awareness of nutrition Gillespie S *et al.*, in 2019 [9]. Children's consumption of micronutrients has improved dramatically as a result of fortified foods programs, which has decreased rates of stunting and anaemia Bird FA *et al.*, in 2019 [11]. Furthermore, food security and children's nutritional outcomes have been strengthened by women's empowerment programs Vir, in 2016 [12]. These results highlight the significance of customised interventions to meet the unique needs of populations throughout South Asia. Children's consumption of micronutrients has improved dramatically as a result of fortified foods programs, which has decreased rates of stunting and anaemia Bird FA *et al.*, in 2019 [11]. Furthermore, food security and children's nutritional outcomes have been strengthened by women's empowerment programs Vir, in 2016 [12]. These results highlight the significance of customised interventions to meet the unique needs of populations throughout South Asia.

**Table 5:** Impact of Strategies on Food Security and Nutritional Status

| Strategies | Impact on Food Security | Impact on Nutritional Status |
|---|---|---|
| Direct Food Assistance | Improved food access | Variable impact on nutritional status |
| Agricultural Interventions | Improved dietary diversity | Mixed results; variable impact on nutritional outcomes |
| Integrated Approaches | Enhanced resource management | Positive impact on overall food security and nutrition |
| Women's Empowerment | Enhanced food security | Improved nutritional outcomes, reduced child stunting |
| Fortified Foods | Increased micronutrient intake | Reduced anemia and stunting rates |
| Mobile Health Interventions | Improved nutrition knowledge | Positive impacts on child nutrition practices |
| Social Safety Nets Programs | Enhanced food access | Improved nutritional status through increased food availability |
| Nutrition-Specific Interventions | Addressed specific deficiencies | Reduced stunting and anemia, improved dietary intake |

## DISCUSSION

In South Asia, significant rates of malnutrition and food insecurity are common. There are an estimated 412.9 million South Asians who are at risk of food insecurity. Only 3.4% of the world's land area is occupied by this region, which is home to 25% of the world's people Rehman A *et al.*, in 2024 [14]. Food insecurity in South Asia is caused by a number of variables, including economic problems, agricultural obstacles, climate change, increased urbanisation, and pandemics like COVID-19 Khalid, in 2020 [15]. Amidst already prevailing acute food insecurity in the region, challenges such as climate change, rising temperatures, extreme weather events, and soil degradation negatively affect the agricultural productivity. This impacts long terms as well as short term availability of food, hence exacerbating food insecurity in the region Rehman A *et al.*, in 2024 [14]. Declining arable land, farm sizes and insufficient fundings for research and development are main factors accounting for decline in agricultural productivity.Moreover, water-induced degradation has degraded around 25% of pasture and cropland in South Asia. Food inflation, trade restrictions and elevated oil prices has further destabilized the situation of food security Khalid, in 2020 [15]. This calls for the formulation of realistic polices and strategies that would address the challenge of food insecurity and its related diseases. The potential solutions include: ramp up of agricultural research and development; improvement of agricultural technology; development and implementation of food security and safety net policies; contribution to rural economy; investment on climate-smart agriculture technologies and infrastructure; access to formal agricultural credit for farmers; and promotion of use of renewable energy Rehman A *et al.*, in 2024 [14]. Literature review of health administration strategies illustrates a system of framework, which has been used to analyze issues concerning nutritional problems and food insecurity in South Asia. While programs like 'Direct Food Assistance Programs' do help in the short run for a particular period say for a month yet such strategies do not rely much on lessons that are learnt from medium and/or long-term nutrition impact. Bird FA *et al.*, in 2019 notes that while such programs increase food access, their effects on food quality and long-term nutritional development are mixed: more supplementary interventions are required besides food provision [11]. It is remarkable that agricultural interventions are more effective according to the studies conducted to enhance dietary diversity and nutritional status among the populations of the less developed countries. From the research done by Gillespie S *et al.*, in 2019, practices which focus on nutrition-sensitive agriculture, like pushing for crop diversification, assists in increasing dietary intake eliminating malnutrition [9]. This is in accordance with the study carried out by Dewey, in 2016, where he points out that while these interventions have a positive influence in the nutritional status of populations, however their effectiveness is increasingly hindered by many factors. South Asia is inflicted with numerous issues, specifically abiotic stresses like drought, flooding, and salinity [13]. Various previous work has been carried out before aimed to promote the notion of 'climate-smart crops. The production of flood tolerant rice varieties, drought resistant maize hybrids, and salt-tolerant rice and

DOI: https://doi.org/10.54393/df.v5i04.151

wheat varieties are some of previous success the in this domain. Other than this, enhancing the nutritional content of staple crops through breeding and genetic modification also aids in addressing concerns pertaining to food insecurity in the region. Vitamin A enriched golden rice, and iron and zinc biofortified rice and wheat are some potent examples Glover D and Poole N *et al.*, in 2019 [16]. Synergic approaches dealing with food, water, and energy needs at once are the effective ways to respond to malnutrition. According to Rasul, in 2016, these strategies that work on the policy process of harmonization of resources management across various sectors, count significant advantages whereby, there is enhanced synergy that enhances the overall food security [10]. However, according to Rasul, in 2021, there are also some limitations; thus, the integration might face some difficulties in synchronizing in several sectors. Another study carried out by Li G *et al.*, in 2019 also adheres to the integrated approach of water, energy and food nexus, aiming to maximize synergies and minimize trade-offs amongst these sectors [17]. It further highlights the importance of sector interdependencies, optimizing usage of resources, and insurance of policy coherence. This nexus aims to align strategies with Sustainable Development Goals (SDGs) to promote integrated, efficient, and sustainable development across water, energy, and food sectors Li *et al.*, in 2019. Therefore, it is crucial to have robust institutional infrastructure to realize these integrated measures.Projects aimed at empowering women especially in the aspect of health have been shown to produce a positive impact on the overall nutritional status of people by directly addressing some cultural, economic practices that negatively influence nutrition status.As highlighted by Vir, in 2016 as well as Alam MB *et al.*, in 2022 education of women coupled with their financial independence led to increased ability to feed families healthy foods [12, 18]. Moreover, their children also enjoy nutritional benefits. These programmes are most effective where they are accompanied by definite nutrition interventions, which clearly marks the paramount importance of these programmes as a permanent solution for poor nutritional and vulnerable communities. Policy makers need to adopt a holistic approach to empower women inn agriculture by ensuring access to land, addressing over-loaded work burdens, and catering issues like childcare, poverty, and education. Such effective programs that include livestock transfer, agricultural training, and financial literacy have proven successful in enhancing food availability and access Quisumbing A *et al.*, in 2022 While each strategy has its own strengths and weaknesses, it may be most effective to use the combination of these strategies to give a comprehensive solution for the numerous issues of food insecurity and malnutrition in South Asia [19]. In South Asia, effectiveness of regional approaches and initiatives on enhancing food security and more nutritional outcomes range significantly because of the situation and problem associated with the different countries of the region Chowdhury SK *et al.*, in 2017 [20]. Several strategies have been implemented in India to improve the status of food Security and nutrition. The program Integrated Child Development Services (ICDS) has been implemented in many schools as a way of providing children with nutrition, health,and early education services, have recorded varying success rates. Leyvraz M *et al.*, in 2016 have pointed out that, although ICDS has addressed the problem of access to fortified foods and supplements, various challenges such as, inadequate logistic management, and an uneven service coverage have constrained the effectiveness of the program [21]. Harris-Fry H *et al.*, in 2022 also notes that while ICDS has resulted in some changes to child malnutrition, the efficacy of this programme again differs from state to state because of variation in the rate of implementation and environmental factors [22]. In Bangladesh, the National Food Policy or NFP is the key strategy through which the country is aiming to lower child malnutrition. For instance, Bhatnagar RS and Padilla-Zakour OI in 2021 indicate that, through the NFP that aims at expanding food availability, and improving the nutritional standard, the reductions in malnutrition among children have been recorded [23]. However, there is always room for problems to occur such as problems arising from economic stability and problems in the implementation of the policy making the policy a less effective one. It is quite conscious here to realize the need to revise policies from time to time to suit the dynamic nutritional requirements.Various programs such as Benazir Income Support Program (BISP) has been launched in Pakistan to help the poor families. Studies on economic stress and socio-economic deprivation during COVID-19 lockdown in South Punjab reveals that BISP have been useful in mitigating some of the harms of economic adversity on prices of food. However, the effectiveness of such a program is limited by regional inequality and the amount of financial aid received; this is why further targeted measures are needed Khalid, in 2020 [15]. In Nepal, The School Feeding Program and the National Multi Sectoral Nutrition Plan has played a major role to solve the Nutritional problems. As pointed out by the document review on food systems policies in India (source needed), these programs have been effective in promoting school attendance among children as well as enhanced dietary diversification. The National Multi-Sectoral Nutrition Plan seeks to focus on the need to coordinate efforts of different sectors to combat the vice of malnutrition. Nevertheless, there is a number of issues that still persist, for example, dealing with such obstacles as logistical concerns and the availability of the resources needed to support these programs Pradyumna A *et al.*, in 2022 [24, 25]. In general, the above analyzed regional strategies have positive trends in increasing the population's food security and nutrition level; however, these tendencies depend on regional peculiarities.

DOI: https://doi.org/10.54393/df.v5i04.151

Adapting interventions to suit certain regional contexts and circumstances is important to ensure that they yield maximum return. Further empirical research and assessment are required to strengthen these approaches and achieve better impacts toward handling the heterogeneity of susceptible groups in South Asia Rehman A *et al.*, in 2024 [14, 26]. Based on the analysis, it is possible to state that there are some crucial differences in food security and nutritional approaches in South Asia. Studies have found mixed success in implementation of India's integrated child development services (ICDS) due to implementation challenges Leyvraz M *et al.*, in 2016 [21, 22]. The National Food Policy (NFP) of Bangladesh has enhanced the child malnutrition index, but this policy is influenced by economic and executive challenges Bhatnagar RS and Padilla-Zakour OI in 2021 [23]. Early initiatives like the Bangladesh's food security and the Benazir Income Support Program (BISP) in Pakistan have lessened the strings attached in terms of economic pressures on the food security except the hurdles of regional disparities (Khalid, Mukhtar and Murtaza, 2020. In Nepal, the School Feeding Program and the National Multi-Sectoral Nutrition Plan have made strides in improving child nutrition but face challenges due to limited resources. These programs' efficacy varied according to the setting, resources available, and techniques implemented.

**Policy and Practice Recommendations for Improving Food Security**

The assessment identifies a number of policy and practice implications which are imperative to address the multifaceted issues of food poverty and malnutrition in South Asia. First, in order to encourage innovation and increase productivity more resources should be allocated to agricultural research Rehman A *et al.*, in 2024 [14]. However, to sustain agricultural production and reduce the impact of climate change, there is a need for climate-smart agriculture practices to be adopted Khalid, in 2020 [15]. Strengthening food-based social protection instruments remains critical because these initiatives can significantly positively impact the food security of vulnerable groups Avula R *et al.*, in 2022 [22, 27]. Moreover, promoting rural growth and enhancing infrastructure is crucial in supporting agriculture growth and increases resourcefulness to the countryside in particular Gillespie S *et al.*, in 2019 [9, 28]. These strategies should be implemented with the aim of enhancing the effectiveness of food security system of the region in the future. However, it is necessary to pay attention to some limitations of this systematic review, primarily because its design is exceptionally rigorous. In particular, it was found that many of the included studies had incomplete or inconsistent reporting, which limited the detail provided in the analysis of all the related interventions. Furthermore, issues of study quality were another factor; the methods used, sample size and measurement tools used in the studies influenced the quality, standardization and comparability of the results. Such limitations might affect the stability of the conclusions made, bring possible biases and limit transferability of the results. Therefore, although this review adds to the knowledge, it also pinpoints the lack of more rigorous and systematic studies to support policy and practice. Subsequent researches on nutrition and food security in South Asia should focus on some major gaps identified in the existing literature. One of the topics which require additional research is the question of long-term effectiveness of health management strategies. More so, cohort designs that track people over time can provide valued knowledge on several aspects of various interventions and help to identify the factors that may affect their outcome or lack of it. However, in order to assess these multiple outcomes and other less easily quantifiable effects of health policies and measures more comprehensive systematic reviews that incorporate both qualitative and quantitative data are also needed. Such evaluations must, in order to satisfy specific requirements, consider socioeconomic situations and regional differences. Emphasizing these topics of study shall enhance understanding of the effectiveness of existing strategies and guide the establishment of better and culturally relevant solutions [29-35].

## CONCLUSIONS

The aim of this systematic review was to presented an evaluation of strategies implemented in health administration in relation to food security and nutritional issues in South Asia. Some conclusions are very significant in demonstrating the extent to which the population in the area is food insecure, factors that have been made worse by poverty, degrading environmental factors and rapid population growth. The studies reveal that many of the existing programmes are not equally effective, where the programmes involving women's participation, direct food transfers, combined approaches and the agricultural support show varying levels of effectiveness. Future policy recommendations highlight the need to increase funding for agricultural research and development, the promotion of climate smart agriculture, enhancement of the social protection programs for the vulnerable and development of the rural areas. Despite such progress, there are significant differences between the regions and resources constraints.

## Authors Contribution

Conceptualization: AA, ZZ
Methodology: AA, ZZ
Formal analysis: AA, ZZ
Writing, review and editing: AA, ZZ

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

DOI: https://doi.org/10.54393/df.v5i04.151

## Conflicts of Interest

All the authors declare no conflict of interest.

## Source of Funding

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

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