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Diet Factor is committed to maintaining the highest standards of professional ethics, accuracy, and quality in all matters related to handling manuscripts and reporting scientific information. The journal welcomes empirical and applied research, viewpoint papers, conceptual and technical papers, case studies, meta-analysis studies, literature reviews, mini reviews and letter to the editors which take a scientific approach to the topics related to health and nutrition.

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- Commentaries
- Perspectives and Opinion
- Meta Analysis
- Case Reports
- Case Studies
- Case Control Studies

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INTRODUCTION
Provide a context or background for the study (i.e.,the nature of the problem and its significance). State the specific purpose or research objective of, or hypothesis tested by, the study or observation; the research objective is often more sharply focused when stated as a question. Both the main and secondary objectives should be made clear, and any pre-specified subgroup analyses should be described. Give only strictly pertinent references and do not include data or conclusions from the work being reported.

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Conclusion should elucidate how the results communicate to the theory presented as the basis of the study and provide a concise explanation of the allegation of the findings.

ACKNOWLEDGEMENT

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DIET FACTOR
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The Impact of Ultra-Processed Foods on Diet Quality and Health Outcomes

Riffat Mehboob[1*]

[1]Lahore Medical Research Center[LLP], Lahore, Pakistan
riffat.pathol@gmail.com

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In recent decades, there has been a notable shift in dietary patterns, with an increased consumption of ultra-processed foods. These foods, characterized by their high levels of added sugars, unhealthy fats, and chemical additives, have become a prevalent part of modern diets. However, mounting evidence suggests that the excessive intake of ultra-processed foods can have profound implications for diet quality and long-term health outcomes. Ultra-processed foods are often low in essential nutrients while being energy-dense, leading to overconsumption and imbalanced diets. The high levels of added sugars, sodium, and unhealthy fats contribute to the rising rates of obesity, cardiovascular diseases, and metabolic disorders. Studies have shown a strong association between the consumption of ultra-processed foods and an increased risk of developing chronic conditions such as type 2 diabetes and certain types of cancers.

Moreover, the processing techniques used in the production of these foods can result in the loss of natural nutrients and the formation of potentially harmful substances. The reliance on refined grains, artificial additives, and preservatives in ultra-processed foods further compromises their nutritional value. These factors, combined with the addictive nature of some ultra-processed foods, make it challenging for individuals to maintain a healthy and balanced diet.

The impact of ultra-processed foods extends beyond individual health to societal and environmental dimensions. The high demand for these products has led to unsustainable agricultural practices, increased food waste, and a strain on natural resources. Addressing the pervasive influence of ultra-processed foods requires a multifaceted approach that includes public awareness campaigns, improved food labeling, and policies aimed at promoting the availability and affordability of healthier options.

As the consumption of ultra-processed foods continues to rise, it is imperative to recognize the detrimental impact they have on diet quality and long-term health outcomes. Efforts should focus on promoting whole, minimally processed foods and educating individuals about the importance of a nutrient-rich diet. By addressing the root causes of excessive ultra-processed food consumption, we can empower individuals to make informed dietary choices, improve diet quality, and mitigate the burden of chronic diseases. Additionally, policy interventions that support sustainable and healthier food systems are crucial in creating environments that promote the availability and accessibility of wholesome food choices.

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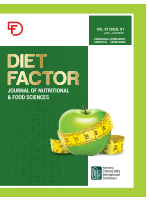

Review Article

Cashew Nut Allergy: A Review

Madiha Khan Niazi[1*] and Farooq Hassan[2]

[1]Institute of Diet and Nutritional Sciences, The University of Lahore, Lahore, Pakistan
[2]Punjab Healthcare Commission, Lahore, Pakistan

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***Corresponding Author:**
Madiha Khan Niazi
Institute of Diet and Nutritional Sciences, The University of Lahore, Lahore, Pakistan
dr.madihaniazi@gmail.com

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ABSTRACT

A severe health problem brought on by increased cashew nut use and dietary and cooking changes is cashew nut allergy. It is brought on by the use of trace amounts of Ana-o 3, Ana-o 1, and Ana-o 2 allergens from cashew nuts. Enzymatic processing, oral immunotherapy, and adrenaline auto-injector devices are effective treatment options. The labelling of foods containing cashew nuts is crucial for prevention.

INTRODUCTION

Different foods that include proteins in their primary, secondary, and tertiary forms can result in allergic reactions [1]. Most deadly food-induced responses are caused by tree nuts and peanuts. Tree nut allergies are widespread and frequently cause severe reactions, ranging from minor mouth irritation to anaphylaxis. This form of allergy varies in prevalence according to age and location and seems to have become more common in kids [2]. Due to their high levels of unsaturated fatty acids and low levels of saturated fatty acids, cashew nuts are a significant food allergy. Due to the potential for severe allergic reactions, cashew nut allergies are on the rise worldwide, particularly in Singapore. An underappreciated healthcare issue is this allergy, particularly in kids [3, 4].

Cashew Nuts

Anacardium occidentale L., a native of Brazil and a common tree around the world, is an evergreen South American nation [5]. Its average annual production over the past ten years has been 547,371 metric tons, making it the third most popular tree nut in the United States. It has been discovered that the cashew nuts are a rich source of hydrolysable tannins, with polymeric proanthocyanidins serving as the main polyphenol [6]. When compared to raw cashews, high temperature (HT)-treated cashew skin showed 3-fold greater gallic acid levels, indicating that gallic acid is released during heat processing. The TPC and

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antioxidant activity of the HT-treated cashew skin was higher than that of the low-temperature-treated samples. To determine the flavonoid concentration of cashew co-products, more research should be done. Cashew nuts' nutritional makeup was determined by their bioactive chemical content, which includes oleic and linoleic acids, phytosterols, arginine, tocopherols, magnesium, and phenolic compounds. The largest quantities of phenolics and tocopherols were found in the wrapped nuts, whereas thiamin, carotenoids, and unsaturated fatty acids were noticeable in the raw cashew nut kernels [7]. The cashew tree is one of the most productive tree crops for recovering lost land. It is also used to treat psychiatric issues, increase bone mineral density, and decrease depression rates. Due to their high nutritional and energy content, nuts have played a significant role in numerous cultures and civilizations for centuries [8]. They are well recognized for having a high quantity of unsaturated fatty acids, fiber, vitamins, minerals, and amino acids. Consuming nuts has been linked to lower risks for metabolic syndrome as well as lower risks for cardiovascular disease and mortality. Cashew trees are widely distributed throughout tropical regions near the equator, and their nutritional makeup may vary depending on where they were grown [9]. According to recent study, milk chocolate recipes can include 25% roasted cashew kernels in place of powdered milk. Many processed items, including confections, butters, baked goods, and snacks, contain cashew nuts. They contain 21% protein and 40–57% oil respectively. Because of their fragility, they are utilized in sweets. 3.58 million tons of cashew nuts were produced in 2021, marking a sharp increase in production. Cashews, however, have not been the subject of much study [10].

Allergens present in Cashew Nuts

Because eating cashew nuts can cause a number of allergy disorders, sensitivity to them has the most clinical impact. An individual's cashew allergy is confirmed via a competitive inhibition test. Three well-known allergies for cashew nuts are Ana o 1, A vicilin, an Ana o 2, an 11S globulin that resembles a legumin, and an Ana o 3, a 2S albumin. Western immunoblotting was used to examine patients with cashew nut allergies. It was discovered that 81% of them were allergic to recombinant Ana o 3, 62% to recombinant Ana o 2, and 50% to recombinant Ana o 1. IgE-immunoblot can be used to identify IgE-binding proteins in the protein extracts of these nuts [11]. In addition to location-based mutations brought on by allergies, recombinant DNA technology is employed to affect the different allergens. Using soluble protein extract, defatted cashew nut flour is produced. A sample's protein content can be estimated using the Bradford protein test [12]. Use of PCSH, also known as pyrrole-2-carboxaldehyde salicyl hydrazone, in immunotherapy for cashew allergy. Because there is basic pepsin digestion present, it reduces IgE reactivity while maintaining T cell boosting properties [13]. Avoiding allergens is the best way to cure a seed allergy or any other kind of allergy. Children with food allergies are encouraged not to share their lunch with their peers and to pack their own lunch for school. In the case of allergens, pepsin digestion shows to reduce allergenicity, especially in the context of oral allergy syndrome and for food allergens. Due to their dietary sensitivities, people with increased HRQL may seek medical attention [14].

Clinical Features

Cashew allergies are becoming more common, and their clinical symptoms can range from itchy mouth to catastrophic anaphylactic shock. A simple clinical reaction could be the result of a modest amount of cashew nut allergen [15]. Every five years, the Royal Children's Hospital in Melbourne receives reports of approximately 117 anaphylactic events, with cashew nut allergies being more common than peanut allergies. Skin or mucosal contact alone caused significant allergic reactions in 5 out of 27 patients with cashew nut allergies as shown in table 1 [16].

Table 1: Patients with cashew nut allergies

Allergic reactions	Symptoms	Number of cases
Anaphylaxis	Respiratory/skin/GIT	5 (25)
	Respiratory	3 (15)
	Respiratory and skin	8 (40)
	Respiratory and GIT	3 (15)
	CVS/skin/GIT	0 (0)
	Respiratory/CVS/skin	1(5)
Non-anaphylaxis	Skin/GIT	0 (0)
	GIT	0 (0)
	Skin	7 (100)

Anaphylaxis symptoms and non-anaphylaxis symptoms are separated in terms of medical terminology. Anaphylaxis, a multisystem allergic reaction, is a term used to describe conditions affecting the skin, gastrointestinal tract, respiratory, and cardiovascular systems. Skin conditions and gastrointestinal problems without respiratory or cardiovascular symptoms are examples of non-anaphylaxis symptoms. To avoid anaphylaxis, infants must consume nuts in the recommended doses. Due to its high content of saturated fatty acids, cashews have been exempted from nut and heart health claims [17]. There is, however, a dearth of clinical evidence linking cashews to blood lipids. This suggests that cashews might have similar results to other nuts however there is a paucity of clinical evidence. Consumption of cashew nuts has been connected to higher blood levels of high-density lipoprotein, cholesterol, and triglycerides, which is related to the reduction of diabetes risk factors. Additionally, the anacardic acids found in cashew nuts may one day be used

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to treat a variety of illnesses. Mediterranean diet and add a few handfuls of nuts to their daily diet have a 30% decreased incidence of severe cardiovascular events and mortality [18].

Diagnosis

History and in vitro testing are some of the criteria used to diagnose cashew nut allergy. According to studies on the diagnosis of cashew nut allergies, the majority of kids who had anaphylaxis symptoms had eaten the particular relevant nut. For measuring the challenges' effects, skin prick tests are analyzed to have high rates in relation to sIgE. The symptoms of cashew nut allergy cannot yet be investigated using a purported allergy illustration that can replace a double-blind, placebo-controlled food challenge test [19].

Management

According to studies, preparing cashew nuts enzymatically can lessen allergy reactivity by blocking IgE from binding to nut allergens. The Aspergillus genus is playing a bigger role in the food processing sector, and *A. niger* and *A. oryzae* are two natural pollutants found in cashew nuts. For the treatment of allergies, oral immunotherapy is being developed, along with dietary restrictions against plant-based meals and the substitution of similar foods. For a better understanding of the treatment of cashew nut allergy, more research is required [20]. A popular meal that can induce severe anaphylaxis is tree nuts. Adrenaline is a hormone that is safe and effective against food allergies, and accurate food labelling is required to manage label compliance. Tree nuts have a wide range of applications, such as a garnish for salads, ice cream toppings, baked goods, and Asian cuisine [21]. Tree nuts are among the top eight food allergies, and according to the 2004 Consumer Protection Act (FALCPA) they must be disclosed on product labels. In the Far East and Indian subcontinent, cashews are utilized in cooking, and tree nut oils can be discovered in lotions, soaps, and hair care products. Asian restaurants, baking, confectionary, sweets, ice cream, and chocolates should all be avoided by anyone with severe nut allergies [22].

CONCLUSIONS

Anaphylaxis can result from a significant allergy to cashew nuts. It can be identified via a test on a meal challenge that is double-blind and placebo-controlled and is brought on by 2S albumins and proteins that resemble legumes. It is advised to limit the consumption of pistachio nuts and other associated allergies.

Authors Contribution

Conceptualization: MKN, FH
Writing-review and editing: MKN, FH

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

Conflicts of Interest

The authors declare no conflict of interest.

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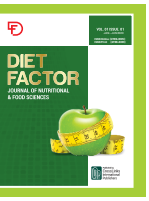

Original Article

Development and Quality Evaluation of Non-Dairy Yogurts

Hina Naz[1], Nighat Raza[1*], Shamas Murtaza[1], Ambreen Naz[1] and Umar Farooq[1]

[1]Department of Food Science and Technology, MNS-University of Agriculture, Multan, Pakistan

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***Corresponding Author:**
Nighat Raza
Department of Food Science and Technology, MNS-University of Agriculture, Multan, Pakistan
nighat.raza@mnsuam.edu.pk

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ABSTRACT

Development of fermented vegetarian milk based food will be important to fulfill nutritional value of both elderly and individuals that require more energy intake. **Objective:** To develop non-dairy vegan yogurts from soy milk, oat milk and coconut milk in conjunction with lactic acid fermentation. **Methods:** Soy yogurt, oat yogurt and coconut yogurt was analyzed for crude protein, crude fat, crude fiber, carbohydrates, ash contents, moisture contents, titratable acidity, total soluble solids and pH analysis and to check its quality and acceptability by sensory evaluation for color, aroma, taste, consistency and acidity. **Results:** The mean values of crude protein of yogurts showed that soy yogurt contain more protein contents than other yogurts that was 6.0±0.1. The mean values for crude fat contents showed that maximum value 8.5±0.65 was noticed in the coconut yogurt and lowest value 3.1±0.1 was observed in soy yogurt. Mean values of crude fiber showed that fiber contents are present in more amount in soy yogurt (1.93±0.152). The mean maximum value for moisture contents was 84.43±4.007 that was noticed in soy yogurt and lowest value 66.69±0.164 was observed in oat yogurt. Mean values for carbohydrate in soy, oat and coconut yogurt was 9.28±0.01, 20.76±0.659 and 16.16±1.258. Mean results of overall acceptability of soy yogurt, oat yogurt and coconut yogurt was 7±0.35, 7.25±0.36 and 8±0.4 respectively. **Conclusions:** The study's findings demonstrated that it is possible to make plant-based yoghurt to meet the organoleptic needs of consumers, particularly those who are lactose intolerant or follow a vegan diet.

INTRODUCTION

Development of non-dairy yogurt is important to fulfill nutritional value of both elderly and individuals as there is gap in market of energy dense and protein enriched nutritional food. In recent years, soybean, oat and coconut have been accepted as a functional food as these are source of protein, dietary fiber, minerals, antioxidants, vitamins and energy [1]. Soy bean and oats are good and inexpensive source of protein especially for many vegetarians or vegan and for those who cannot buy meat and milk [2]. Non- dairy yogurt contains unsaturated fatty acid that help to reduce incidences of cardiovascular diseases. In case of lactose intolerance, consumption of vegan milk is beneficial. Non-dairy yogurts have high nutrients and minerals level and it will work as a synbiotic food which is important for human gut, intestine and increase antibodies in human body so it boosts immunity [3]. Soya milk, oat milk and coconut milk can replace animal milk, in the manufacturing of dairy products. Fermentation of milk reported to reduce antinutritional factors and increase mineral's bioavailability [4, 5]. Yogurt is a fermented dairy product and probiotic carrier. It is rich in protein, magnesium, potassium, fat and vitamins [6]. Yogurt have many health benefits than simple milk, like it can be use by lactose intolerance patients that have allergy to lactose which is sugar of milk as in yogurt lactose is transformed into lactic acid and do not cause allergy in lactose intolerance people. It has probiotic characteristics so prevent antibiotic associated diarrhea, help to improve

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gastrointestinal conditions. On the other hand, calcium of yogurt is absorbed faster than milk in body, as lactic acid turns calcium to solution. It contains vitamins of A, B, C, D, all ingredients of milk that help in digestion of food, strengthen abdomen and relax nerves. Yogurt is recommended to use with antibiotics as majority of antibiotic are fatal for beneficial bacteria of digestive system [7]. Non-dairy yogurts have low fat milk that coagulates to a custard like consistency. It contains *Lactobacillus bulgaricus* and *Streptococcus thermophilus* cultures [8]. Soy yogurt fermentation is done with friendly bacteria mainly *Lactobacillus bulgaricus* and *Streptococcus thermophilus.* It contains other sugars such as starchyose and raffinose not lactose. Soy protein have equal nutrients that are present in meat and eggs and require for human health [2]. Animal milk have more saturated fats and cholesterol level while vegan milk have less saturated fats and cholesterol which is good for human health so this is also a factor that promote selection of vegetable substitute for animal milk [9]. Fibers in oat yogurt act as prebiotics that improve beneficial bacterial growth while lactic acid bacteria act as probiotics that improve human intestinal balance and have antagonistic action against pathogens. Oat milk yogurt is free of many allergens found in other milk. Oat yogurt contains protein, fat, carbohydrates, dietary fibers, riboflavin, calcium, phosphorus, iron, potassium, calories, vitamin A and D [10]. Oat milk yogurt have many health benefits such as it is vegan, lactose free, nut free, gluten free so it can be use by people that have gluten intolerance or celiac people. It contains vitamin B2 and B12, low blood cholesterol so good for heart health as well as bone health. Coconut milk contain protein, fats, fiber, carbohydrates, iron, folate, magnesium, potassium, copper, manganese, selenium, vitamin C and vitamin content calories [11]. Coconut yogurt have many health benefits like it reduce inflammation as it has anti-inflammatory effect, decrease stomach ulcer size, fight viruses and bacteria, improve heart health. Coconut milk yogurt contain healthy fatty acids, it reduces LDL and raises HDL which is good for health. It also improves brain functions in Alzheimer's disease and burn abdominal fat. Non-dairy yogurts can be produce in large scale on industry level and chances of jobs will be increase. Moreover, investors can use and enjoy the benefit produce by soy yogurt, oat yogurt and coconut yogurt on large scale [12].

METHODS

The current study was conducted in the central laboratories of MNS-University of Agriculture, Multan. Soybeans and oats was procured from department of Agronomy, MNSUAM. Belle-bella company's non-dairy yogurt starter was taken from market. Unripen coconut was purchased from local market. Soybeans was washed and soaked in water overnight. It was then boiled for 5 minutes. After blanching, the soybeans were crushed in a blender, and the resulting slurry was filtered through cheese cloth at a ratio of 7:1 water to slurry. The filtrate was then boiled for 20 minutes to produce soymilk [2]. 5g of yogurt culture was added in 1 liter of milk that has been heated to 82℃ for 15 minutes and quickly cooled to 43℃. To generate proper acidity, the inoculation mix was incubated for 4 hours at 40-45℃. After that, it was cooled and stored at a temperature of 6℃ [13]. To begin, rolled oats was milled into finely granulated oat flour. Then a slurry was formed with the oat flour and water. To obtain oat milk, this slurry was filtered through muslin fabric [10]. First, oat milk was cooked for 5 minutes at 70℃. It was then chilled to 40℃ and injected with 2 percent yogurt culture or probiotic pills. This sample was kept at 39℃ for 16 hours until it coagulates. The fermented samples were stored at 4℃ [14]. Coconut milk was made by shattering the shells of coconuts and removing the nuts with a knife. Nuts skin was removed and washed. Then for 20 minutes, mixed these nuts with warm water to homogenize it. The extract was discarded after passing through muslin cloth [15]. The extracted coconut milk was cooked for 10 minutes at 90℃ and then allowed to cool gradually. Yogurt culture was introduced and incubated at 39℃ for 12 hours or until coagulation occurs. Fermented probiotic yoghurt was kept at 4℃ [16]. Crude protein of yogurts was analyzed by kjeldhal method as explained by AOAC (2005). It was analyzed by Gerber method as explained by AOAC (2005). It was analyzed by same protocol as explained by AOAC (2005). Moisture contents in non-dairy yogurts was analyzed by using oven at 105℃ for 6 hours as explained by AOAC (2005). Ash contents was analyzed by heating yogurt samples in muffle furnace at 630℃ for 3 hours as described by AOAC (2005). It was determined by calculating percentage remaining after subtraction of protein, fat, moisture and ash contents from hundred as explained by AOAC (2005) [17]. In this titratable acidity, pH and total soluble solids was analyzed. 3g of sample was dissolved in 10ml of distilled water in a flask and was titrated against 0.1N NaOH using 1% phenolphthalein as indicator. Pink color was the end point. Final readings were noted by method prescribed by Soukoulis *et al.*, [18]. pH values were analyzed by direct measurement with digital pH meter as given in AOAC (2016) [19]. Total soluble solids were determined by using refractometer that was expressed in degree brix as described by Larriguadiere *et al.*, [20]. Syneresis and water holding capacity was determined by centrifugal acceleration test as described by Ares *et al.*, [21]. Sensory parameters (color, texture, taste, aroma and overall acceptability) were analyzed for sensory evaluation

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by using methods described by Wichchukit and O' Mahonye [22]. The obtained data were subjected to statistical analysis by following the guidelines explained by ANOVA Montgomery.

RESULTS

After preparation of soy, oats and coconut yogurt, it was analyzed for different tests like protein, fat, fiber, moisture, ash, carbohydrates. Mean values of protein, fat, fiber, moisture, ash and carbs content for different yogurt samples are given in Table 1. The mean value of protein contents of control sample was 8.83±0.152 while protein contents of other different treatments was 6.0±0.1(T_1), 3.2±0.1 (T_2) and 1.06±0.66 (T_3). Mean results of fat contents of different treatments T_o, T_1, T_2 and T_3 were 4.9±0.1, 3.1±0.1, 3.5±0.1 and 8.5±0.65 respectively. The maximum value 8.5±0.65% was noticed in the treatment T_3 (coconut yogurt) and lowest value 3.1±0.1 was observed in T_1 (soy yogurt). The mean value of fiber contents of control sample was 0.01±0.015 while fiber contents of other different treatments was 1.93±0.152(T_1), 1.1±0.1 (T_2) and 1.76±0.665 (T_3). ANOVA results of moisture contents of vegan yogurts showed that highly significant ($p<0.01$) difference was observed among the treatments of different yogurts. Mean results of moisture contents of different treatments T_o, T_1, T_2 and T_3 were 80±1, 84.43±4.007, 66.69±0.164% and 82.17±1.268 respectively. The maximum value 84.43±4.007 was noticed in the treatment T_1 (soy yogurt) and lowest value 66.69±0.164 was observed in T_2 (oat yogurt). ANOVA results of ash content of vegan yogurts also showed that highly significant ($p<0.01$) difference was observed among the treatments of different yogurts. Mean results of ash contents of different treatments T_o, T_1, T_2 and T_3 were 0.24±0.243, 0.52±0.08, 0.29±0.060 and 0.32±0.015 respectively. The maximum value 0.52±0.08 was noticed in the treatment T_1 (soy yogurt) and lowest value 0.24±0.243 was observed in T_o (dairy yogurt). The mean value of carbohydrates contents of control sample was 3.91±0.104 while carbohydrates contents of other different treatments was 9.28±0.01(T_1), 20.76±0.659 (T_2) and 16.16±1.258(T_3).

Table 1: Mean values of proximate composition of soy, oats and coconut yogurts

Treatments	Protein	Fat	Fiber	Moisture	Ash	Carbs
T0	8.83±0.152^a	4.9± 0.1^b	0.016±0.015^b	80 ± 1^a	0.24±0.243^b	3.91±0.104^d
T1	6.0± 0.1^b	3.1± 0.1^c	1.93 ± 0.152^a	84.43±4.007^a	0.523±0.087^a	9.28± 0.01^c
T2	3.2 ± 0.1^c	3.5 ± 0.1^c	1.1 ± 0.1^a	66.69±0.164^b	0.29 ± 0.060^b	20.76±0.659^a
T3	1.06±0.665^d	8.5±0.65^a	1.76± 0.665^a	82.17±1.268^a	0.32 ± 0.015^b	16.16±1.258^b

T_0=(Control dairy yogurt sample)
T_1=(Soy yogurt sample)
T_2=(Oat yogurt sample)
T_3=(Coconut yogurt sample)

After proximate analysis, physico-chemical analysis of yogurts was done. ANOVA results for TSS of vegan yogurts are given in Table 2, which showed that highly significant ($p<0.01$) difference was observed among treatments of different yogurts. Mean results of TSS of different treatments T_o, T_1, T_2 and T_3 were 12.26±1.330, 13.5±1, 34.04±3.214 and 17.5±1 respectively. The maximum value was of treatment T_2 (oat yogurt) which was 34.04±3.214 and lowest value was observed in T_o (dairy yogurt) which was 12.26±1.330. Mean squares of pH of different yogurt samples showed non-significant difference between different treatments of yogurt samples. The mean value of pH of control sample was 4.5±0.1 while pH of other different treatments was 4.6±0.1(T_1), 4.4±0.1 (T_2) and 4.73±0.493(T_3). ANOVA results of titratable acidity of vegan yogurts showed that highly significant ($p<0.01$) difference was observed among the treatments of different yogurts. Mean results of titratable acidity of different treatments T_o, T_1, T_2 and T_3 were 1±0.264, 0.68±0.1, 0.008±0.001 and 0.48±0.01 respectively. The maximum value was of treatment T_o (dairy yogurt) which was 1±0.264 and lowest value was observed in T_2 (oat yogurt) which was 0.008±0.001.

Table 2: Mean values of Physico-chemical analysis of soy, oats and coconut yogurts

	TSS	pH	TA
T0	12.26± 1.330^c	4.5 ± 0.1^a	1±0.264^a
T1	13.5 ± 1b^c	4.6 ± 0.1^a	0.68±0.1^{ab}
T2	34.04± 3.21^{4}a	4.4± 0.1^a	0.008±0.001^c
T3	17.5± 1^b	4.73±0.493^a	0.48±0.01^b

T_0=(Control dairy yogurt sample)
T_1=(Soy yogurt sample)
T_2=(Oat yogurt sample)
T_3=(Coconut yogurt sample)

Mean values for syneresis of T_o, T_1, T_2 and T_3 were found to be 33.9±1.25, 36.7±0.23, 12.0±0.5 and 23.1±0.01(Figure 1).

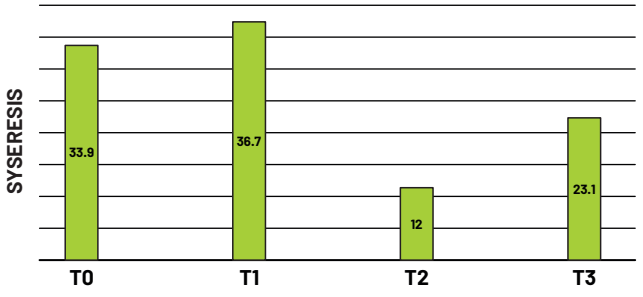

Figure 1: Graph representing syneresis of soy, oat and coconut yogurts

Figure 2 represents water holding capacity of different yogurts. Mean results for water holding capacity were 86.1±1.20(T_o), 85.0±1.40(T_1), 89.0±0.02(T_2) and 76.6±0.01(T_3) respectively for soy, oat and coconut yogurts.

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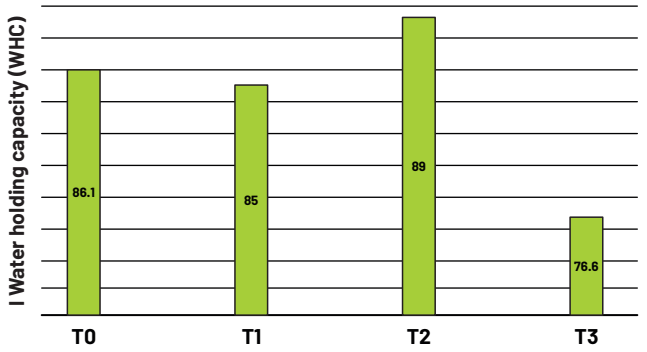

Figure 2: Graph representing water holding capacity of soy, oat and coconut yogurts

Eight participants were asked to taste and answer questions on each of the three yoghurt samples. The hedonic ratings of qualities such as color/appearance, taste, aroma/odour, texture and overall acceptability of yoghurts were included in the questions. The samples were completely random. ANOVA results of color of dairy, soy, oat and coconut yogurts showed that there was non-significant ($p>0.05$) difference among the treatments of these yogurt samples. Regarding color/appearance of yogurt samples, the mean values for dairy, soy, oat and coconut yogurts were found as 6.75±0.33, 6.87±0.34, 6.87±0.34 and 6.5±0.325 respectively according to 9-point hedonic scale (Table 2).

Table 2: Mean values of sensory evaluation of soy, oats and coconut yogurts

Treatment	Color	Taste	Texture	Aroma	Overall acceptability
T0	6.75±0.33^{a}	8± 0.4^{a}	7.5±0.37^{a}	6.62±0.33^{ab}	8.12±0.40^{a}
T1	6.87±0.34^{a}	6.87±0.34^{a}	6.87±0.34^{a}	5.87±0.29^{b}	7±0.35^{a}
T2	6.87±0.34^{a}	7.12±0.35^{a}	6.5±0.32^{a}	6.37±0.31^{ab}	7.25±0.36^{a}
T3	6.5±0.325^{a}	8±0.4^{a}	7.37±0.36^{a}	7.37±0.36^{a}	8±0.4^{a}

T_0=(Control dairy yogurt sample)
T_1=(Soy yogurt sample)
T_2=(Oat yogurt sample)
T_3=(Coconut yogurt sample)

Mean squares for taste of dairy, soy, oat and coconut yogurt samples showed that there is significant difference between treatments of yogurt samples. The mean sensory score regarding taste of dairy, soy, oat and coconut yogurt samples were found to be 8±0.4, 6.87±0.34, 7.12±0.35 and 8±0.4 respectively according to 9-point hedonic scale (Figure 3).

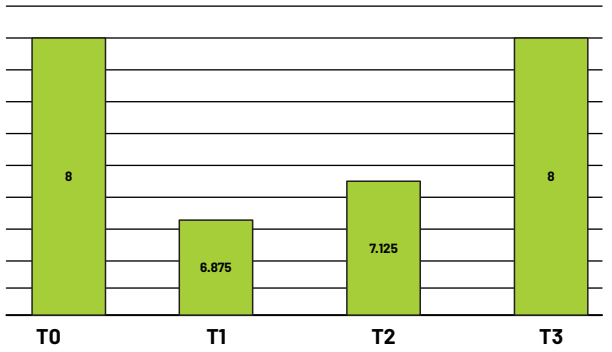

Figure 3: Graph representing taste of soy, oat and coconut yogurts

Mean squares of texture of dairy, soy, oat and coconut yogurt samples showed non-significant difference between treatments of yogurt samples. The mean sensory score regarding texture of dairy, soy, oat and coconut yogurt samples were found to be 7.5±0.37, 6.87±0.34, 6.5±0.32 and 7.37±0.36 respectively according to 9-point hedonic scale (Figure 4).

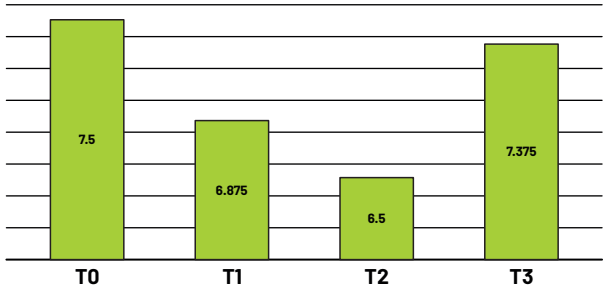

Figure 4: Graph representing texture of soy, oat and coconut yogurts

ANOVA results of aroma of dairy, soy, oat and coconut yogurt samples showed that there was significant ($p<0.05$) difference among treatments of different yogurts. Regarding aroma of samples of yogurt, the analysis shows the sensory score for soy, oat and coconut yogurts were found to be 5.87±0.29, 6.37±0.31 and 7.37±0.36 respectively according to 9-point hedonic scale while keeping dairy yogurt as control treatment which gained 6.62±0.33 mean score. It shows that coconut yogurts gained more score regarding its good color while soy and oat yogurts gained less scores than coconut yogurts (Figure 5).

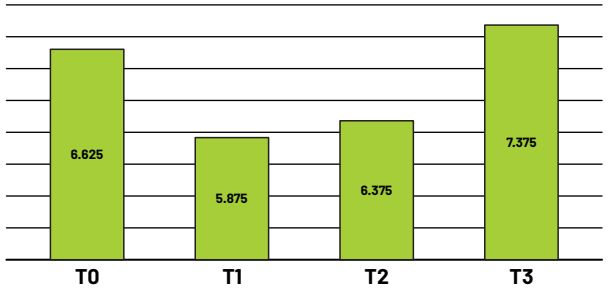

Figure 5: Graph representing aroma of soy, oat and coconut yogurts

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Eight participants were asked to taste and answer questions on each of the three yoghurt samples. The hedonic ratings of qualities such as color/appearance, taste, aroma/odour, texture and overall acceptability of yoghurts were included in the questions. The samples were completely random. ANOVA results of color of dairy, soy, oat and coconut yogurts showed that there was non-significant ($p>0.05$) difference among the treatments of these yogurt samples. Regarding color/appearance of yogurt samples, the mean values for dairy, soy, oat and coconut yogurts were found as 6.75±0.33, 6.87±0.34, 6.87±0.34 and 6.5±0.325 respectively according to 9-point hedonic scale (Table 2).

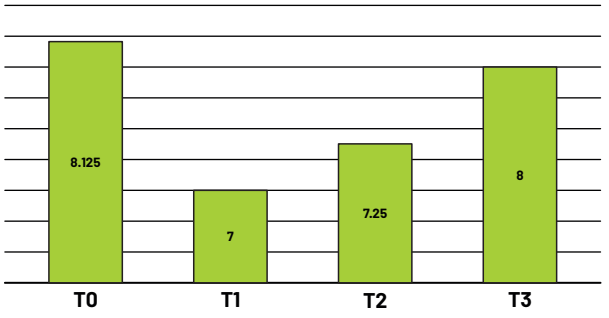

Figure 6: Graph representing overall acceptability of soy, oat and coconut yogurts

DISCUSSION

Plant-based milk substitutes are becoming increasingly popular. In general, they can serve as low-cost alternatives for cow's milk for individuals who cannot afford it due to its high price and restricted availability, or for those who are allergic to cow's milk. The current study was aimed to develop healthy plant-based yoghurts with high overall acceptability as an alternative to bovine milk that is both tasty and nutritionally appropriate to suit current consumer demands. For this purpose, soy, oat and coconut yogurts were prepared first and then analyzed for crude fat, crude fiber, crude protein, carbohydrates, ash, moisture contents, titratable acidity, total soluble solids, pH, syneresis and water holding capacity analysis. After proximate and physico-chemical analysis, yogurts were analyzed for sensory evaluation to check its quality and acceptability for color, aroma, taste, texture and overall acceptability. After that, all obtained data were subjected to statistical analysis. The results of protein content of different yogurts found in the current study are similar to the results of Craig and Brothers, who reported 6g protein contents in soy yogurt, 3g protein contents in oat yogurt and 0-1.5g protein contents in coconut yogurts. The results of fat contents of different yogurts found in the current study are also similar to the results of Craig and Brothers, who reported 2.5-3.5g fat contents in soy yogurt, 3-4.8g fat contents in oat yogurt and 5.5-12.5g fat contents in coconut yogurts. The results of fiber contents of different yogurts found in the current study are similar to the results of above study, who reported 1-2g fiber contents in soy yogurt, 1-2g fiber contents in oat yogurt and 0.6-2g fiber contents in coconut yogurts [23]. The results of moisture contents of different yogurts found in the current study are similar to the results of Osundahunsi *et al.*, who reported 87.8±0.01 moisture contents in soy yogurt, while moisture contents reported by Malki *et al.*, in oat yogurt was 65.79±1.0 and moisture contents in coconut yogurt was 83.52±0.00 as reported by Ezeonu *et al.*, [3, 24, 25]. The results of ash contents of different yogurts found in the current study are similar to the results of Osundahunsi *et al.*, who reported 0.52±0.23 ash contents in soy yogurt, while ash contents reported by Malki *et al.*, in oat yogurt was 0.37±0.3 and ash contents in coconut yogurt was 0.36±0.01 as reported by Ezeonu *et al.*, [3, 24, 25]. The results of carbohydrates content of different yogurts found in the current study are similar to the results of another study, who reported 18.5-23.5g carbohydrates contents in soy yogurt, 19-20g carbohydrates contents in oat yogurt and 10-22g carbohydrates contents in coconut yogurt [23]. The results of total soluble solids of different yogurts found in the current study are similar to the results of Osundahunsi *et al.*, who reported total soluble solids in soy yogurt are 14.5±0.21 while total soluble solids reported by Malki *et al.*, in oat yogurt was 36.38±0.3 and total soluble solids in coconut yogurt was 10.47**±**1.93 as reported by Nidife *et al.*, [3, 16, 24]. The results of pH of different yogurts found in the current study are similar to the results of Grasso *et al.*, who reported 4.38-4.56 pH of soy yogurt and 4 pH of coconut yogurts while pH of oat yogurt was 4.5 that was reported by Rani *et al*. The results of titratable acidity of different yogurts found in the current study are similar to the results of Grasso *et al.*, who reported 0.78% of titratable acidity of soy yogurt and 0.49% of titratable acidity of coconut yogurt while titratable acidity of oat yogurt was 0.009% as reported by Rani *et al*. The results of appearance of different yogurts found in the current study are also similar to the results of Grasso *et al.*, who reported 6.82±0.01 appearance value for soy yogurt and 6.93±0.30 appearance value for coconut yogurt while appearance value for oat yogurt was 6±0.00 that was reported by Rani *et al.* The results of taste of different yogurts found in the current study are similar to the results of Grasso *et al.*, who reported 5.75±0.21 taste value for soy yogurt and 4.79±0.16 taste value for coconut yogurt while taste value for oat yogurt was 7 that was reported by Rani *et al.* The results of texture of different yogurts found in the current study are similar to the results of Grasso *et al.*, who reported 6.49±0.31 texture value for soy yogurt and 6.37±0.23 texture value for coconut yogurt while texture value for oat yogurt

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was 6±0.00 that was reported by Rani *et al.* The results of aroma/odour of different yogurts found in the current study are similar to the results of Grasso *et al.*, who reported 6.29±0.05 aroma value for soy yogurt and 6.43±0.35 aroma value for coconut yogurt while aroma value for oat yogurt was 6±0.00 that was reported by Rani *et al*. The results of overall acceptability of different yogurts found in the current study are similar to the results of Grasso *et al.*, who reported 5.95±0.21 overall acceptability value for soy yogurt and 5.19±0.27 overall acceptability value for coconut yogurts while overall acceptability value for oat yogurt was 6.2±0.00 that was reported by Rani *et al*, [26, 27].

CONCLUSIONS

The study's findings demonstrated that it is possible to make plant-based yoghurt to meet the organoleptic needs of consumers, particularly those who are lactose intolerant or follow a vegan diet. Development of vegan yogurts will be important to fulfill functional nutritional values for children, adults as well as old ages as there is a gap in market of products made from non-dairy sources. Soy bean and oats are good and inexpensive source of protein especially for many vegetarians or vegan and for those who cannot buy meat and milk. Fat and cholesterol contents are present in low amount in vegan milk than animal milk. Non-dairy yogurt contains unsaturated fatty acid that help to reduce incidences of cardiovascular diseases. In case of lactose intolerance, consumption of vegan milk is beneficial. Non-dairy yogurts have high nutrients and minerals level and it will work as a synbiotic food which is important for human gut, intestine and increase antibodies in human body so it boosts immunity.

Authors Contribution

Conceptualization: HN, NR
Methodology: SM, NR
Formal Analysis: AN, SM
Writing-review and editing: UF, HN

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

Conflicts of Interest

The authors declare no conflict of interest.

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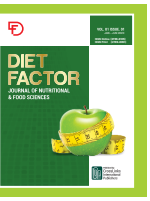

Original Article

Insomnia related to Stress and Anxiety in Adolescence

Mashal Khan[1], Maria Aslam*[1], Alveena Naqvi[1], Hooria Baloch[1], Aiman Rafique[1], Sabahat Bukhari[1]

[1] University Institute of Diet and Nutritional Sciences, Faculty of Allied Health Sciences, The University of Lahore, Lahore, Pakistan

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***Corresponding Author:**
Maria Aslam
University Institute of Diet and Nutritional Sciences, Faculty of Allied Health Sciences, The University of Lahore, Lahore, Pakistan
mnarz.aslam@gmail.com

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ABSTRACT

Insomnia, anxiety, and sadness are all linked in adolescence, according to the research. Anxiety and depression disorders are common and are linked with undesirable consequences. **Objective:** To better understand the interconnection between sleeplessness, anxiety disorders, and major depression in a community-based sample of adolescents. **Methods:** A cross sectional study was conducted at The University of Lahore, Lahore, during 4 months. A pre-tested questionnaire was used to collect data from 100 students through convenient sapling technique. SPSS version 21.0 was used to store and analyze the data. **Results:** The results revealed that insomnia is not common in mostly students but they have sleep issue due to exam disturbance and their diet. Late night phone usage is also a major cause in this study. Many students are worried about their current life situation and mostly have snore and choking issues due to worries. In general, the link between sleeplessness and depression was stronger and more constant in patients with serious depression than in those with depression symptoms. **Conclusion:** Adolescent insomnia has a significant impact on future health and functioning, and it is thought to cause and perpetuate a variety of emotional and behavioral issues, notably anxiety and sadness.

INTRODUCTION

Insomnia is the most common sleep disorder in the elderly clinical population and adolescents. The main complaint is difficulty falling maintaining sleep or asleep or being unable to return to sleep, resulting in obvious daytime symptoms, containing difficulty focusing and temper disorders [1]. Anxiety and depression disorders are common and are linked with undesirable consequences, such as increased hospitalization, decreased adherence to treatment, poor function, and increased mortality. However, despite the harmful effects of these disorders, depression, and anxiety in patients with heart failure have not been fully diagnosed and treated [2]. Some mental illnesses, such as anxiety and depression, have been shown to be closely related to insomnia. More severe symptoms of anxiety, depression, and stress before treatment have also reported increased daytime fatigue, the severity of insomnia, and sleepiness [3]. Although the prevalence of insomnia in adolescents is increasing. 30% of adults from different countries have symptoms of insomnia. However, in Pakistan, Nepal, India, and Bangladesh, the prevalence rates are 59.04%, 35.4%, 70%, and 69.5%, respectively [4]. Sleeping pills are widely used to treat sleep disorders and insomnia. Users with short sleep time are three times more likely to have metabolic equivalent than non-users with short sleep time [5]. Insomnia is a composite interaction of mental and cognitive awakening and changes circadian tempo and self-balance apparatus. The decrease in sleep-wake switch function may also cause insomnia. During sleep, there is a slow transition from non-rapid eye movement (non-REM) to rapid eye movement (REM) sleep. AASM

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divides sleep into 5 progressive stages, such as W (awake), N1 (relaxed awake), N2 (light sleep), N3 (deep slow-waves wave sleep), and R (rapid eye movement sleep or dreaming). Stages N1-N3 are non-rapid eye movement sleep stages. In this stage, cerebral cortex activity is low, while in the REM sleep stage, brain activity is very active [6]. Sleeping and eating behavior are important lifestyle factors to safeguard the health of adolescents [7]. The eating and sleeping habits of adolescents are important factors affecting the health of adolescents. Nutritional intake levels are an important part of adolescents' daily lifestyle, and studying its comprehensive role in specific adolescent groups can provide information for the improvement of prospective widespread interventions to prevent mental illness [8]. Proper family meals can serve as a role model for healthy ingestion behaviors [9]. Diet quality may also be a predictor of children's growth and development [10]. There is increasing evidence that modifiable lifestyle factors, such as proper nutritional status, are particularly helpful in preventing mental illness [11]. In terms of nutrition, studies have found that adolescents' eating behavior is related to sleep time and sleep quality [12]. In addition, a large-scale survey (n = 5,003 Chinese adolescents) confirmed that healthy eating patterns are negatively correlated with the prevalence of depression and anxiety symptoms [13]. Therefore, adolescents with unhealthy eating behaviors may have an increased risk of metabolic syndrome, obesity, and poor mental health [14]. Therefore, in developed countries, the eating behavior of adolescents is considered an important social issue [15]. It is worth noting that most previous studies on this topic behavior on the impact of lifestyle behavior (e.g., nutritional intake) on sleep quality, depression, and/or anxiety [8]. Therefore, to better understand this topic, the first purpose of this research is to separately investigate the relationship between lifestyle behavior (nutrition) and insomnia, depression, or anxiety. Disorders of eating behavior during adolescence lead to nutritional deficiencies and developmental delays and are even related to poor academic performance [16]. National Health and Nutrition Survey reported, from 1999 to 2000, 20.5% of 36.1% of young people aged 14-18 and children aged 9-13 did not eat breakfast [17]. Adolescents' eating behaviors tend to persist throughout their lives [18].

METHODS

A cross sectional study was conducted at The University of Lahore, Lahore, during 4 months, from September to December 2022. Ethical approval was taken from IRB of The University of Lahore, Lahore, Pakistan. A pre-tested questionnaire was used to collect data from 100 students through convenient sapling technique. Prior written inform consent were taken from all the participants. SPSS version 21.0 was used to store and analyze the data.

RESULTS

Data of 100 students were gathered for the current investigation. The findings showed that 65% of the population were male students, and 35% were female students. 35 students were graduated and 65 were undergraduate. Most students were between the weights of 40 and 50 as shown in Table 1.

Variables		Frequency (%)/Mean ± SD
Gender	Male	65 (65%)
	Female	35 (35%)
Education	Graduated	35 (35%)
	Undergraduates	65 (65%)
Age	18-28	22.95±1.919
Weight	39-95 Kg	59.67±13.80

Table 1: Demographics of the respondents

The Table 2 displays the percentage of students who report having trouble falling asleep, getting enough sleep, sleeping like a baby, having snoring problems, have taking sleeping pills, lost interest in their daily work, mind races thoughts, insomnia issues and breathing or choking issues during sleep.

Variables	Never	Sometimes	Yes
Do you think you have enough sleep?	42%	51%	7%
Do you have trouble falling asleep?	48%	51%	1%
Do you face difficulty with while staying awake during day?	36%	58%	6%
Do you have trouble in sleeping during night after awakening?	35%	59%	6%
Do you have sleep problems as a child?	58%	7%	35%
Do you snore?	36%	58%	6%
Do your mind races thoughts while going to sleep?	29%	60%	11%
Does your insomnia occur three times a week?	58%	32%	10%
Do you sleep better in unfamiliar bedrooms like hotel?	7%	58%	35%
Have anybody said that you stop breathing or choke while sleeping?	37%	67%	6%
Have you lost interest in your daily activities or work?	26%	51%	23%
Do you have medical condition that disturbs your sleep?	45%	49%	6%
Do you take any sleeping pill?	47%	52%	1%
Are you satisfied with your current sleep patterns?	9%	63%	28%
How noticeable are your sleeping problems in terms of impairing quality of life?	12%	60%	28%
To what extent sleep problems interfere with your daily issues?	40%	-	60%
How worried are you about current problems?	31%	57%	12%
Do you feel sleep disturbance during exam?	56%	-	44%
Do you eat healthy?	30%	50%	20%
Do you feel lack of sleep because of your diet?	19%	55%	26%

Table 2: Response of the participants to questions asked

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Table 3 shows the response of participants to the questions about cravings and diet. Most of the students liked homemade foods. The major percentage of girls liked desserts.

Questions	Desserts	Homemade	Fast food
What your cravings mostly consists?	12%	49%	39%
What you prefer in your diet?	22%	54%	24%

Table 3: Response of participants to the questions related to diet

DISCUSSION

Insomnia is the most usual sleep disorder in the older clinical population and adolescents. The main complaint is difficulty falling sleep, maintaining sleep or being unable to return to sleep, resulting in obvious daytime symptoms, containing difficulty focusing and temper disorders. We also noticed that the prevalence of insomnia in adolescents is increasing. 30% of adults from different countries have symptoms of insomnia [19]. However, in Pakistan, Nepal, India, and Bangladesh, the prevalence rates are 59.04%, 35.4%, 70%, and 69.5%, respectively. According to the report of National Health and Nutrition Survey, from 1999 to 2000, 20.5% of 36.1% of young people aged 14-18 and children aged 9-13 did not eat breakfast. Adolescents' eating etiquettes tend to continue all over their lives. Alvaro *et al.*, conducted research in 2017 that found a link between sleeplessness and depressed symptoms [20]. The research was carried out in eight different schools. It was completed by 318 and 255 students, respectively. According to the findings, sleeplessness symptoms are linked to depression symptoms. In another study conducted by Blake *et al.*, there is a high prevalence of negative impacts among adolescents. Insomnia was predicted by an increase in emotional reactivity and a decrease in emotion management capacity, according to the findings [21]. In our study data was collected from 100 students. The results revealed that the majority of students were male i.e. 65 and 35 were females of the total population. 35 students were from graduated and 65 were undergraduate. Mostly students were from weight range of 40-50. As per many studies, mostly students like fast foods that disturb their sleep cycle. Our results showed a link between sleeplessness and serious depression in adolescents, showing that the two are linked. We also concluded that the more research into the paths of sleeplessness and major depression in childhood and adolescence is needed [22].

CONCLUSIONS

Our results are the first to show a link between sleeplessness and serious depression in adolescents, showing that the two are linked. More research into the paths of sleeplessness and major depression in childhood and adolescence is needed.

Authors Contribution

Conceptualization: MK, MS
Methodology: AN, SB
Formal Analysis: HB, AR
Writing-review and editing: MK, AN, HB, MS

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

Conflicts of Interest

The authors declare no conflict of interest.

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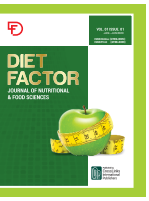

Original Article

Knowledge, Attitude and Practices Regarding Dietary Salt Intake Among University Students

Ayesha Zafar[1], Bahisht Rizwan[1*], Hafiza Madiha Jaffar[1], Asad Ullah[1], Hamza Akhtar[1], Saiwa Ghulam Ghous[1], Syeda Samia Ali[1] and Zain Ali[1]

[1]University Institute of Diet and Nutritional Science, The University of Lahore, Lahore, Pakistan

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***Corresponding Author:**
Bahisht Rizwan
University Institute of Diet and Nutritional Science, The University of Lahore, Lahore, Pakistan
bahisht.rizwan@dnsc.uol.edu.pk

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ABSTRACT

High salt intake is associated with an increased risk of hypertension, which is a major risk factor for cardiovascular diseases. **Objective:** To investigate the knowledge, attitudes and practices (KAP) for dietary salt intake among university students. **Methods:** A cross-sectional study was carried out from The University of Lahore. Total 100 students were selected through non-probability convenient sampling technique. SPSS Version 25.0 was used for data analysis. Inclusion Criteria included participants between the ages of 18 to 30 and also healthy individuals. **Results:** 40 participants were underweighted, 44 normal-weight, and 16 were over-weight of BMI. 80 respondents were under-graduated and 20 respondents were post graduated, 90 unmarried and 10 married respondents, 83 belonged to middle class, 23 participants felt warning signs before the start of blood pressure. 60 was affecting the quality of life, 28 were affecting on daily activities. In knowledge based, 95% agreed high salt cause health problems, 23 consumed high salt. In Attitude based, 50 participants were right amount of salt, 35 participants consumed salty snacks and 32 consumed pickle foods. In practices based, 31 participants rarely tried to reduce spices, 41 participants were trying to buy low salt foods, 23 consumed or added soy sauce to food at the table, and 20 consumed pasta and noodles less than 1-2 times weekly, 59 consumed fast foods. **Conclusions:** The study showed that all of the participants consume sauces, pickles and salty snacks which are major dietary sources of salt. Public education initiatives should promote less use of "hidden salt".

INTRODUCTION

Common salt, also known as sodium chloride, is frequently used in packaged foods. Salt has a big impact on the taste, quality, and overall structure of solid meals. The most popular way to consume salt, which is an essential nutrient that contains 40% sodium and is recognised as sodium chloride. The meat, fish, and eggs group, together with the snacks items and pizza group, are the leading source of salt, along with additional salt, cereals, cereal-based products, and starchy tubers [1]. The majority about 75% of the salt consumed in affluent nations comes from industrially processed foods [2]. Salt is used to preserve and treat food, such as pickles, processed meats like sausages, and salted fish, to lengthen their shelf lives [3]. Salt is the main dietary sodium source (95%), with the exception of nations that employ large quantities of monosodium glutamate as a taste enhancement [4]. The majority of salt consumption comes from baked goods because they have a comparatively higher salt content than other foods. Also rich in salt include dairy products, soups, snacks, spices, and sauces [5]. The World Health Organization (WHO) found that humans worldwide intake more salt than is physiologically necessary [6]. One Of the

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World Health Organization's voluntarily globally health strategies is a 30% decrease in population level salt by 2020. If salt consumption were reduced to the daily recommends limit of < 5g, it has been calculated that 1.7 million lives may be saved globally each year [7]. The World Health Organization estimates that 62% of all strokes and 49% of cardiovascular events are caused by excessive blood pressure. In addition, a high salt intake in the diet is a well-known risk factor for high blood pressure. It has been hypothesized that limiting salt will lower blood pressure in all age groups and in both men and women. There is evidence to support the idea that small dietary salts reductions could significantly lower cardiovascular events and medical expenses as well as prevent serious vascular problems [8]. According to estimates, 77% of fatalities in the United Arab Emirates (UAE) are attributable to NCDs, and 40% of these deaths are attributable to CVDs. One of the most economical ways to improve population health has been shown to be salt restriction. Clinical studies have demonstrated that limiting dietary salt intake can minimize the incidence of CVDs by lowering blood pressure [9]. According to information from the National Health and Nutrition Examination Survey 2015–2016, hypertension affects about one-third of Americans. This is concerning because hypertension is a risk factor for both stroke and cardiovascular disease, which are the first and fifth leading causes of mortality in the US, respectively. Many factors, including biological and behavioural ones, affect a person's risk for hypertension. The three key lifestyle elements that can be adjusted are smoking, eating, and exercise [10]. Restricting salt may lower blood pressure in all age groups and in both men and women. High-blood pressure is unquestionably one of the key risk factors for the emergence of CVDs [11]. Due to the high salt content of the sauce and soup, this had an indirect impact on blood pressure. Individuals' knowledge, attitude, and practises (KAP) regarding salt intake had an impact on it. Due to a lack of knowledge regarding the link between high salt consumption and hypertension, people still consume excessive amounts of salt [12]. According to previous researchers, there was a significant increase in both knowledge and attitude towards salt immediately after the intervention, but this improvement was not fully maintained four weeks later. After 4 weeks, 5 of the 13 practices evaluated showed improvement: trying to buy low-salt items rose from 10% to 19% (P = 0.022); adding salt to food only rarely rose from 5% to 16% (P = 0.019); adding salt to food only Occasionally rose from 29% to 42% (P = 0.011); reducing salt intake rose from 26% to 41% (P= 0.014); and using spices to reduce salt rose from 31% to 45% (P = 0.044). The educational intervention increased participants' salt-related knowledge, attitudes, and practices; however, these gains were not maintained over time. Educational interventions should be considered on a periodic basis to update knowledge and reinforce behaviors [13]. Furthermore, different studies had been conducted in 2019 Dalal Alkazemi et al., investigated the research on six hundred and fifteen KU undergraduates were recruited between November 2013 and March 2014 for this cross-sectional study. The research involved the use of a self-report questionnaire, a body mass index calculation, and a Healthy Eating Score (HES) calculation. The HES was used to assess how people think about and approach food. The results showed that the prevalence of overweight and obesity among males was higher than among women. Both sexes have unhealthy eating habits, as shown by their low mean HES scores. Gender variations in diet are evident, with males reporting consuming more than 6 ounces (168 g) of animal protein daily compared to women (p 0.001). Female students were more likely to report more than twice-daily use of sweets (p = 0.041) and frequent consumption of potato chips and other fatty, salty snacks p = 0.12 [14]. In 2021, Mansor et al., was to determine the prevalence of hypertension and the associations between participants' socioeconomic status, anthropometric blood pressure measurements, and Participants' Knowledge, Attitude, and Practise (KAP) regarding the relationship between salt intake and hypertension. When asked how essential it was to them to reduce their salt intake, only 62.2% answered it was very important, while 35.1% and 2.7% respectively indicated it was not at all important. 8% or more of those with hypertension believed their salt consumption was normal. The correlation between gender and diastolic blood pressure DBP (the lower number) was statistically Significant (p=0.003) [15]. People continue to consume excessive salt intake due to lack of awareness about the effect of excessive salt intake towards hypertension. They need to develop culture-specific awareness campaigns on salt intake, and their association with health. To make a good attitude and behaviour towards the salt intake nutrition education is necessity so that the chances of high blood pressure and chronic diseases like CVD can be reduced. There is need to arrange seminars and workshops to encourage people to modify their dietary practices and they should be guided regarding the sources of hidden salt.

METHODS

A cross-sectional study was carried out from The University of Lahore, total 100 students were selected through non-probability convenient sampling technique. The ethical approval was signed by the ethical committee of the University of Lahore. In this study, all data were collected randomly through a survey using a detailed self-constructed questionnaire after approval from experts.

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The consent was taken from the participants before data collection. Questionnaires were distributed among participants, and they were asked to fill them. Inclusion Criteria includes participants between the ages of 18 to 30 and also healthy individuals including in the study. In Exclusion criteria includes Participants below and above the selected age groups. The first section included demographic information of the participants, i.e., their gender, occupation, socioeconomic status, marital status, and family type. In the same section, there were also questions regarding participant's anthropometric measurements. It included height, weight, age, and BMI. The level of knowledge, attitudes and practices (KAP) towards salt intake among university students. Knowledge questions were added to assess participant's perception about high salt intake health problems, low salt intake reduce blood pressure, salt content in foods, nutrition fact table in the whole package of food, and consumption of foods high in salt. It covered almost ten questions. For Attitude questions were added to assess participant's perception about salt consume, importance of reducing consumption of processed foods, regular basis of salt intake, usual taste for foods, consuming pickle foods and consuming salty snacks. It covered almost ten questions. And some practices questions were added about added salt to food at the table, salt added in cooking or preparing foods, use spices to reduce salt, read nutrition labels on food packages, trying to buy low salt foods, Intention to reduce salt and salt intake per day. It covered almost twelve questions. A table (like a food frequency questionnaire) was added to assess which food groups are mostly preferred by participants usually. Processed foods were also added like ketchup, mayonnaise, sauces, chicken spread, chips, Nimko and also fast foods were added. The servings of the foods group per day, per week and monthly frequencies were added to the table. Data collected from this study were analyzed using SPSS version 25.0. The qualitative variables were expressed as mean ± S.D, results were expressed in mean S.D, frequency and percentages. Descriptive and inferential statistics was used to report the data. The association between the variables was found by using chi-square. Level of significance is set as p-value ≤ 0.05.

RESULTS

As shown in Table 1, participants' age ranged between 18-30 years. Majority of students were male (60%), and (40%) females, (90%) unmarried and (10%) married. Most of the students were under-graduated (80%) and (20%) post-graduated. Students belongs to (4%) lower class, (83%) middle class and (13%) upper class. More than 44% of the study were classified as normal weight, (40%) under-weight and (16%) over-weight.

Table 1: Socio Demographic Characteristics of the students

Demographic Profile	Frequency (%)
Gender	
Male	60(60)
Female	40(40)
Marital Status	
Unmarried	90(90)
Married	10(10)
Age	
18-25	83(83)
26-29	14(14)
Above 30	3(3)
Education Level	
Under Graduated	80(80)
Post Graduated	20(20)
BMI	
Under-Weight	40(40)
Normal Weight	44(44)
Over-Weight	16(16)
Socio-Economic Status	
Lower class	4(4)
Middle class	83(83)
Upper class	13(13)

Table 2, indicates that virtually all students (95%) had knowledge that high salt diet can cause a serious health problem while those who had no knowledge were (5%). Almost (93%) students had knowledge that low salt intake helps reduce blood pressure while those who had no knowledge were (7%). Almost half of the students agreed that low salt intake would make people limb weakened. (79%) had knowledge about eating a low salt diet help breathe easier while (21%) denied it. Most of the students had knowledge that nutrition fact table tells you the no. of mgs of salt in the whole package of food while (22%) had no knowledge. (23%) had knowledge that consumption of high salty foods almost every day, (34%) consumed 1-2 times per day, (21%) had consumed 3-4 times per day and (22%) had no knowledge about consumption of high salty foods.

Table 2: Knowledge Regarding Salt Intake

Knowledge Regarding Salt Intake	Frequency (%)
Do you agree high salt intake would cause health problems?	
Agree	95(95)
Disagree	5(5)
Do you agree less salt intake helps reduce blood pressure?	
Agree	93(93)
Disagree	7(7)
Do you agree low salt intake would make people limb weakened?	
Agree	55(55)
Disagree	45(45)
Agree	79(79)
Disagree	21(21)
Agree	78(78)
Disagree	22(22)

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Do you agree low salt intake would make people limb weakened?	
Almost every day	23(23)
1-2 times per week	34(34)
3-4 times per week	21(21)
Never	22(22)

The Table 3, shows that half of the respondents (50%) believed that they were consuming right amount of salt, and (15%) were unsure the amount of salt that they think they are consumed. Most of the respondents (49%) believed that lowering salt in the diet is very important. Almost (76%) agreed that reducing consumption of processed foods is important. (60%) of the respondents think that they either do anything on regular basis to control their salt intake. (35%) of the respondents were consumed salty snacks 1-2 times per week. (32%) respondents were consumed pickle foods 1-2 times per week.

Table 3: Attitude Regarding Salt Intake

Attitude Regarding Salt Intake	Frequency (%)
How much salt do you think you consume?	
Too Much	15(15)
Right Amount	50(50)
Too Little	20(20)
Don't Know	15(15)
How important to you is lowering the salt in your diet?	
Very Important	49(49)
Somehow important	39(39)
Not Really Important	12(12)
Reducing consumption of processed foods is important to you?	
Agree	76(76)
Disagree	24(25)
Do you do anything on regular basis to control your salt intake?	
Yes	60(60)
No	40(40)
How often have you consumed salty snacks in the past months?	
Almost every day	20(20)
1-2 days per week	35(35)
3-4 days per week	22(22)
Once per week or less	23(23)
How often have you consumed pickled foods in the past month?	
Almost every day	16(16)
1-2 days per week	32(32)
3-5 days per week	11(11)
Once per week or less	41(41)

In Table 4, majority of the respondents (34%) rarely add salt to food at the table. Most of the respondents add salt in cooking or preparing salt in their household. Most of them never tried to use spices to reduce salt. About (47%) never add salt to food at the table. On the other hand, majority of them (41%) sometimes tried to buy low salt foods.

Table 4: Practices Regarding Salt Intake

Practices Regarding Salt Intake	Frequency (%)
Do you add salt to food at the table?	
Never	24(24)
Rarely	34(34)
Sometimes	28(28)
Often	7(7)
Always	7(7)
How often is salt added in cooking or preparing foods in your household?	
Never	12(12)
Rarely	20(20)
Sometimes	23(23)
Often	21(21)
Always	24(24)
Did you try to use spices to reduce salt?	
Never	31(31)
Rarely	28(28)
Sometimes	25(25)
Often	13(13)
Always	3(3)
Do you add soy sauce to food at the table?	
Never	47(47)
Rarely	17(17)
Sometimes	23(23)
Often	8(8)
Always	5(5)
Did you try to buy low salt foods?	
Never	29(29)
Rarely	17(17)
Sometimes	41(41)
Often	10(10)
Always	3(3)

Figure 1, showed that, 54 respondents consumed processed foods less than 1-2 times monthly whereas 23 respondents consumed 4-6 times monthly.

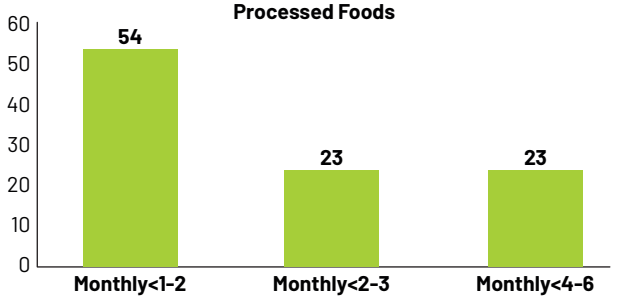

Figure 1: Daily Consumption of Processed Foods among participants

Figure 2 showed that, 21 respondents consumed different types of sauces less than 1-2 times monthly, 16 respondents consumed 2-3 times weekly and 13 respondents consumed 1-2 times daily.

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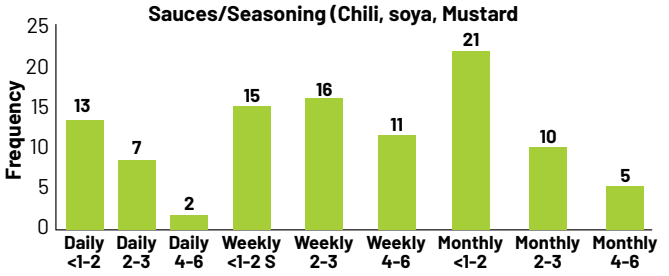

Figure 2: Daily Consumption of Sauces/Seasoning(Chili, Soya and Mustard)

Figure 3, showed that, 58 respondents consumed monthly less than 1-2 times, 23 respondents consume 2-3 times monthly and 19 respondents consume 4-6 times monthly.

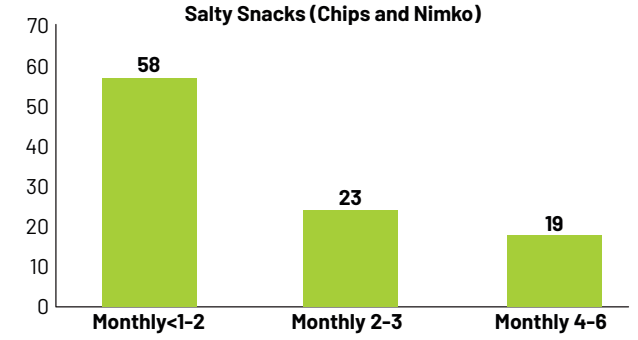

Figure 3: Daily Consumption of Salty Snacks (Chips and Nimko) among participants

DISCUSSION

The knowledge, attitude and practices of the students studying at University of Lahore to salt were investigated in this research. Moreover, the participants were selected through a non-probability convenient sampling technique. The current study demonstrates a cross-sectional survey which was conducted with 100 individuals whose ages ranged from 18 to above 30. Bhattacharya *et al.*, in 2018, undertook a cross-sectional study with 300 participants, ages 18 to 69 [16]. Another similar study was conducted by Kenao *et al.*, in 2023, a cross-sectional survey with 400 participants between the ages of 25 and 44 that was conducted in 2022 knowledge, attitudes, and practises (KAP) about dietary intakes of salt and potassium [17]. According to a recent study, a cross-sectional survey included 60 male participants and 40 female participants. In a cross-sectional study conducted by Ahmad in 2020, 358 female (89.1%) and 44 male (10.1%) participated [6]. According to this study, 90 respondents were unmarried and 10 respondents were married. In a cross-sectional study conducted by Santos *et al.*, 2022, (17.5%) were unmarried and (68%) were married [1]. According to the current study, 40 of these individuals were underweight, 44 were of normal weight, and 16 were overweight. According to Herrera-F *at el.*, in 2021, 44% of them were overweight and 19% of them were obese [18]. Other than this, we focused on the salt intake related knowledge, attitudes, and practices among graduated and under-graduated students. In a research conducted by Cheikh Ismail *at el.*, in (2019) ninety university non-medical students were enlisted to look at KAP in relation to dietary salt consumption. According to our study, under-graduated students of University of Lahore were (80%) and post-graduate students were (20%)[13]. Another similar study was conducted by Ahmad in 2020, a total of 402 undergraduate students participated in this cross-sectional survey. Another similar study was conducted by Ahmed *et al.*, in 2016, the majority of participants were women, just 19% and 21% of medical and dentistry students' respectively [19]. The current study shows that 44 participants had family history of hypertension and 56 participants had no history of hypertension. According to Hu *et al.*, in 2017, no significant difference was seen with respect to family history of hypertension [20]. The current study showed that 95 participants agree that high salt intake cause health problems. According to Sarker *et al.*, in 2018, more than three out of every five respondents (61.9%) said consuming too much salt could result in major health issues [21]. Another similar study conducted by Herrera-F *et al.*, in 2021, only 38.4% of people systematically limit their salt intake, despite the fact that 99% are aware that a diet heavy in salt contributes to health issues [18]. The current study showed that 93 participants had awareness of low salt help reduce blood pressure. According to the Zhang *et al.*, 2013, (25%) know that less salt help reduce blood pressure [22].The current study showed that 55 respondents agreed low salt intake limb weakened. According to the Zhang *et al.*, 2013, (18%) know that less salt intake made people limb weakened [22]. The current study shows that 79 respondents were aware about low salt diet helps breathe easier. According to the Zhang *et al.*, 2013, more than 65% know that less salt intake helps breathe easier [22]. According to the current study, 34 respondents consumed high salt diet in 1-2 times per week. According to the Zhang *et al.*, 2013, (22%) was consuming high salty diet (< 3times per week) [22]. According to the current study, 60% of the respondents think that they either do anything on regular basis to control their salt intake. According to Grimes *et al.*, 2020, more than (78%) thought on regular basis to control dietary salt consumption [23]. According to the current study, 95 participants concur that consuming too much salt has negative health effects. According to Sarker *et al.*, in 2018, more than three out of every five respondents (61.9%) stated that ingesting too much salt could lead to serious health problems [21]. According to another study conducted by Dahalim *et al.*, in 2020, all people (95.4%) were aware that excessive salt consumption can lead to hypertension[3]. According to the

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current study, 49 individuals thought reducing salt in our diets was very important, while 39 thought it was important in some other way. According to Bhattacharya *et al.*, in 2018, the majority of respondents (64%) said it was not vital to minimize their salt intake [16]. Another study was conducted by Mansor *et al.*, in 2021, only 62.2% of respondents said consuming less salt in their everyday meals was extremely important, while 35.1% and 2.7% said it was only somewhat or not at all important [15]. Reducing processed food consumption, as shown by the current study's 70 participants, is crucial. Bhattacharya *et al.*, found that in 2018, the majority of people there (96%) ate processed food and that 99% didn't care how salty it was [16]. According to the current survey, 50 respondents said they consumed the appropriate amount of salt. According to Bhattacharya *et al.*, in 2018, the majority of them (75%) thought they were taking in the recommended amount of salt. They had no idea what the daily recommended allowance was. 43 percent of people were not aware of the harmful effects of dietary salt [16]. Another similar study conducted by Sarker *et al.*, in 2018, more than four out of five respondents (82.8%) thought they used to consume the proper quantity of salt, and one-fourth (26.0%) thought it was crucial to reduce salt intake [21]. Another similar study conducted by Herrera-F *et al.*, in 2021, 97 percent of individuals believed they consumed too much salt [18]. The study's findings of the present investigation demonstrated that 28 individuals occasionally added salt to their meals. In 2019, Cheikh Ismail *et al.*, studies that knowledge and attitudes about salt have greatly improved, and more people now occasionally season their food at the dinner table up from (29 to 42%) [13]. The results of the current investigation revealed that 20 participants rarely used salt when preparing meals at home. A recent investigation found that 28 respondents tried using spices instead of salt. Cheikh Ismail *et al.*, attempted to use spices in 2019 to reduce salt consumption, which reached from (31 to) 45% [13]. A recent study found that 24 respondents occasionally looked at labels to see how much salt they contained. Wicaksana *et al.*, (2017), who conducted the study, found that the total salt amount listed on the label was favoured (42% vs. 31.1%) and that only a small percentage of participants frequently examined the packaging indication (7.2% vs. 11.3%) [24]. According to a recent survey, 41 respondents were attempting to purchase low-salt items. In 2019, Cheikh Ismail *et al.*, study, after four weeks, five of the 13 practises that were reviewed improved: attempting to purchase low-salt foods increased from 10% to 19% [13]. Another similar study conducted by Hadgu *et al.*, in 2016, Most of the participants (83%) know why they should buy iodized salts because it is good for well-being while 2% of respondents choose not to purchase it because they are unsure of its necessity [25]. This research found that 38 individuals intended to cut back on their intake of salt. Cheikh Ismail *et al.*, in 2019 attempted to reduce salt consumption by (26 to 41%) [13]. According to the current study, 35 participants eat salty snacks 1-2 days per week, and 2-3 servings of chips. According to Alkazemi *et al.*, (2019), female students were more likely to say they routinely ate chips and other fatty, salty snacks ($p = 0.12$) [14]. According to the current study, 54 respondents consumed processed foods monthly 1-2. According to Leyvraz *et al.*, (2018), several processed food items with known or presumably high salt content (such as pizzas and savory snacks) were consumed rather infrequently <3 times/week [26].

CONCLUSIONS

Salt consumption is increasing among the university students which is alarming for increasing the risk of hypertension and cardiovascular diseases among them. It was assessed that during the study majority of the students didn't know about the harmful effects of excess sodium intake. Their knowledge about salt was very doubtful that they even don't know about its consequences, and most of the respondents have awareness about nutrition fact table. Their attitude regarding salt is that students were consuming too much salt in the form of fast foods, pickles food and processed foods due to social culture or in peer influence. Their poor practices due to consumption of salty snacks, fast foods (burger, pizza, rolls etc.) was very high and that results in poor dietary practices regarding salt intake. They have inadequate healthy dietary practices. They are overly consuming salt and the form of different sauces, fast foods, and processed foods. Few of them were trying to use spices to reduce salt and some of them are trying to buy low salt or alternatives foods.

Authors Contribution

Conceptualization: AZ, BR
Methodology: HMJ, AU, AZ
Formal analysis: HA, SGG
Writing-review and editing: SSA, ZA, AZ

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

Conflicts of Interest

The authors declare no conflict of interest.

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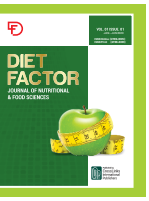

Original Article

Evaluating the Gelling Properties of Red Kidney Beans Protein Isolates with Different Gums

Nighat Raza[1*], Adeel Hakim[1], Muhammad Shahbaz[1] and Mujahid Farid[2]

[1]Department of Food Science and Technology, MNS-University of Agriculture, Multan, Pakistan
[2]Department of Environmental Science, University of Gujrat, Gujrat, Pakistan

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***Corresponding Author:**
Nighat Raz
Department of Food Science and Technology, MNS-University of Agriculture, Multan, Pakistan
nighat.raza@mnsuam.edu.pk

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ABSTRACT

Due to the higher consumption, increased demand of animal based hydrocolloids and problems associated with animal based hydrocolloids are religious beliefs and mad cow disease, researchers are looking for alternative sources of hydrocolloids like marine and plant based hydrocolloids. **Objective:** To evaluate the gelling properties of red kidney beans protein isolates with different gums. **Methods:** The gelling powder developed with red kidney bean protein (KPI)-carrageenan (CG) and protein-xanthan (XG) gum with six different concentrations. **Results:** Added protein increased the plasticity of the gel and showed a higher blooms strength and hardness in all treatments except T_1. KPI-CG gel had bloom strength values 198.67 ±1.53g, 249.67 ±1.53g and 282.33 ±1.56g and respectively KPI-XG gel bloom strength values were 170.33 ±1.6g, 232.67 ±2.08g and 256.67 ±2.52g; while hardness of KPI-CG gel shows 23.5 ±0.5N, 37 ±1N, 42.33 ±1.54N and 22 ±1N, 34 ±1N, 40 ±1N of KPI-XG gel respectively. The lower G'' values than G' indicate that there is gelling ability in all the concentrations. Added carrageenan-protein gelling agent with maximum gum concentration showed the highest gel strength of 1629.99±16.12 pa which is double the amount of KPI-XG gel elasticity 878.043±8.08 pa. **Conclusions:** These results indicate that the KPI-CG mixed gel has a better gelling strength. The outcomes of this work will be used to provide the groundwork for developing a novel designed plant protein-based gel system and the use of gel in yoghurt, which might increase functionality over protein or gums alone and replace the animal-based gelling component.

INTRODUCTION

Proteins derived from animal sources like gelatin, ovalbumin, casein and whey, now dominate the protein ingredient sector. However, the replacement with plant derived materials is increased in food industry as customers feel fear of spreading diseases from animal source (e.g. prion), dietary preferences and food choices based on moral, religious beliefs (Muslim and the Jewish communities represent nearly 23 percent of global population) and some traditional restrictions that prohibits the consumption of products derived from animals [1, 2]. Increasing trend of vegetarianism in developed nations and growing demand for food products without soy and wheat protein, red kidney beans (*Phaseolus vulgaris* L.) are attracting more attention for the development of nutritious foods [3]. Moreover, foods prepared with soy as ingredients attracted the consumers concern due various allergies and off-flavorings. Possible potential substitutes for protein can include oilseed crops and various legumes but they are poor in functional characteristic than the current available proteins. Researchers are trying to improve the functional properties of plant base proteins from many years through enzymes and chemicals means with little achievements [4]. Red beans are known with different names like common bean, "Lal lobia" and "rajma" Haricot bean in English, Haricot commun in French. The red kidney bean, scientifically known as *Phaseolus vulgaris* L., is an excellent

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source of a variety of nutrients, including proteins, carbs, minerals, and vitamins [5]. These are well-known for having exceptionally high levels of dietary fiber, minerals, and protein respectively. They are also good sources of several vitamins (riboflavin, thiamine, folic acid, niacin and vitamin B6) and minerals Mg, K, Zn, Cu, Fe, P, and Ca [6]. Non-nutritional components, such as antioxidants and phenolics, are also abundant in kidney beans [7-9]. A protein extract of red kidney beans has been found to gel better than those from mung or other beans because of the unique vicilin feature [10]. This bean's principal storage protein, vicilin (also known as phaseolin), has a unique mechanical characteristic, e.g. minimum vulnerable to trypsin digestion, and also higher subunit uniformity, in contrast to other vicilin constituents [10]. An oligomeric protein with molecular weights ranging from 43 to 53 kDa, it contains 2 or 3 polypeptide subunits, based on the generic type [11]. In contrast to soy bean protein, the protein composition of *Phaseolus* family legumes are more homogeneous (having major vicilin content about 83 to 86 percent, compared to the collective legumin and vicilin), therefore these protein isolates can be easily processed to act as functional components. The gelling ability of the protein isolate from kidney bean was found to be the best of the three *Phaseolus* legume protein isolates evaluated [10]. Depending on the protein-polysaccharide ratio, several effects can be observed. There are two possibilities for how gum content affects the final product. As the amount of gum added increases, the thermodynamic incompatibility between proteins and gums tends to increase because the uncertainty of the structure occurs when the quantity of water is deficient, leading to struggle for hydration particles, which in turn leads to higher interaction among the biopolymers and the resulting repulsive interactions [12]. Due to the nature and density of their charge, if gums are compatible with the protein, compatibility tends to increase as the gum content increases. A depletion process occurs when these big hydrophilic macromolecules begin to combine, which causes gum to flocculate. This behavior, however, is only seen up to a particular concentration [13, 14]. As a result, it is advantageous to cross-link the biopolymers within the particles in order to enhance their stability. It's possible to crosslink biopolymers using either chemical or enzymatic methods, depending on the unique properties of the biopolymers involved [15, 16]. Therefore, this study was conducted to evaluate gelling properties of red kidney beans.

METHODS

Red kidney beans were purchased from the local grain market of Multan. The required chemicals to be used in this study was of Merck and Sigma-Aldrich were procured from a local scientific store of Multan. Analysis of red kidney beans samples for crude protein, moisture, crude fat, crude fiber and ash were done conferring to the protocols mentioned in AACC (2000). The moisture content of red kidney bean sample was analyzed using a hot air oven according to the standard procedure of AACC (2000). A clean petri-dish already dried at 98 °C for 60 minutes contained about 2 to 10 grams of sample. A hot air oven at 100°C was used to dry the sample for 2 to 3 hours. The % of moisture in the sample was determined by comparing the starting weight of the sample to the final weight after drying(final weight).

$$\text{Moisture \%} = \frac{\textit{Initial weight of sample-final weight of dried sample}}{\textit{inital weight of sample}} \text{ x100}$$

Crude protein value of the malted and un-malted flour was calculated according to (Method no. 46-30) prescribed in AACC (2000). In this method kjeldahl's apparatus was used to calculate the crude protein content. Two to three gram of sample was taken in the digestion tube that was placed in distillation unit for 3 to 4 hours with 1 digestion tablet and 30ml of sulphuric acid until the yellowish or transparent color will appear. After distillation, the sample was cool down and placed in the volumetric flask (500 ml) and markup to 250ml by using distilled water. Took 10ml solution from the sample which was diluted and place it in the digestion flask and pour 15 ml of 40% solution of sodium hydroxide in it. Ammonia started releasing from the sample and comes in the flask with 4% of boric acid solution while methyl red was used as an indicator. Moreover, the resultant solution was then titrated against the 0.1 N sulphuric acid. Protein calculation was done by using the following formula:

$$\text{Nitrogen \%} = \frac{\textit{Volume of 0.10N H2S04 used}\times 0.0014 \times \textit{volume of diluation(250)}}{\text{Weight of sample} \times 10} \text{ x100}$$

Crude protein value of the malted and un-malted flour was calculated according to (Method no. 46-30) prescribed in AACC (2000). In this method kjeldahl's apparatus was used to calculate the crude protein content. Two to three gram of sample was taken in the digestion tube that was placed in distillation unit for 3 to 4 hours with 1 digestion tablet and 30ml of sulphuric acid until the yellowish or transparent color will appear. After distillation, the sample was cool down and placed in the volumetric flask (500 ml) and markup to 250ml by using distilled water. Took 10ml solution from the sample which was diluted and place it in the digestion flask and pour 15 ml of 40% solution of sodium hydroxide in it. Ammonia started releasing from the sample and comes in the flask with 4% of boric acid solution while methyl red was used as an indicator. Moreover, the resultant solution was then titrated against the 0.1 N sulphuric acid. Protein calculation was done by using the following formula:

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Protein % = Nitrogen percentage × 6.25

Determination of fat content of the sample was done according to the method No (30-25) of AACC (2000). In this method solvent extraction method was applied to calculate the total amount of fat and n-hexane was used as a solvent extraction. Took 5g sample (Pre-dried) and warped in a filter paper to make thimble and the weight the thimble. The thimble was then placed in the soxhlet apparatus. 250 ml of petroleum either was added in the receiving flask. Set the rate 1 to 2 drops after condensation. The fat content that present in the sample were removed and then placed the sample in the dried oven for 10 to 15 minutes at 65 to 75℃ and then weigh the sample. Fat was calculated by using the formula:

$$\text{Crude Fat \%} = \frac{\textit{weight of sample+Thimble)-(Weight of dried fat free sample)}}{\textit{Weight of sample}} \times 100$$

Method No (08-01) of AACC (2000) was used to estimate the ash % in red kidney beans. In this method sample were placed in the crucible for ashing and burned by using hot flame. After charring the sample, it was employed in the muffle furnace at a temp about 550℃ for a time duration of 5 hours until ash of grey color appeared. At the end the sample was placed in the desiccator and allowed them to cool. By using the formula, we can determine the ash content.

$$\text{Crude Ash \%} = \frac{\textit{finial weight}}{\textit{Weight of sample}} \times 100$$

Fiber content was calculated according to the method of (Method no. 32-10) of AACC (2000). 1.25% concentrated sulphuric acid was used to boil the 5g of red kidney beans sample for about 25 to 30min. The boiled sample was than filtered and washed two to three times to remove to acid content present in the sample. The residue sample was again boiled with 200 ml of sodium hydroxide (1.25) % for 25- 30 minutes. Filter the sample and washed with distilled water. Place the sample in hot air oven at 100℃ for about 24 hours. Then the sample was burned in muffle furnace for 5 hours at 550℃. After that, sample was weighed. Fiber content was determined by using this formula:

$$\text{Fiber \%} = \frac{\textit{Weight of oven dried sample (g)-Weight of sample after ashing(g)}}{\textit{Weight of sample (g)}} \times 100$$

Nitrogen Free Extract (NFE) value of sample was obtained by subtracting the proximate analysis of flour from 100. NFE was calculated according to the following expression:

NFE = 100 – (% Crude Protein+% Crude Fiber + % Crude Fat + % Ash) Anti-nutrients were inactivated in red kidney beans according to the method adopted by Shimelis and Rakshit. Red kidney beans were sifted to remove the broken beans, dust and other foreign particles. Soaking and cooking were the processing methods utilized to remove the anti-nutritional elements. Further the samples were freeze dried, grinded and sieved [17]. Red kidney bean sample of 500 (g) were dipped in water at ambient temperature for 12 hours for hydration, water having a pH of 6.9 and 0.05%, solution of sodium bicarbonate having a pH of 8.2. The solution to seed ratio was 3:1 (w/v). The extra unabsorbed water was removed, and rinsing of seeds was done twice with distill water [17]. After 12 hour of hydrations red kidney bean seeds were washed with distill water and then boiled/cooked (double the amount of water than the weight of soaked red kidney beans). The cooking of hydrated red kidney beans was done at 97°C. After boiling the extra water was removed and red beans were washed with distill water twice. Further, freeze drying was done using a bench top freeze drier at 60 °C under a pressure of vacuum 0.03 bar. The freeze dried red kidney bean samples were than grinded using 60mesh size and until further use it is stored in air tight jar at temp 4°C [17]. Red kidney bean protein isolates (KPI) were extracted using the process of Kusumah *et al.*, (2020) with little modifications. The freeze dried and grinded red kidney bean powder is defatted by solvent extraction method by with n-hexane. The sample was wrapped in the thimble and placed in the soxhlet apparatus and set 1 to 2 drop after condensation. Repeated washings were done for complete removal of fat from the samples. After complete washing of fat, the sample were removed from the soxhlet apparatus and oven dried for 10 to 15 minutes at 65 to 75℃ for the complete removal of n-hexane. The red kidney bean protein isolation was done in alkaline environment and continues settlement process of protein at isoelectric point. The 100g red kidney bean defatted flour solution was made with distill water in a ratio 1:10. Then stirred about 10mins and checked for pH. Then the solution pH was regulated to 9 by careful addition of 0.5N NaOH solution with a dropper and continuous stirring was done for 20min. Then the solution was loaded in the centrifuge and first centrifugation was to extract the supernatant. The first centrifugation was done at 4500rpm at 10°C for 30min. The supernatant was free from all the excess materials like carbohydrates and fiber and concentrated with protein [18]. To separate the protein from the supernatant second centrifugation was done but before that pH was calibrated to 4.5 by adding HCl of 1N to reach the isoelectric pH. The supernatant was then centrifuged at 4500rmp at 4°C for 30min. The sludge sticked to the bottom of the falcon tube is protein free from all the other impurities. The obtained protein was then freeze dried for 2 hours at 50°C. The wet protein was then converted in to powdered form [18]. The protein isolates were then stored in air tight container for further use. Hua *et al.*, (2003) method was used to prepare the KPI and gums gel according to the treatment plan shown in Table 1.

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Table 1: Treatment plan for gel preparation

Treatments	Protein isolates (%)	Carrageenan gum (%)	Xanthan gum (%)
T0	0	50	50
T1	75	25	0
T2	50	50	0
T3	25	75	0
T4	75	0	25
T5	50	0	50
T6	25	0	75

Lopes-da-Silva and Monteiro, (2019) methodology was used to measure the rheology and viscosity of gels. Gelation was examined utilizing a controlled-stress rheometer and oscillatory rheological experiments performing small deformation amplitudes with a plate to plate geometry (20 mm, gap 1mm). The operating conditions of instrument for oscillation frequency are, 1 HZ and a stain of 0.3%. Solutions were directly transferred on to the plate at 25°C (room temperature). Heating of samples for 5min at 90°C and then again cooled to 25 °C and kept at this temp for 20 min. The rise and fall of temperature was done at a frequency of 1°C/min. Gel samples were covered with castor oil to avoid the evaporation at high temperature while measurement. To fit the size of the geometries, the samples were sliced with a sharp knife; a spatula was used to shift the samples of weak gel on the plate. The sample was carefully examined to confirm that the gel was free of air bubbles. All samples were taken in triplicates and results mentioned are mean of the replicates [19]. Gel strength was determined by the bloom strength by using the method of Hafidz *et al.*, and Sarbon *et al.*, (2013). The different samples of gel were prepared and poured in the bloom jar and the jar was covered. Gel in the bloom jar was placed in the refrigerator to cool at 10 °C overnight about (16 to 18 hour) to mature and stabilize the gel for further testing. Texture analyzer was used with standard cylinder of 1.25mm diameter. The bloom jar was placed underneath the plunger in the center and when the power was applied and the plunger penetrated 4mm deep in to the gel. The maximum force (the resistance to penetration) applied to penetrate the cylinder in the gel was noted as the Bloom Strength (g) of the gel. All the samples were analyzed in triplicate and readings were note [20, 21]. All the samples were prepared in triplicates and analysis of variance (ANOVA) two-way factorial design was applied for the determination and evaluation of results.

RESULTS

The proximate compositions of red kidney beans are shown in Table 2. The crude protein, ash, moisture, crude fiber, crude fat and carbohydrate quantity were described in % of dry weight.

Table 2: Proximate analysis of red kidney beans

Chemical Parameter	Mean ± SD
Crude Fat%	2.27 ±0.15
Ash	4.03 ±0.10
Crude Fiber%	4.1 ±0.11
Moisture Content %	10.03 ±0.15
Crude Protein %	25.21 ±0.20
NFE %	64.22 ±0.24

The gelation characteristics of KPI and hydrocolloids were evaluated as a characteristic of concentration of CG and XG in Figure 1 and 2. The bloom strength and hardness two parameters are evaluated; a clear increase can be observed in gel hardness as the hydrocolloid concentration increase. The KPI- CG gel shows a higher gel hardness than KP-XG gel at all concentrations. Same trends can be observed in the bloom strength of KPI-CG and KPI-XG gel. At minimum concentration KPI and CG gel shows a hardness of 23.5±0.5 (N) and with the increase in gum concentration hardness shows a clear increasing trend. The protein-XG gel also shows a growing trend with the increase in gum concentration and shows a maximum hardness of 40 ±1 (N) at maximum concertation, which is lower than the KPI-CG gels maximum hardness 42.33±1.54 (N).

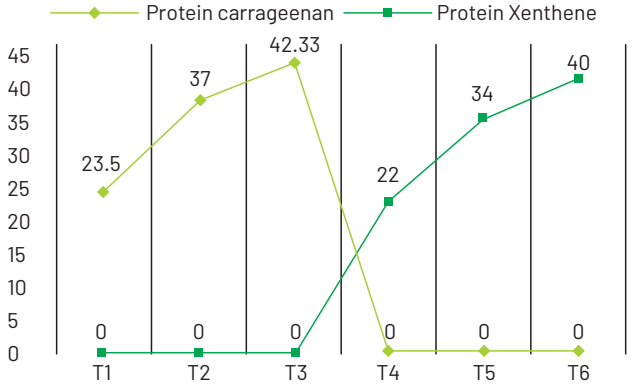

Figure 1: Hardness of protein hydrocolloid gel

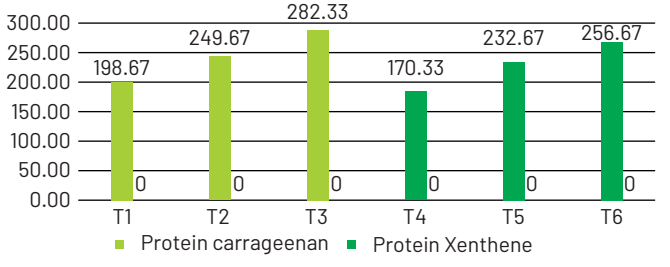

Figure 2: Bloom strength protein hydrocolloid gel

The KPI-CG and KPI-XG samples were examined through frequency sweep tests and results are shown in Table 3. All samples, showed a gel-like structure. The G' elastic modules was higher than the G'' which indicates the proper gelling in all the formulations. The KPI-CG G' was higher than the KPI-XG formulations. At lower concentration of

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KPI-CG the storage modules were low as well as in the case of KPI-XG gel.

Table 3: Rheological properties (G', G") of red kidney bean protein-carrageenan and protein-xanthene gum mixtures under varied conditions

Treatment	T gel (°C)	G' pa	G" pa
T0	45.20 ±1.06	910.67 ±5.62	135.03 ±2.04
T1	38.83 ±1.04	133.69 ±6.16	137.75 ±7.20
T2	44.17 ±1.26	1647.2 ±16.12	234.96 ±6.47
T3	45.43 ±1.01	1629.99 ±13.54	182.95 ±6.07
T4	73.5 ±0.5	584.17 ±10.39	132.09 ±8.60
T5	71 ±1	1483.79 ±11.41	208.75 ±6.74
T6	74 ±1	878.043 ±8.08	182.46 ±6.95

DISCUSSION

Total moisture content of raw sample of red kidney beans was found to be 10.3±0.15%. In other studies, moisture content of raw sample of red kidney beans were found to be in a range of 10.12% which is quite similar to the moisture content assessed in this study [22]. Results of some other studies show similarly moisture content of kidney beans 13.30 per 100 g [23]. Ash content in raw sample of red kidney beans was 4.03±0.10 % respectively. Rui *et al.*, reported the similar ash content ranged from 4.25% to 5.09% which can be correlated with present findings [24]. Whole red kidney beans had 3.57 % ash [18]. Red kidney beans had lower fat content 2.27±0.15 % which is slightly higher than the 1.97% previously reported by Kusumah *et al.*, [18] and lower than the 3.84 % reported by Moreno *et al.*, [25]. Fat content of the kidney bean is small making these beans a foodstuff with positive nutritional implications. Fiber content of red kidney beans was 4.1±0.11%. Almost similar results of fiber content 4.6% can be seen in literature [26]. A wide range of protein content in the red kidney bean has been reported in the literature like 28.31% which is more than the observed results by Roy *et al.*, [27] and 22.36 % less than the obtained values by Rui *et al.*, [24]. 24.25% protein was reported by Olanipekun *et al.*, [28]. Amount of ash, fat, protein, moisture, and fiber is normally subtracted from 100 to determine nitrogen-free extraction. In current situation the NFE readings were 64.22±0.24% in raw red kidney bean. These results have similarity with the results of Rui *et al.*, [24]. Previous studies have reported the use of polysaccharides such as gellan gum to increase the gel strength and functional properties of animal protein [29]. Bloom strength results shows an increase in the gelling strength of KPI-CG gel with the highest value 282.33±1.56 (g) and the same concentration of KPI-XG exhibits a value of 256.67±2.52 (g) which is much lower than the former one. KPI-CG gel seemed more firm, while KPI-XG gel had moderate hardness. Similar results were observed when fish gelation-xanthene and fish gelatin-gum arabica gel hardness was checked gelatin xanthene gum gel showed the less hardness and bloom strength [30]. Studies showed that kappa-carrageenan and other hydrocolloids improved the gel strength and texture. Results showed that composites protein polysaccharide gel increase the gelation up to certain concentration and shows minimum syneresis [31]. While the G' follows an increasing trend with KPI-CG and KPI-XG concentration up to a certain limit. Researchers found greater G′ values by increasing the concentration of polysaccharide [32]. The G'' modules also flows the same trend in viscoelastic behavior. The Gelling temp also increase with concentration of CG and XG. Similar results were reported in different studies that shows the gelling temp become high when protein and kappa-carrageenan concentration increased in the gel [33]. Literature shows that whey protein and xanthan exhibit similar condition of gelling with the increase in gum concentration [34]. Protein-carrageenan and protein-xanthene shows a maximum G' and G'' value respectively at when the protein concentration is medium and gums molecular weight is not high. Comparably, it is evaluated that gelatin and CG shows that the higher the amount of CG used, the gel's G′ was enhanced because electrostatic interactions between the two biopolymers led to the creation of junction zones [35]. When the CG and XG amount cross the certain limit, the G' and G'' show a reverse phenomenon and G' decrease up to certain limit. The G' and G'' of KPI-XG gel is much lower than the KPI-CG gel. Similar results suggest that gum concentration effect the preparation of protein-polysaccharide gels. In this situation it is observed that high amount of XG in egg white gel makes it less coarse [36]. Despite these findings, a zeta potential analysis demonstrated that at greater gum concentrations, the gel formed a sheet-like microstructure with less protein-gum interactions. As the gum content increased, the density of the network at gelatin-tara gum increased, becoming coarser, indicating an excessive weighting of polysaccharide in the gel [37].

CONCLUSIONS

Gel formation via regulated protein-polysaccharide interactions may provide a method for increasing their function as components without requiring enzymatic or chemical treatment. The outcomes of this work will be used to provide the groundwork for developing a novel designed plant protein-based gel system and the use of gel in yoghurt, which might increase functionality over protein or gums alone and replace the animal-based gelling component.

Authors Contribution

Conceptualization: NR, AH
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All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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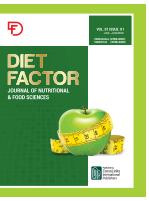

Original Article

Effectiveness of Atenolol on the Basis of Pattern of Side Effects in Hypertensive Patients

Maria Fareed Siddiqui[1*], Mehreen Rasheed[1], Ahmad Alwazzan[2] and Sadia Sarwar[3]

[1]Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan
[2]Department of Obstetrics and Gynecology, King Abdul Aziz University, Jeddah, Saudi Arabia
[3]Department of Zoology, Lahore College for Women University, Lahore, Pakistan

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***Corresponding Author:**
Maria Fareed
Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan
maria.pharmacist@gmail.com

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ABSTRACT

Hypertension plays a significant role in changing Insulin parameter. They are taken as valid diagnostic markers in determining side effects of anti-hypertensive drugs. These parameters are grossly affected by the use of beta blockers which are used to control hypertension. **Objective:** To determine the effects of atenolol, a beta blocker on insulin when taking alone in hypertensive patients. **Methods:** A total of 80 patients participated in this study of which 43 patients were taking atenolol alone while 37 were taking atenolol in combination with other anti-hypertensive drugs and 20 healthy controls were included of all age groups population to make a comparison and to find variation in values of insulin levels, in patients experiencing hypertension. Medical efficacy was evaluated on the basis of variations in insulin levels upon use of anti-hypertensive medications. ELISA technique was used for conducting insulin levels. **Results:** We found significant results of insulin values because atenolol cause hyperglycemia Atenolol was non-significant in both groups of patients having low insulin levels due to use of atenolol alone or use of atenolol in combination therapy. Other anti-hypertensive drugs did not affect the insulin levels therefore variation is basically because of atenolol so the main focus of our study was atenolol. **Conclusions:** Atenolol prove efficacy but it also causes the disturbance in insulin levels therefore we recommend use of any other drug in conjunction with atenolol to avoid insulin variation due to atenolol. Further these results may be employed on large patient population for strengthening our evidences.

INTRODUCTION

According to Chobanian, hypertension (HTN) is a chronic medical disorder marked by elevated blood pressure in the arteries [1]. The heart has to work harder to pump blood through the blood arteries, which puts more strain on it. Systolic and diastolic readings, which represent the heart's contraction and relaxation stages between beats, are used to calculate blood pressure. The average range of normal resting blood pressure is 60-90mmHg diastolic and 100-140mmHg systolic. Hypertension is characterized by a blood pressure level that is consistently at or higher than 140/90 mmHg [2]. However, rather than being completely attributable to high blood pressure itself, these symptoms are more likely linked to concomitant worry [3]. Numerous pieces of evidence suggest that genetic factors have a role in controlling blood pressure [4]. Blood pressure between related people is more similar than it is between unrelated people, indicating that there may be some type of heredity [5]. Mendelian variations of high and low blood pressure have occasionally been linked to a single gene mutation [6]. According to Lifton *et al.*, and Wilson *et al.*, these types of hypertensions are caused by about 10 genes [7, 8]. By altering renal salt processing, these mutations interfere with the control of blood pressure [9]. The control of pressure, volume, and chemoreceptor signals is another

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important function of the autonomic nervous system in preserving cardiovascular homeostasis [10]. The peripheral vasculature and kidneys are modulated to do this, which results in an increase in cardiac output, vascular resistance, and fluid retention [11]. Blood pressure elevation and the onset and maintenance of hypertension are caused by sympathetic nervous system dysfunction, which is characterized by overactivity [12]. Renin-angiotensin-aldosterone regulation of blood pressure is another function of this system. Renin is an enzyme that contributes to the maintenance of arterial vasoconstriction and extracellular volume. It does this by converting the liver's angiotensinogen into the peptide angiotensin I. Angiotensin-converting enzyme (ACE), which is largely found in the pulmonary circulation and is linked to endothelium, further cleaves angiotensin I to create angiotensin II, the most powerful vasoactive peptide [13]. According to Singh and Haldar, angiotensin II is a powerful blood artery constrictor that raises blood pressure and increases peripheral resistance [14]. Additionally, angiotensin II stimulates the adrenal glands to generate aldosterone, which causes kidney epithelial cells to improve salt and water reabsorption, resulting in an increase in blood volume and a rise in blood pressure. As a result, high blood levels of renin, which typically range from 1.98 to 24.6 ng/L in adults sitting erect, can cause hypertension [15]

METHODS

In our case-control study, a total of 80 cases were selected with hypertension taking atenolol alone or in combination and 20 with blood pressure within normal range as control. Patients were selected after taking complete history and medical as well as physical examination who visited Punjab Institute of Cardiology Lahore for the evaluation of hypertension status. Those with hypertension taking atenolol alone or in combination were selected and their insulin levels were checked. All positive hypertensive patients were included and those on multidrug therapy and with concomitant disorder were excluded in this study. Those with normal blood pressure were taken as control for comparison. After collection of 5 cc blood from these hypertensive patients, blood was centrifuged and serum were separated at 3000 rpm for 5 minutes at room temperature and then insulin test was done and effects of medicines were recorded on hypertension profiling. Simple random sampling technique was employed to collect the sample. Informed consent was filled and signed by the subject on the consent form for the collection of blood sample. Relevant history and general physical examination were recorded on the performa (Annexure- B). The lab reports of insulin report indicate whether to include them in the study or take them as controls. The cases that were within our criteria of study, blood was drawn from these patients through 5cc disposable syringe by random sampling. Blood was drawn from cubical vein of the fore-arm and a period of 6 month was employed to collect samples and conduct the study. A sample of 80 individuals of hypertension taking atenolol and 20 with normal blood pressure was taken as a control. Insulin microplate ELISA test is intended to be used for the quantitative determination of insulin levels in human serum. SPSS version-20 was used for result analysis.

RESULTS

The assessment of insulin levels was done on the hypertensive people who made up the control group. Each participant's gender, age, and insulin levels (measured in U/L) are included in the data (Table 1).

Table 1: Evaluation of Insulin in control population

Sr. No.	Gender	Age	Insulin (0.7-9µU/L)
1	Male	35	2.6
2	Male	45	4.7
3	Male	37	3.9
4	Male	48	6.4
5	Male	51	7.5
6	Male	36	5
7	Male	42	4.3
8	Male	41	4.5
9	Male	55	5.4
10	Male	34	3.2
11	Female	35	0.9
12	Female	44	3.6
13	Female	45	5.6
14	Female	46	4.5
15	Female	44	5.6
16	Female	55	6.8
17	Female	35	1.6
18	Female	33	2.6
19	Female	57	6.2
20	Female	42	5.6

An analysis of insulin levels in hypertensive individuals using just atenolol is shown in Table 2. The table contains information about the subjects' gender, age, administration period, atenolol dosage (in milligrammes), and insulin concentrations (measured in U/L).

Table 2: Evaluation of insulin in hypertensive patients taking atenolol alone

Sr. No.	Gender	Age	Duration of Administration	Dose (mg)	Insulin (0.7-9µU/L)
1	Female	34	2 years 2 months	100	-0.48
2	Female	35	1 week	50	0.03
3	Female	38	8 years	50	4.84
4	Female	38	1 years	50	11.42
5	Male	39	4 months	100	-0.23
6	Female	42	1 year 1 month	50	8
7	Female	43	7 months	50	-0.35
8	Female	43	7 years	100	2.05

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Sr. No.	Gender	Age	Duration of Administration	Dose (mg)	Insulin (0.7-9µU/L)
9	Female	45	1 week	100	2.94
10	Male	45	9 months	100	4.84
11	Female	45	4 months	100	-3.27
12	Male	47	6 months	25	56.99
13	Male	47	1 year	100	16.99
14	Female	47	1 year 3 months	100	-0.35
15	Female	48	2 years 10 months	100	17.87
16	Female	48	1 year 1 month	50	4.46
17	Male	48	7 months	25	-1.37
18	Male	50	1 year 3 months	100	5.22
19	Male	50	1 year 1 month	50	-1.37
20	Female	50	3 years	100	14.33
21	Female	50	1 year 2 months	50	4.58
22	Female	50	6 months	100	6.73
23	Female	50	5 months	50	4.71
24	Female	51	7 months	20	9.77
25	Female	52	9 months	100	14.58
26	Female	54	1 year 3 months	50	2.3
27	Male	56	2 years	50	3.19
28	Male	60	1 month	20	7.49
29	Male	60	1 month	25	7.49
30	Female	60	6 years 1 months	50	1.42
31	Female	60	10 months	100	1.42
32	Female	60	2 years 2 months	100	5.97
33	Female	60	1 year 9 months	50	8.51
34	Female	60	5 months	100	13.82
35	Female	60	7 years	50	5.09
36	Male	61	7 years	100	10.28
37	Female	62	4 months	100	4.46
38	Male	65	2 years 7 months	50	1.67
39	Female	67	6 months	50	-0.61
40	Male	70	1 year	50	1.67
41	Female	70	5 months	100	3.19
42	Female	70	1 year 2 months	50	22.3
43	Male	77	2 weeks	100	10.91

In figure 1 20% control and remaining diseased population is shown with 42 % patients with normal insulin values, 22 % with higher insulin values and 16% with lower insulin values.

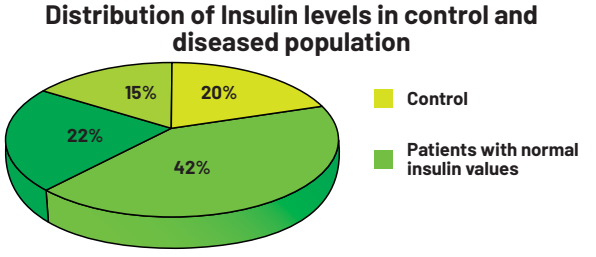

Figure 1: Distribution of Insulin levels in control and diseased population

DISCUSSION

The results of this study and its comparison with earlier studies in the area are the focus of the discussion on the effects of atenolol, a beta blocker, on insulin when taken alone in hypertensive individuals. We can better understand the effect of atenolol on insulin regulation and its implications for controlling hypertension by analyzing and contrasting the data. According to the results of the current study, hypertensive patients who received atenolol as a stand-alone therapy saw considerable changes in their insulin levels, mostly as a result of the onset of hyperglycemia [15]. These results are consistent with other studies that suggested beta blockers, such as atenolol, may contribute to abnormalities in insulin control and glucose metabolism. The impact of beta blockers on insulin and glucose metabolism has been investigated in a number of research. For instance, research by Palatini and Julius found that nonselective beta blockers like propranolol can cause reduced glucose tolerance and insulin resistance [16]. Comparably, research by Bühler *et al.*, found that beta-blocker-treated hypertension individuals had higher insulin levels and worse insulin sensitivity [17, 18]. These findings and others support the idea that beta blockers may have a negative impact on insulin control. In contrast to other research, the current study paid particular attention to atenolol's impact on insulin levels in hypertensive individuals. According to the study, atenolol alone was linked to considerable fluctuations in insulin levels, which is consistent with beta blockers' overall effects on the control of insulin. However, it is notable that regardless of whether patients were receiving atenolol alone or in combination treatment, the effects of atenolol on insulin were non-significant among those who already had low insulin levels. It is important to recognize its limitations and take into account the larger body of research in this field since the current study offers insightful information on the effects of atenolol on insulin in hypertensive individuals. First off, the study only evaluated insulin levels; it did not evaluate other indicators of glucose metabolism, such as insulin resistance or glucose tolerance [19, 20]. Future research might look into these areas to have a more complete knowledge of atenolol's impact on glucose control. Second, with just 80 participants, the sample size of the current study was somewhat small. This restricts the findings' applicability to other contexts and necessitates further extensive research to confirm and support the findings. Furthermore, the study did not take into account variables that can affect how atenolol affects insulin levels, such as the length of atenolol administration, dose fluctuations, or unique patient features.

CONCLUSIONS

Atenolol prove efficacy but it also causes the disturbance in insulin levels therefore we recommend use of any other drug in conjunction with atenolol to avoid insulin variation due to atenolol. Further these results may be employed on

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large patient population for strengthening our evidences.

Authors Contribution

Conceptualization: MFS
Methodology: MR, MFS
Formal analysis: SS
Writing-review and editing: MFS, SS

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

Conflicts of Interest

The authors declare no conflict of interest.

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