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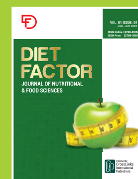

Nutritional Preparedness: Strengthening Immune Defenses against Biological Threats

Naheed Mojgani[1*] and Sumel Ashique[2]

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As the world continues to grapple with the aftermath of the COVID-19 pandemic, it has become increasingly evident how vulnerable we are to Biological Warfare. At the time of crisis, developing effective vaccines at such short notice and distributing them equitably proved to be a significant challenge. Some governments and health policy makers were ill-equipped to handle the task effectively resulting in unnecessary delays in the process of developing effective vaccines and ineffective supply chains that consequently resulted in millions of death worldwide. All this has urged and spurred governments to prioritize bio-preparedness and enhance bio-defense measures.

However apart from the Governments and health policy makers' preparedness, COVID 19 pandemic has taught us invaluable lessons for preparing ourselves at individual level for such threats in future. We have now become more aware of the importance of personnel hygiene and maintaining body's resistance against the foreign agent that could protect us from the biological threat. As seen during the recent pandemic, individuals with underlying health conditions, many of which were linked to poor dietary habits were more vulnerable to the infection. The pandemic has served as a wake-up call, highlighting the importance of prioritizing nutrition as a means of modulating our gut microbiome consequently fortifying our body's defenses against infections and diseases. A comprehensive literature survey has proven how healthy dietary choices including an adequate supply of essential and bioactive nutrients such as vitamins, proteins, Zinc etc and bioactive compounds such as probiotics, prebiotics, antioxidants, polyphenols etc, could reduce inflammations and help maintain a robust immune system.

Nutrition plays a crucial role in maintaining a healthy immune system and warding off infections. Research has consistently shown that a well-balanced diet rich in fruits, vegetables, whole grains, lean proteins, and healthy fats is essential for bolstering our immune response. These nutrient-dense foods provide our bodies with the vitamins, minerals, and antioxidants necessary to support our immune system's function. For instance, vitamin C, found in citrus fruits and leafy greens, is known for its immune-boosting properties. It aids in the production of white blood cells, which are vital for fighting off infections. Similarly, vitamin E, present in nuts, seeds, and vegetable oils, acts as an antioxidant that protects our cells from damage and strengthens our immune system.

As we navigate the post-pandemic landscape, it is imperative that we draw lessons from this experience and prioritize nutrition as a fundamental aspect of public health. Healthcare professionals, educators, and policymakers must work collaboratively to integrate nutrition education into public health initiatives and empower individuals to make informed food choices.

Furthermore, media outlets play a crucial role in disseminating information about the link between diet and immunity. By featuring expert opinions, sharing immune-boosting recipes, and debunking common myths surrounding nutrition, the

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media can help foster a culture of health-conscious eating habits.
In conclusion, the COVID-19 pandemic has underscored the critical role of diet in supporting our immune system's ability to combat infections and diseases. It is imperative that we seize this opportunity to prioritize nutrition education and promote healthy eating habits as essential components of public health initiatives. By doing so, we can empower individuals to take proactive steps towards bolstering their immune health and ultimately contribute to a healthier population. Personal preparedness complements government and health policy efforts. By taking responsibility for our own health and well-being, we can create a more resilient society that is better equipped to handle future biological threats.

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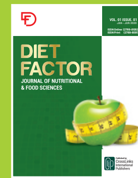

Original Article

Development and Characterization of *Bauhinia variegata Linn* Leaves Powder Biscuits

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ABSTRACT

Iron deficiency anemia is the major public health problem all over the world especially in children under 5 and pregnant females. **Objective:** To develop and explore the nutritional and sensory quality of iron and Vitamin C enriched biscuits by using leave powder of *Bauhinia variegata* and lemon juice for the study period of two months. **Methods:** Experimental research was performed to determine the levels of macronutrients, micronutrients and overall acceptability of *Bauhinia variegata l*eaves powder biscuits. For this purpose, *Bauhinia variegata* leaves were collected, washed with clean water, sun-dried and finely grinded to form powder. *Bauhinia variegata* leaves powder was analyzed for proximate analysis, iron and Vitamin C levels. Functional biscuits with treatments (T_0, T_1 and T_2) were made by using 10 g and 10 mL of *Bauhinia variegata* leaves powder and lemon juice respectively. The iron-enriched biscuits were evaluated for proximate composition, iron, Vitamin C contents and sensory traits such as color, flavor, taste, texture and overall acceptability. One-way Anova was applied on the obtained results. **Results:** The consequences found that *Bauhinia variegata* leaves powder was rich in protein and iron contents. Incorporation of *Bauhinia variegata leaves* powder and lemon juice in the biscuits significantly increased the nutritional composition of biscuits. The results related to sensory parameters proved that *Bauhinia variegata* leaves powder biscuits had high sensory acceptability as compared to control. **Conclusions:** It is concluded that by adding dried leaves powder of *Bauhinia variegata* and juice of lemon improved the nutritive value and consumer acceptability of the functional biscuits.

INTRODUCTION

Globally, malnourished population is more severely affected from the deficiency of micronutrients. Protein-energy deficiency is more common among all the deficiencies from micronutrients. Vitamins and minerals that are included in micronutrients are essential for proper metabolism of foods and needed in minute amounts as compared to macronutrients. Amongst all the micronutrients' deficiencies, iron deficiency is the predominant public health problem which results in countless issues in children under 5 and pregnant females. According to latest studies, more than 2 billion people are facing the issue of iron deficiency anemia across the globe especially in developing countries [1]. Utilization of iron supplements is mostly practiced to overcome the deficiency from iron but they are mostly high in cost and have low absorption rate as compared to natural sources. Therefore, to prevent and treat iron deficiency anemia natural sources can be used without any side effects as they are mostly safe and non-toxic [2]. *Bauhinia variegata Linn* commonly known as Kachnar (mountain Ebony) is medium-sized deciduous tree and belong to family of Leguminosae (Caesalpinioideae). The different parts of Kachnar such as stem, leaves, seeds, roots, bark and flowers contain numerous macronutrients (protein, fat and fiber), micronutrients (calcium, iron, phosphorous & vitamin C) and phytochemicals (saponins, terpenoids, kaempferol, cardiac glycosides, tannins, flavonoids, and quercetin has vital role in promoting human health.

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Traditionally, Kachnar is a popular medicinal tree that is largely utilized to cure different diseases due to its countless therapeutic properties like anti-inflammatory, anti-diabetic, haematinic, immunomodulatory, haemagglutinating, anti-tumour, anti-microbial, hepatoprotective, anti-bacterial and anti-ulcer activity) [3].

Worldwide, cereals are considered as chief staple diet for the population. Wheat is majorly consumed in the form of bakery products as compared to other cereal grains. Among all the bakery products, biscuits are ideal for fortification of micronutrients to tackle several chronic and nutrition-related diseases [4, 5]. To prevent and treat iron deficiency anemia, fortification of food items with iron is an effective method to meet the requirement of iron level in the diet. Biscuits are considered as a snack rather than a meal. They have better nutrition, palatability, acceptability, easy availability, affordability and long storage stability [6, 7].

Due to current status of iron deficiency anemia worldwide, the aim of present investigation was to develop the functional biscuits by the incorporating finely ground leaves powder of *Bauhinia variegata* and juice of lemon. Furthermore, Kachnar biscuits were determined for chemical analysis, iron, vitamin C contents and sensory attributes.

METHODS

The present study was performed in National Institute of Food Science and Technology (NFSAT) and Institute of Home sciences, University of Agriculture, Faisalabad. Kachnar (*Bauhinia variegate Linn.*). Leaves were procured from the Ayub Agriculture Research Institute (AARI), Faisalabad. After collection, the Kachnar leaves were washed with tap water, dried under sun and then kept in air tight jars [8]. Iron and Vitamin C enriched biscuits were made by using white flour, leaves powder of *Bauhinia variegata* and juice of lemon with formulation of (100: 0:0), (90: 10: 0) and (80 :10: 10) in T_0, T_1 and T_2 respectively according to the methodology of AACC Method No. 44-15 A, 44-40 with slight modifications [9]. The proximate composition (moisture, ash, fat, protein, fiber contents) and nitrogen free extract (NFE) content of sun-dried Kachnar leaves powder and functional biscuits were determined by using the AOAC official Method No. 925.10, 923.03, 935.38, 979.09 and 991.43 respectively [10]. Iron and Vitamin C levels of Kachnar leaves powder and biscuits were estimated by AOAC methodology 960.0 and 961.21 accordingly [10]. The sensory traits of the functional biscuits such as color, flavor, taste, texture and overall acceptability were evaluated voluntarily by the trained judges by using 9-hedonic scale and consent was taken prior to evaluation[11]. The study period of present investigation was two months. The findings related to all the parameters studied were statistically investigated by using SPPS software version 23.0 with the application of One-way ANOVA [12].

RESULTS

The result of chemical composition of Kachnar (*Bauhinia variegata*) leaves powder presented in Table 1 found that the values of crude moisture, crude ash, crude protein, crude fat and NFE contents were found to be 8.79 ± 0.02 %, 4.60 ± 0.02%, 14.7 ± 0.1%, 4.06 ± 0.04 %, 4.23 ± 0.01 % and 38.67 ± 0.02 % respectively.

Table 1: Chemical Composition of Kachnar Leaves Powder

Proximate Composition	Quantity (%)
Crude Moisture Content	8.79 ± 0.02
Crude Ash	4.60 ± 0.02
Crude Protein	14.7 ± 0.1
Crude Fat	4.06 ± 0.04
Crude Fiber	4.23 ± 0.01
NFE	63.43 ± 0.01

Mean ± SD of overall proximate composition of *Bauhinia variegata* leaves.

In the present study, the results showed that the iron and Vitamin C contents of dried Kachnar (*Bauhinia variegate*) leaves powder were 22.08 ± 0.02 and 5.5 ± 0.04 mg/100g respectively (Table 2).

Table 2: Iron and Vitamin C Contents of Kachnar Leaves Powder

Micronutrients	Quantity (mg/100g)
Iron content	22.08 ± 0.02
Vitamin C	5.5 ± 0.04

Lemons carry an ample number of beneficial micronutrients especially Vitamin C that is present in appreciable amount and iron content in less quantity. The consequences displayed in Table 3, depicted that lemon contained 0.38 ± 0.33 mg/100mL iron and 53.2 ± 0.38 mg/100mL Vitamin C.

Table 3: Iron and Vitamin C Content of Lemon Juice

Micronutrients	Quantity (mg/100 mL)
Iron Content	0.38 ± 0.33
Vitamin C Content	35.77 ± 0.38

The outcomes of chemical composition of the functional biscuits are shown in table 4. According to results, the Kachnar leaves powder biscuits had more moisture content than the control cookies (T_0). However, the crude ash, crude protein, crude fat, crude fiber and nitrogen free extract (NFE) contents of functional biscuits significantly increased from (2.37 ± 0.05-2.75 ± 0.03 %), (17.98 ± 0.21-18.85 ± 0.22 %), (20.23 ± 0.35 - 20.35 ± 0.18 %), (4.01± 0.03-4.08 ± 0.02 %) and (48.24 ± 0.41- 49.52 ± 0.44) in T_0 to T_3 respectively.

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Table 4: Chemical Composition of *Bauhinia variegata* Leaves Powder Biscuits

Treatments	Crude Moisture Content (%)	Crude Ash Content (%)	Crude Protein Content (%)	Crude Fat Content (%)	Crude Fiber Content (%)	Nitrogen Free Extract
T_0	4.15 ± 0.04^c	2.37 ± 0.05^c	17.98 ± 0.21^c	20.23 ± 0.35^c	4.01 ± 0.03^c	48.24 ± 0.41^c
T_1	4.32 ± 0.1^b	2.52 ± 0.03^b	18.54 ± 0.21^b	20.29 ± 0.35^b	4.02 ± 0.02^b	45.91 ± 0.74^b
T_2	4.55 ± 0.12^a	2.75 ± 0.03^a	18.85 ± 0.22^a	20.35 ± 0.18^a	4.08 ± 0.02^a	49.52 ± 0.44^a

Values with different superscripts show statistically significantly different (p<0.05).

The results represented in figure 1 described that the iron and vitamin C contents of functional biscuits significantly increased from T_0 to T_2 (3.92 ± 0.06-7.02 ± 0.03 mg/100g) and (1.94 ± 0.03-10.2 ± 0.08 mg/100g) respectively.

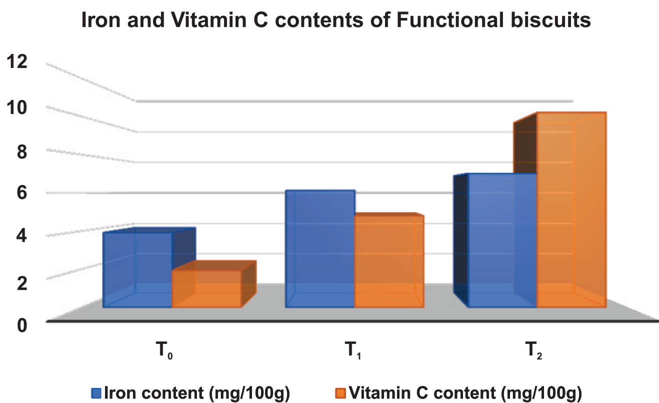

Figure 1: Graphical Representation of Iron and Vitamin C Contents of Functional Biscuits

The outcomes related to sensory evaluation of functional biscuits as shown in table 5 found that color, flavor, taste, texture and overall acceptability of T_0 were given the maximum scores 7.04 ± 0.44, 7.02 ± 0.44, 7.0 ± 0.44, 7.00 ± 0.44 and 7.01 ± 0.44 out of 9 respectively. T_1 was given 6.68 ± 0.42/9 and T_2 were assigned /9. Lesser scores (6.52 ± 0.47, 6.30 ± 0.44, 6.12 ± 0.54, 6.09 ± 0.54 and 6.25 ± 0.54) were assigned to T_2 as compared to T_0.

Table 5: Sensory Evaluation of Kachnar Leaves Powder Biscuits

Treat-ments	Color	Flavor	Taste	Texture	Overall Acceptability
T_0	7.04 ± 0.44^a	7.02 ± 0.32^a	7.00 ± 0.48^a	7.00 ± 0.42^a	7.01 ± 0.23^a
T_1	6.68 ± 0.42^b	6.42 ± 0.37^b	6.24 ± 0.56^b	6.22 ± 0.30^b	6.39 ± 0.33^b
T_2	6.52 ± 0.47^c	6.30 ± 0.54^c	6.12 ± 0.54^c	6.09 ± 0.39^c	6.25 ± 0.46^c

Values with different superscripts show statistically significantly different (p<0.05).

DISCUSSION

The findings of present study found that dried Kachnar (*Bauhinia variegata*) leaves powder contained high amount of crude moisture, crude ash, crude protein, crude fat, NFE and iron contents. While, lemon had ample amount of Vitamin C. The values obtained by this analysis were very close to the values given by [13, 14]. Furthermore, the outcomes depicted that Kachnar leaves powder biscuits prepared with and without the supplementation of lemon juice had more moisture, crude ash, crude protein, crude fat, crude fiber, nitrogen free extract (NFE), iron and Vitamin C contents than the control cookies (T0) at time interval of two months. The leaves powder of Kachnar improved the nutritional profile of functional biscuits by enhancing their macronutrients and iron levels. Furthermore, addition of lemon juice improved the Vitamin C level of biscuits. Vitamin C affects the absorption of iron in different food items, Ascorbic acid (vitamin C) show a significant role in improving the absorption of iron [15]. The previous studies proved that daily intake of Ascorbic acid significantly enhanced the absorption of non-heme iron from plant-based food items [16]. The outcomes derived from the current research are in collaboration with the study done by Galla *et al.*, who found out that addition of spinach powder had significant effect on chemical composition of functional biscuits [17]. According to another research, supplementation of cookies with mushroom powder significantly enhanced proximate and mineral composition as compared to control cookies [18]. The sensory parameters of the biscuits were evaluated on the basis of colour, flavour, taste, texture and overall acceptability and results found that all the parameters of the functional biscuits significantly affected by the adding leaves powder of Kachnar and juice of lemon at the study period of 8 weeks. The results of the study were similar to the previously done work by Dwivedi and Bhatt who depicted that scores for all the sensory parameters of Niger seed flour fortified cookies significantly differed with the addition of Niger seed flour as compared to control cookies but remained acceptable [19]. Another previous investigation showed that sensory attributes of biscuits prepared from blend of wheat, soybeans and orange flesh sweet potato improved significantly according to the scores given by trained panel of judges [20].

CONCLUSIONS

The supplementation of sun-dried kachnar leaves powder and lemon juice significantly enhanced the nutritional profile and consumer acceptability of functional biscuits. Biscuits developed by utilizing Kachnar leaves powder (rich in iron) and lemon juice (natural source of Vitamin C) may play significant role in the prevention and treatment of iron deficiency anemia due to their strong therapeutic potential.

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Authors Contribution

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Methodology: SA
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Writing-review and editing: AM

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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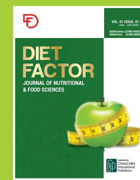

Original Article

Eating Habits and Lifestyle Practice of Young Adults in Karachi, Pakistan; A Cross-Sectional Survey

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ABSTRACT

Each year in Pakistan about 35% to 40% of deaths occur due to cardiovascular disease that is mainly caused by decreased physical activity and unhealthy food consumption. Obesity among young people is a serious public health issue because it is frequently associated with multiple metabolic syndromes. **Objective:** To determine young adults' eating habits and lifestyle practices and compare this among male and female genders. **Methods:** A total of 249 participants aged between 18 to 25 years young adults were recruited from different universities using a non-probability convenient sampling technique. The participants' eating habits and lifestyle practices were analyzed using a self-designed questionnaire. **Results:** Among 249 students, 43.8% were male 56.2% were female. Statistically, there was a significant difference found between the two genders in the consumption of water ($p<0.001$), and meal consumption daily ($p=0.007$), Eating habits after joining university ($p=0.44$), thinking of physically strong ($p=0.001$), Physical inactivity ($p<0.001$) and their preferred living place in leisure time ($p<0.001$). **Conclusions:** This study concluded that most of the female were involved in unhealthy dietary habits concerning skipping meals and having fast food, whereas there is no difference found between genders in their physical activity. However further research should be conducted to explore the relation of eating habits and lifestyle in gender.

INTRODUCTION

Physical activity is defined as any action of the body that causes an increase in energy expenditure. It can range from sedentary to very active and it is a necessary component of a healthy lifestyle [1]. A healthy lifestyle is defined as a set of positive lifestyle patterns, habits, and practices that contribute to an individual's overall health, a sedentary lifestyle, on the other hand, is well-defined as a pattern of behavior in which people spend more time in sitting or reclining back with little or no energy expenditure (less than 1.5 according to METS)[2, 3]. A person's nature is the influence of their relationship with food, social life, and culture with the environment which are normally referred to by their eating habits. The eating habits of an individual are marked as healthy when they follow the national dietary plans and recommendations that are purely based on international guidelines. If not, so they will be considered unhealthy dietary plans like eating junk food which includes; snacks, fast food, and supplement drinks in excess amounts which can cause various types of health-related issues. In young and middle adulthood, people skip their meals and use high carbs and junk foods [4]. According to the United Nations' World Health Organization

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(WHO), Health risk behaviors that begin in adolescence are the primary cause of disease in adults (e.g., unhealthy eating practices). Each year in Pakistan about 35% to 40% of deaths occur due to cardiovascular disease that is mainly caused by decreased physical activity and unhealthy food consumption [5]. Childhood and adolescents' poor dietary choices and lifestyle patterns will continue till their adulthood and they have a great risk of various nutritional problems in their lives [6]. Obesity is one of the most common chronic diseases in young adults; on the other hand, it is the most common nutritional disorder in children worldwide, with prevalence increasing in both developed and developing countries, affecting all social and economic groups, both sexes, all ages, and ethnic groups [7]. Obesity among young people is a serious public health issue because it is frequently associated with metabolic syndrome, type II diabetes mellitus, hypertension, dyslipidemias, and more frequent sleep disturbance, US students showed a significant difference in their weight with men being more obese as compared to women [8,9]. Being active is slightly different between male and female populations, because males are more involved in physical activities to perform their recreational work, while most of the females do activities because they mostly want to reduce their weight or to maintain it [10,11]. There is a difference in food consumption between the two genders according to studies, like female population used to eat more vegetables and fruits but they also ate more sweets as compared to the male population, while the male used a diet which is high in protein as well as rich in fat, they also drink wine, cold drinks [12]. According to psychological factors, many adults are used to eating more when they feel bored or even when they are happy, this figure is significantly higher in females than males [13]. Men with more physical work mostly like to consume more meat, they show a positive relation about the intake of meat but on the other hand feminine traits are more likely to eat a vegetarian diet [14]. The female population has an increased chance of greater nutritional insecurities as compared to the male population. In poor Asian and African countryside females are 2% more involved in food insecurities than males [15]. Fast food consumption gives 58% of energy demand and 89% more added sugar which is related to more weight gain and this connection is more pronounced in females So, to establish a Healthy lifestyle, a person should adopt good eating habits and do physical activities daily [16, 17].

This study aimed to determine the eating habits and lifestyle of young adults and also to compare these patterns concerning gender. This might have a favorable or detrimental influence on young adults' health, promoting or discouraging healthy lifestyles.

METHODS

A Cross-sectional study was conducted among university-going students in Karachi, Pakistan from February 2023 to July 2023. Data were collected from young adults, including males and females in the age group of 18-25 residing in Karachi, Pakistan. All those participants who were using any medications that may impact eating habits had a history of any psychological eating issues, and did not provide consent to participate in the survey were excluded from the study. A Non-probability convenient sampling technique was used. The sample size for the study was calculated using Open Epi version 3.0 (18) with an error margin of 5 % and a 95 % confidence interval of 249. Data were collected with the help of self-designed questionnaires that included fifteen close-ended questions to assess the general eating habits among young adults and the lifestyle practices of participants (the questions were related to the number of meals consumed each day, water intake, meal choices related to home cooked or junk food preferences, change in eating habits, lifestyle pattern, engagement in physical activity and sleep pattern). The research team surveyed different universities to approach young adults in the age group of 18-25 years. The purpose of the study was explained to the participants. The data were analyzed statistically using IBM SPSS (Statistical Package for Social Sciences) version 20.0. To determine the distribution of data, a test of normality was applied. To provide a comprehensive overview of the variable, descriptive statistics were used to evaluate the mean frequency and percentage. An Independent sample t-test was applied to compare the test values. Institutional review board approval was attained in February; reference number (ASC-PT-007/02/2023) and participants were asked to fill out a consent form for voluntary participation. Following the written informed consent taken by participants, they were enrolled in the study and asked to fill out the questionnaire. Confidentiality was maintained throughout the study.

RESULTS

The survey was collected from 249 young adults in Karachi, Pakistan. The response rate was 100%. Among 249 students, n=109 (43.8%) were male whereas n=140. (56.2%) were female. The participants with age 18-20 were 65(26%), in the 21-23 age group were 148 (59.4%) students whereas, in the 24-25 years of age group, participants were only 36 (14.5%). Table 1 represents the response of the questionnaire on water consumption, it is found that the majority of the females consume 6 glasses of water per day and most of the males consume 8 glasses of water per day. Regarding the question of consuming three meals a day, it was found that only 39 (35.8%) males regularly consume 3 meals daily whereas females who consume habitually were

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84 (60%). In response to the question regarding breakfast during the study period, it was calculated that only 49 (45.0%) males and 79 (56.4%) females had breakfast at home and we also found that 12 (11.0%) males whereas 44 (31.4%) females who neither have their breakfast at home nor canteen/cafe. By using a questionnaire, it was found that there were 63 (57.8%) males and 92 (65.7%) females who followed unhealthy lunch patterns and consumed restaurant fast food during their study hours. It was also found that there were 35 (32.1%) males and 34 (24.3%) females who consumed fast food every day during their study period. In response to the question regarding the change in participants eating patterns after getting into university, it was concluded that there were 71 (65.1%) males and 108 (77.1%) females whose eating patterns changed a lot since they started university (Table 1).

Table 1: Response of Participants Associated with Eating Habits

Questions	Options	Responses		p-Value
		Male N (%)	Female N (%)	
Water Consumption Per Day	2 liter (8 Glasses)	64 (58.7)	32 (22.9)	<0.001
	1.5 liter (6 Glasses)	29 (26.6)	59 (42.1)	
	1 liter (4 Glasses)	16 (14.7)	49 (35.0)	
Consumption of Three Meals Per Day	Regularly	39 (35.8)	84 (60.0)	0.007
	Sometimes	65 (59.6)	38 (27.1)	
	Not at all	5 (4.6)	18 (12.9)	
Consumption of Breakfast During Studies	Home	49 (45.0)	79 (56.4)	0.879
	Canteen	48 (44.0)	17 (12.1)	
	Never	12 (11.0)	44(31.4)	
Lunch During the Period of the Lesson	Home	19 (17.4)	35 (25.0)	0.600
	Canteen	27 (24.8)	13 (9.3)	
	Fast Food Restaurant	63 (57.8)	92 (65.7)	
Consumption of Lunch During Studies	Everyday	35 (32.1)	34 (24.3)	0.109
	More than Once in a Month	68 (62.4)	93 (66.4)	
	Not At All	6 (5.5)	13 (9.3)	
Consumption of Fast Food During University Hours	Yes, A Lot	71 (65.1)	108 (77.1)	0.049
	Yes, But Not Much	35 (32.1)	27 (19.3)	
	Not At All	3 (2.8)	5 (3.6)	

Table 2 represents the responses associated with weight analysis; it was found that 20 (14.3%) females are more prone to being underweight rather than 13 (11.9%) males. and it was also found that 69 (63.2%) males and 86 (61.4%) females were normal weight. In the lifestyle patterns, 36 (33.0%) males and 46 (32.9%) females are living sedentary lifestyles while other participants 67 (61.5%) males and 74 (52.9%) females are active while the remaining is highly active. Regarding the question of physically strong it was found that 44 (40.4%) males and 22 (15.7%) females are physically strong while others are not much at all. In leisure time the extracurricular activities participants are 37 (33.9%) males and 22 (15.7%) females daily. In other questions about being physically inactive, it is found that 58 (53.2%) males and 37 (26.4%) females are physically inactive (Table 2). Responses on the questionnaire in the weight category, it is found that 20 (14.3 %) females are underweight as compared to males, and 13 (11.9 %) males are underweight. The frequency of females who are of normal weight is 86 (61.4 %) whereas 69 (63.2 %) are male. It is observed that there is no greater difference in living a sedentary life among males and females but there is a remarkable difference in activity level among both genders as shown in Table 2 there are 67 (61%) males are active whereas only 74 (52.9%) are active females. It is noted that 37 (33%) of males are involved in extracurricular activities in their leisure time whereas only 22 (15.7%) females are involved in activities daily according to table 2.

Table 2: Participant Responses Associated with Lifestyle Patterns

Questions	Options	Responses		p-Value
		Male N (%)	Female N (%)	
Weight Analysis	Under Weight	13 (11.9)	20 (14.3)	0.729
	Normal Weight	69 (63.3)	86 (61.4)	
	Over Weight	27 (24.8)	34 (24.3)	
Lifestyle Patterns	Sedentary	36 (33.0)	46 (32.9)	0.355
	Active	67 (61.5)	74 (52.9)	
	Highly Active	6 (5.5)	20 (14.3)	
Physically Strong	Yes, a lot	44 (40.4)	22 (15.7)	0.001
	Yes, but not much	51 (46.8)	102 (72.9)	
	No, not at all	14 (12.8.)	16 (11.4)	
Extracurricular Activities in Leisure Time	Everyday	37 (33.9)	22 (15.7)	<0.001
	More than Once a Month	64 (58.7)	77 (55.0)	
	Never	8 (7.3)	41 (29.3)	
Being Physically Inactive	Yes, a lot	58 (53.2)	37 (26.4)	<0.001
	Yes, but not much	37 (33.9)	77 (55.0)	
	No, not at all	14 (12.8)	26 (18.6)	

According to table 3, the response to the questionnaire about worrying about everyday problems showed that females are more prone to stress than males and males can manage everyday problems nicely as compared to females. It is found that 84 (60%) females get worried easily about everyday problems whereas males are only 56 (51.4%) Regarding the question period of lessons they live, it was found that 63 (57.8%) males live with their families during the period of lessons whereas 122 (87.1%) female lives with their families during study hours, Regarding the question make an effort to feel happy and content, it was found that 65 (59.6%) males make an effort to feel happy whereas, 72 (51.4%) females make an effort to be happy, regarding the question about how many hours do you sleep on an average at night 37 (33.9%) males sleep 3 to 5 h on an average at night whereas 53 (37.9%) females sleep 3 to 5 h on an average at night. The statistically significant difference between males and females was found in the consumption of water (p<0.001), consumption of three meals daily (p=0.007), change of eating habits after joining university (p=0.49), thinking of being physically strong (p=0.001),

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extracurricular activities in leisure time (p<0.001), physically inactive (p<0.001) and living place during study (p<0.001).

Table 3: Participant Responses on Sleeping Habits

Questions	Options	Responses		p-Value
		Male N (%)	Female N (%)	
Worrying about Everyday Problems	Yes, A Lot	56 (51.4)	84 (60.0)	0.073
	Yes, But Not Much	36 (33.0)	47 (33.6)	
	No, Not At All	17 (15.6)	9 (6.4)	
Living While Study	With Your Family	63 (57.8)	122 (87.1)	<0.001
	With Your Relatives	10 (9.2)	7 (5.0)	
	With Your Friends	36 (33.0)	11 (7.9)	
Feel Happy and Content	With Your Family	65 (59.6)	72 (51.4)	0.737
	With Your Relatives	26 (23.9)	61 (43.6)	
	With Your Friends	18 (16.5)	7 (5.0)	
Sleeping Hours	3 to 5 hours	37 (33.9)	53 (37.9)	0.271
	6 to 8 hours	57 (52.3)	76 (54.3)	
	9 to 10 hours	15 (13.8)	11 (7.9)	

DISCUSSION

The purpose of this study was to access and analyze young adults' lifestyle practices to educate them about a good and healthy lifestyle, as well as to assess the effectiveness of how gender-based eating patterns affect young people. It was found that there was no significant difference in water consumption among genders but there was a significant difference in eating habits among both genders. There is a huge amount of scientific evidence that suggests eating fast food frequently can be bad for one's health. This is due to the high levels of sugar, salt, saturated fat, processed components, and calories in the majority of fast food. Additionally, it typically has low levels of fiber, antioxidants, and many other minerals. The main findings are that eating breakfast regularly and eating home-cooked meals are preventative factors against a poorer QOL across all of its aspects. However, eating fast food and sugary snacks are risk factors for low QOL. [19]. A survey study conducted by Ushula *et al.*, on the students, revealed that missing meals especially breakfast is prevalent among university students (63.1% of them missed breakfast on less than one day). Missing breakfast is common in young adults, especially students [20]. Many experts have asserted that eating breakfast must be healthy. Numerous studies indicated that those who eat breakfast are more likely to be in better health, but they cannot establish that breakfast consumption was the direct reason. Breakfast eaters probably have other healthy living practices that can account for this admission to a university may cause significant changes in the pattern of exposure to health risks Sahrin S *et al.* proposed this in their study. She also stated that some males and females have changed their eating habits after attending university while other males and females have changed but not much while others have not changed their eating habits. Healthy eating leads to a healthy person and a beautiful peaceful mind which makes a person healthy [22]. The main objective of this study was to assess and inquire about young adult males and females perceptions regarding their weight and in which category they fall. There is a rich literature devoted to the role women play in ensuring the food security of the household and other household members. However, relatively little attention has been paid to their food security situation. This was studied in detail by Broussard NH *et al.*, where almost all females and males consider themselves as having normal weight while others having an overweight female are more than males and others underweight [15]. Sahrin S *et al.*, discussed in detail the relationship between physical activity, sedentary behavior, and the subjective and objective indications of quality of life as well as life satisfaction among university students, whose education is related to different dimensions of health. Having a good lifestyle makes a difference between a healthy person and an unhealthy person this study has shown that some males and females are active and few males and females are highly active while others have a sedentary lifestyle without having any regret. This sedentary lifestyle affects them physically, mentally, emotionally, and socially. Sedentary behavior during the week related positively with the subjective quality of life and its intimacy dimension, but sedentary behavior at the weekends was negatively related to the objective and subjective quality of life as well as dimensions including intimacy, safety, and communicative aspects of life. Neither physical activity nor sedentary behavior demonstrated a significant relationship with the level of life satisfaction [22]. In this study, it has been observed that some males and females consider themselves physically strong while some of them do not. Activities in our lives play an important role which works as therapy for us we have asked a question to young adult males and females do they indulge in extracurricular activities in their leisure time? this makes a big difference in our life mentally and physically too, which we get answered that few males and females do extracurricular activities in their leisure time while some do once a month and others don't. This study is based on limited exposure and through this study, we have observed that young adult males and females are having an unhealthy lifestyle and having unhealthy eating habits which is not a good sign of a healthy person. Healthy eating habits have a positive effect on students' academic performance. However, other factors, such as sleep habits, may be more important [23, 24]. So, according to this study, young adults should have healthy eating habits and good lifestyle practices that give them good life satisfaction and a healthy body. Also, the majority of the participants regardless of gender; males and females showed that they are physically inactive and worried more about their everyday problems leading to depression and anxiety. Masella R *et al.*, reported findings

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similar to our study and claimed that more than 20 % of students have moderate levels of stress and depression which indicates some psychological issues in students. Our study also reflected that more males and females live with their parents as family participation and physical activity help students to practice a good lifestyle, and the type of living and sleeping hours determine the lifestyle of an individual [25, 26]. A study reported that males show healthy lifestyles and have good quality sleep as compared to females because most of the females have household responsibilities that show sleep changes in females [27].

CONCLUSIONS

Young individuals have a high frequency of unhealthy eating habits and lifestyles, and this is greatly influenced by gender. The findings of this study proved that the female gender has an unhealthy food choice predisposition and it does impact the activity status and lifestyle choices. Also, this study reported that regardless of gender; physical activity status remained unchanged. However, similar studies need to be conducted on a larger scale with reliable outcome tools to draw meaningful results that can be generalized across the globe.

Authors Contribution

Conceptualization: FMI[1], FMI[2]
Methodology: SUR, MA[1], MA[2]
Formal analysis: AF
Writing, review and editing: Ma[1]

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

Conflicts of Interest

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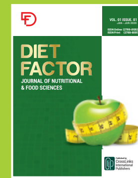

Original Article

Assessment of Nutritional Status among Tuberculosis Patients: A Survey-Based Study

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ABSTRACT

Tuberculosis (TB) remains a formidable global health challenge, representing a significant contributor to illness, mortality, and disability. Despite medical advancements, TB persists as the leading cause of death attributable to treatable infectious diseases, underscoring its enduring impact on public health worldwide. **Objective:** To assess the nutritional status among tuberculosis patients. **Methods:** A cross-sectional study encompassing both male and female participants was conducted. Out of the 205 participants involved, 115 were male and 90 were female, with an average age of 45 years. Our sample pool included both newly diagnosed and retreatment tuberculosis (TB) patients, and data gathering took place across various healthcare facilities within the Sahiwal district of Punjab, Pakistan. **Results:** Our research also found that patients with tuberculosis (TB) symptoms lasting over three months before diagnosis, were more prone to malnutrition compared to those with symptom durations. Additionally, our study indicated that 46.8% of the individuals involved in the research were malnourished. To sum up, our study underscores the link between health and TB among individuals. This study noted multiple factors like family size, income, education level, and other socioeconomic factors that affect tuberculosis outcomes and emphasized the importance of treatment outcomes. **Conclusions:** Detecting TB early and ensuring patients receive diagnosis and treatment is essential to prevent undernutrition from developing in TB patients.

INTRODUCTION

Tuberculosis remains a health challenge causing a significant effect on well-being, mortality, and incapacity worldwide despite developments in health science. Despite advancement, TB remains the cause of death due to curable infectious disorders emphasizing its persistent impact on public health internationally [1]. This challenge is impaired by expanding poverty levels, growing populations, and the existence of HIV/AIDS. The World Health Organization (WHO) has stressed the consequence of this issue leading to a rise in TB cases globally [2]. Many problems like poverty, lack of education, overpopulation, and nutritional status of the subjects, are linked with the incidence of tuberculosis (TB). Recent studies have proved that TB is related to the frequency of diseases with genetic susceptibility [3]. Malnutrition is considered one of the major reasons among them, influencing its impact by the development of latent tuberculosis disease into active disease and increasing the rate of disease recurrence [4].

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Various studies have shown that TB and malnutrition can coexist, both in developed and underdeveloped countries of the world [5]. Tuberculosis patients show poor nutritional status and play a key part in the healing of the disease and prognosis [6]. Poor nutritional status damages the immune system of the patients. There is a necessary to identify the relationship between malnutrition and tuberculosis and develop focused approaches to address both malnutrition and tuberculosis [7]. Moreover, the poor nutritional status contributed to latent tuberculosis infection repetition, strengthening the tuberculosis prevalence, especially when linked with HIV infection [8]. The assessment of nutritional status is necessary for the active therapy of disease and for reducing disease-related difficulties [9]. However, multiple indications shown by tuberculosis patients like reduced metabolism, lack of appetite, and reduced food intake, enhance the risk of death and delay recovery from the disease [6]. It is important to know the association between undernutrition and tuberculosis for the improvement of the treatment outcomes [10].

The objective of this study was to reveal potential risk factors such as age, gender, socioeconomic levels and education that might interact with the nutritional status of tuberculosis patients. The goal of the research was to find these correlations to advance data on the complex relationship between tuberculosis and malnutrition, which would assist develop tuberculosis prevention and management strategies.

METHODS

A cross-sectional study was conducted, including both males and females, for assessment of the members. The desired confidence level 95% which has a z-score of 1.96. A total number of 205 participants, who had an average age of 45, were included 115 males and 90 females. The data were gathered from patients with tuberculosis (TB) disease in the Sahiwal area of Punjab, Pakistan, across several healthcare centers. The study was conducted in 4 months. A questionnaire was designed to get data on nutritional status and its related determinants in TB patients through individually conducted interviews. The survey was categorized into five sections, each comprised of different criteria that were crucial for the study. The first section related to anthropometric measurements like waist circumference, mid-arm circumference, weight, and height. The second section examined the socio-demographic traits, and the third section was associated with questions for evaluating the participant's health. The fourth section identified the symptoms of tuberculosis disease, while the last section included questions about biochemical data. First, the nutritional status of the patients was evaluated by carefully weighing the subjects with the use of a standard balance, which was accurate near 0.1 kg. Similarly, the height of the patients was measured while they were standing, and the results were recorded to the closest 0.5 cm. Body mass index (BMI) was calculated using the formula: BMI = weight in kg / (height in meters)2. BMI charts were then used to identify the results. To further clarify the results of the nutritional health, measurements of the waist and mid-upper arm circumference (MUAC) were made and compared to the standard norms. To ensure data accuracy, definite procedures were applied. The relevant questionnaire was designed to ensure relevance and accuracy. The collection of data was strictly observed throughout the interviews, the procedure was reviewed for uniformity and completeness. The descriptive data were collected including age, sex, type of occupation, level of education, and ethnic group. The data of different variables was collected for statistical analysis using SPSS version 24.0 software. The study provided useful data related to nutritional status and the related variables that impact the health status of the patients.

RESULTS

Total 205 participants included males and females were provided data in this study. The number of males and females included were 115 and 90 respectively. The mean age of study participants was 45 years (18-92 Years). The results were calculated on the basis of objectives of study. Table 1 shows that 115 (56.1%) were males and 90 (43.9%) were females. Males were slightly higher than females. 186 (90.7%) participants were married and 19 (9.3%) were single. Among study participants 141 (68.8%) were uneducated, 30 (14.6%) were got primary education, 16 (7.8%) were got middle and only 18 (8.8%) participants were got matriculation or higher education. 87 (42.4%) females were house wives, 6 (2.9%) were government employee, 30 (14.6%) were farmer, 27 (13.2%) were unemployed, 26 (12.7%) were merchant and 29 (14.1%) choose the other option. Among participants 81 (39.5%) were from urban area and 124 (60.5%) were from rural area. 63 (30.7%) participants had 5 or less family members while 142 (69.3%) had more than 5 family members. Among participants 110 (53.7%) were earning less than 15000 rupees, 82 (40%) were earning from15000 to 30000 and only 13 (6.3%) were earning more than 30000 rupees monthly. 123 (60%) participants were smoking and 82 (40%) were not smoking. 28 (13.7%) participants were immunized with vaccine while 177 (86.3%) were not.

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Table 1: Socio-Demographic Characteristics of the Study Participants

Subject	Category	Frequency (%)
Age	< 25 y	16 (7.8)
	25-35 y	44 (21.5)
	36-45 y	54 (26.3)
	>45 y	91 (44.4)
Sex	Male	115 (56.1)
	Female	90 (43.9)
Marital Status	Married	186 (90.7)
	Single	19 (9.3)
Educational Status	Uneducated	141 (68.8)
	Primary	30 (14.6)
	Middle	16 (7.8)
	Higher Education	18 (8.8)
Occupation	House Wife	87 (42.4)
	Govt. Employee	6 (2.9)
	Farmer	30 (14.6)
	Unemployed	27 (13.2)
	Merchant	26 (12.7)
	Others	29 (14.1)
Residence	Urban	81 (39.5)
	Rural	124 (60.5)
Family Size	≤ 5	63 (30.7)
	>5	142 (69.3)
Income (Rupees)	<15000	110 (53.7)
	<30000	82 (40)
	>30000	13 (6.3)
Smoking	Smoking	123 (60)
	Not Smoking	82 (40)
BCG Vaccination	Vaccine	28 (13.7)
	Not Vaccine	177 (86.3)

Table 2 shows health status of the participants. 6 (2.9%) females were pregnant and 13 (6.3%) were feeding their children. 31 (15.1%) participants were diabetic. 39 (19%) of study participants showed the symptom of a cough or other TB symptoms within less than one month, 162 (79%) study participants were showed the symptom of a cough or other TB symptoms within one to three months and 4 (2%) of study participants were showed the symptom of a cough or other TB symptoms after three months before diagnosis of TB. 176 (85.9%) were new cases and 29 (14.1%) were retreatment cases. Among participants 99 (48.3%) were smear +ve and 106 (51.7%) were smear -ve. Among 166 (81%) patients were in intensive phase and 39 (19%) in continuous phase.

Table 2: Heath Status of Study Participants

Subject	Category	Frequency (%)
Pregnancy	Yes	6 (2.9)
	No	199 (97.1)
Lactation	Yes	13 (6.3)
	No	192 (93.7)
Diabetes	Yes	31 (15.1)
	No	174 (84.9)
CKD	Yes	6 (2.9)
	No	199 (97.1)
Symptoms Before Diagnosis	Less than a Month	39 (19)
	1-3 Month	162 (79)
	Above 3 Month	4 (2)
Form of TB	New Case	176 (85.9)
	Retreatment Case	29 (14.1)
Type of TB	Smear +ve	99 (48.3)
	Smear -ve	106 (51.7)
Anti-TB Rx Status	Intensive Phase	166 (81)
	Continuous Phase	39 (19)

Table 3 shows 200 patients were complaining of cough that lasts three or more months, 45 of coming blood with coughing, 191 were complaining of chest pain, 61 were complaining of night sweats, 139 for loss of appetite and 165 for unexplained weight loss.

Table 3: Clinical Data of Study Participants

Subject	Category	Frequency (%)
Coughing (3 or >3 Weeks)	Yes	200 (97.6)
	No	5 (2.4)
Coughing Up Blood	Yes	45 (22)
	No	160 (78)
Chest Pain	Yes	191 (93.2)
	No	14 (6.8)
Night Sweats	Yes	61 (29.8)
	No	144 (70.2)
Loss of Appetite	Yes	139 (67.8)
	No	66 (32.2)
Weight Loss	Yes	165 (80.5)
	No	40 (19.5)

Figure 1 shows that 96 patients were underweight, 107 were normal and 2 were overweight according to BMI.

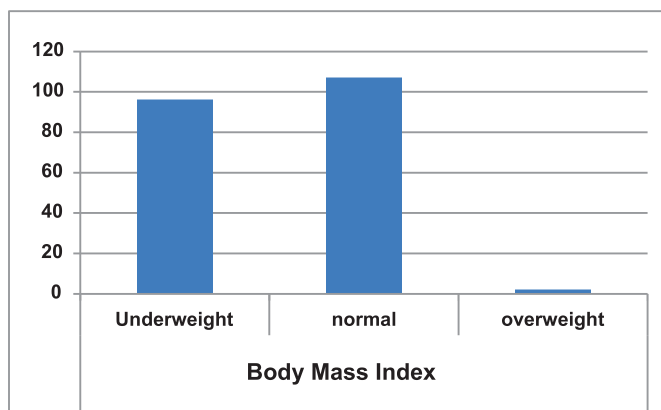

Figure 1: Body Mass Index (BMI)

94 were underweight and 111 were normal in accordance with Mid Upper Arm Circumference (MUAC) as shown in figure 2.

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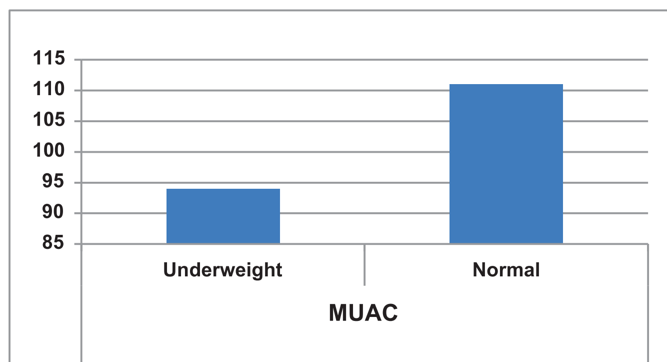

Figure 2: Mid Upper Arm Circumference (MUAC)

According to waist circumference 91 were underweight and 114 were normal (figure 3).

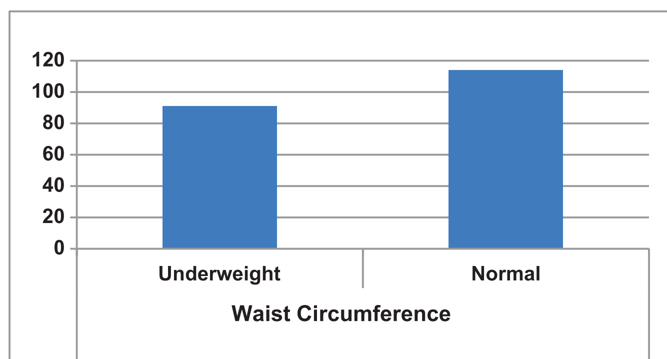

Figure 3: Waist Circumference

DISCUSSION

The main conclusion of our study underscored the implication of nutritional status as an independent variable associated with tuberculosis (TB) among individuals in Timor and Rote Islands [11]. A study has shown that many sociodemographic characteristics like large family size, family history of tuberculosis, non-indigenous ethnicity, and employment status were significantly related to tuberculosis [12]. Particularly, participants (46.8%) of our study were undernourished, which was consistent with results from a previous study conducted in Addis Ababa [13]. The prevalence of the disease may be explained by the region's public health facilities like medical support, high-quality treatment, and counseling services related to dietary choices. Our study is comparable with the results of previous studies. Studies in places like Gonder (Ethiopia), rural India, Uganda, Sekondi-Takoradi (Western region of Ghana), and Malawi reported higher rates of undernutrition, whereas studies conducted in Taipei, Taiwan, and Peru reported lower rates of undernutrition among adult tuberculosis patients compared to our study [14]. Different studies showed different results due to multiple reasons like lifestyle characteristics, dietary habits, socioeconomic and sociocultural factors, and the duration of the data collection [15]. This study reported that individuals were showed disease symptoms for more than three months are more prone to a high risk of undernutrition as compared to those whose symptoms lasted for a shorter duration. It is important to identify problems early, make a diagnosis, and recommend treatment and follow-up for the patients. Poor nutritional status is the major risk factor for the onset of tuberculosis [16]. Moreover, overpopulated and densely living areas are more susceptible to transmitting tuberculosis [17]. The results are consistent with other studies from Ghana and Indonesia that show an association between adult TB patients' undernutrition and the size of their families [18]. Compared to, the person with a high literacy rate shows greater adherence to drug consumption and follows a healthy diet. This shows that education level provides support against the prevention of the disease [19]. Extreme poverty is the major contributing factor to malnutrition, acting as a major risk factor for the development of tuberculosis [20]. This study showed that multiple factors like family size, income, education level, and other socioeconomic factors were associated with malnutrition in tuberculosis patients at the time of registration at the health center for treatment.

CONCLUSIONS

Our study shows a significant correlation between the nutritional status of an individual and tuberculosis. Different sociodemographic characteristics like extended families, nonindigenous ethnicities, employment status, and a family history of tuberculosis, were found to be associated with tuberculosis. This study noted multiple factors that affect tuberculosis outcomes and emphasized the importance of treatment outcomes.

Authors Contribution

Conceptualization: TA
Methodology: TA, HN, SQ, FQ, SM, ASQ
Formal analysis: SQ
Writing-review and editing: ISQ, AA, FQ, ASQ

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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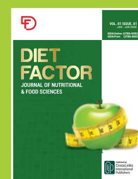

Original Article

Therapeutic Effect of Avishan-e-Shirazi (*Zataria Multiflora* Boiss) Root Extract on Oxidative Stress Markers in Moderate Asthmatic Patients

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ABSTRACT

Avishan-e-Shirazi (*Zataria Multiflora Boiss*) is a plant widely recognised for its medicinal and culinary purposes. **Objectives:** To evaluate the therapeutic effect of plant's root extract on pulmonary function tests and oxidative stress markers among moderate Asthma tic patients. **Methods:** A total participant of 66 patients with pulmonary disease, 30-40 age, were selected for the 2-month research trial. Individuals were enrolled and distributed equally in two groups, 33 each. In the control group, 53.8% of individual were man, and 46.2% were women. In the treatment group, 49.1% of participants were man, and 50.9% were women. The groups G0 was considered as a control group with no root extract, and group G1 where conventional treatment and root extract were advised to participants. The participants were selected from the Jinnah Hospital, Pakistan. The 5mg/kg/day dried root extract supplements 3 times a day were given to the individuals daily for 2 months. **Results:** The mean age group of Asthma tic patients enrolled in the study was 35 ± 2.98 years in G0, and in G1, it was 34 ± 3.05. The mean BMI was 31.34 and 30.98 kgm-2 in both groups, respectively. There was a significant improvement in FEV1 levels and MDA enzyme levels with a p-value less than 0.05. The NO2 levels were also better. Similarly, the enzymes thiolase and SOD levels also improved with a p-value less than 0.05 in the treatment group. **Conclusions:** The current study concluded that Avishan-e-Shirazi root extract could improve the pulmonary function and inflammation among Asthma patients.

INTRODUCTION

Sudden episodes of breath shortening characterise bronchial Asthma. The heaviness is felt around the chest area, and wheezing is caused by temporary constriction of the bronchial airways. This constriction is caused by muscle spasms, swelling of the mucosal lining, and an increase in sticky bronchial secretions, which result from an inflammation reaction in the bronchial walls [1]. Asthma is a common respiratory condition affecting millions of people globally. The current estimate is that around 334 million individuals live with Asthma; according to the latest record, the number of individuals with Asthma will be one hundred million. It will be the approximation for the year 2025. In Pakistan, the incidence of Asthma varies significantly between different regions, with reports showing a range from 4.3% to 31.58% [2]. Individuals with severe Asthma are at higher risk of experiencing a lower quality of life, permanent airway blockage, hospitalization, and even death. To lower the impact of the disease, biologics may be necessary [3]. Asthma symptoms can change over time and become more severe, leading to respiratory failure during episodes of exacerbation [4]. Asthma symptoms can be triggered by various factors,

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including infections, allergens such as pollens and molds, exercise, certain drugs can also trigger, and these drugs are anti-inflammatory and non-steroidal mostly, smoking, exposure to fumes from chemicals or herbs, emotional stress, contact with household pets, dust, and mites [5]. This disease has four stages, ranging from mild to moderate and intermittent to high-risk levels [6]. Asthma can present with symptoms such as wheezing, breathing difficulty, coughing, and heaviness around the chest area. All these symptoms are due to one main reason: the blockage of microtubules of the lung's airways [7]. Asthma is a frequent health issue in Pakistan that has become more prevalent lately. Research in the Journal of Pakistan Medical Association estimates that 5-7% of Pakistan's population suffers from Asthma. The research also discovered that children are more likely than adults to develop Asthma, with an estimated frequency of 9.8% in children ages 13 to 14 [8]. The most common cause of sudden Asthma exacerbations in children and adults, particularly children, is viral infections of the respiratory system. Up to 75% of wheezing episodes in infants and 50-70% in adults may be caused by viruses [9]. Most cellular intrudes include neutrophils, lymphocytes, eosinophils, mast cells, macrophages and basophils. Asthma heterogeneity is shown by the vast variations in these cell ratios across individuals [10]. Avishan-e-Shirazi is a plant widely recognised for its medicinal and culinary purposes and it is mainly grown in countries like Iran, Pakistan and Afghanistan [11]. Conventional medicine has been using the aqueous extract of Avishan-e-Shirazi for various therapeutic purposes, primarily for treating coughs, bronchitis, and other respiratory tract disorders. This native herb also caters the oral hygiene due to its bactericidal properties. Carvacrol, thymol, linalool, and p-cymene are the active compounds present in Avishan-e-Shirazi [12]. Due to their diverse pharmacological properties, natural products and their components are an essential source of potential new drugs. Avishan-e-Shirazi, for example, has been shown to have antimicrobial effects. Its nutrition composition showed that it contains 101 kcal, carbohydrates 24.45g, protein 5.56g, fat 1.68g, fiber 14g, vitamin A 1010 IU, vitamin C 160mg, vitamin K 1714.5µg, calcium 405mg, iron 17.45mg, magnesium 160mg, potassium 609 mg [11]. The use of Avishan-e-Shirazi in traditional medicine has been documented for centuries, and its pharmacological properties have been extensively studied [12]. In recent years, the plant has gained increased recognition for its medicinal properties. Various diseases can be targeted for treatment by this herb as it has anti-fungal and anti-inflammatory characteristics [13]. Avishan-e-Shirazi needs more inquiry through research as many of the valuable effects of this herb are unknown to science. The scientific knowledge is also insufficient on the mode of activity of plants. It is also important to note that Avishan-e-Shirazi should not be used as a substitute for conventional medical treatments, and individuals should always consult with their healthcare providers before using herbal remedies [14]. The use of Avishan-e-Shirazi in conjunction with other medications may also result in adverse effects, so it is essential to monitor its use and evaluate its potential benefits and risks carefully. These compounds have been shown to have relaxant effects on tracheal smooth muscle and to play a role in managing respiratory tract disorders [15]. The therapeutic effects of Avishan-e-Shirazi are likely due to its ability to modulate different physiological processes and its various pharmacological properties [16].

In this study, we focused on the therapeutic effect of Avishan-e-Shirazi root extract on the moderate asthmatic patients, who experienced symptoms and had nighttime awakenings more than once a week.

METHODS

We conducted randomized control trial (RCT) at Jinnah Hospital with the target population of patients with Asthma aged 30 to 40 years. The sample size was estimated using the method of dependent means, with pre and post treatment mean MDA values of -38.40 and 18.91, respectively, resulting in a calculated sample size of 33 in each group. Sixty-six individuals satisfied the criteria for our study and were distributed into two groups. Based on the sample population distribution, patients were assigned to either the treatment (n=33) or control (n=33) groups. The sample size was determined using the following formula:

$$t = \frac{\sum d}{\sqrt{\frac{n(\sum d^2) - (\sum d)^2}{n-1}}}$$

where d: difference per paired value
n: number of samples

Patient baseline characteristics, including anthropometrics and demographics measurements, were taken along with FEV1, oxidative stress, MDA, superoxide dismutase, thiol, nitrite and BMI. The parts of Avishan-e-Shirazi were separated, washed and air-dried. Furthermore, it was milled and extracted by percolation method performing using 1000 ml of ethanol 70% at room temperature for 72 hrs. After filtration, ethanol was evaporated at 40°C in a rotary. After that, solvent evaporation was performed by vacuum desiccator for 24 hrs, and the dried extract was stored at -20c the efficiency of this method was 16.5% [17]. The Avishan-e-Shirazi root extract provided to patients was prepared in the lab following a standard procedure to ensure uniformity in extract intervention as shown in table 1.

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Table 1: Treatment Details

Variables	G_0 Control Group (No Treatment)	G_1 Treatment Group (Avishan-e-Shirazi Root Extract)
Dosage	-	5mg/ kg Avishan-e-Shirazi Root Extract
Duration	8 Weeks	8 Weeks
Frequency	-	3 Times a Day
Target Population	30-40 Years	30-40 Years

Participants were enrolled in the study if they met the inclusion criteria. Blood samples and spirometry levels (which gauge the rate of airflow) were taken from Asthmatic patients and healthy volunteers. The plasma layer was separated by centrifugation and measured oxidative stress markers was collected by the biology of stress tolerance lab, IMBB lab assistant, The University of Lahore, Lahore. All participants gave written informed consent (attached), and the information and data obtained were absolutely confidential. Participants remained anonymous throughout the experiment and were informed that they might withdraw at any time during the procedure. The experiment lasted around nine months.

RESULTS

The mean age of G_0 (control group) individuals was 35 ± 2.98, and that of G_1 (treatment group) participants was 34 ± 3.05. Individuals mean weight in G_0 was 78 ± 3.98, and in G_1 was 75.98 ± 2.67. The maximum weight observed in G_o and G1 were 92 and 95 kg respectively. The study had a total of 66 participants enrolled and distributed equally in two groups with the control group, 53.8% of participants were men, and 46.2% were women. In the treatment group, 49.1% of participants were men, and 50.9% were women respectively. The mean BMI of individual in groups G_0 and G_1 was 31.34 ± 1.56 kg/m2 and 30.98 ± 1.90 kg/m2 respectively. The maximum BMI of individuals observed in groups G_o, and G_1 was 36.4 and 37.6 kg/m2, respectively. The mean FEV1 of patients in G_0 was 37 ± 1.9 as compared to 41 ± 2.1 among patients in G_1 before treatment, with the mean MDA of patients in G_0 being 2.70 ± 0.1 as compared to 2.40 ± 0.1 among patients in G before the treatment (table 2).

Table 2: Characteristics of Asthma Patients Before Intervention

Variables	Control Group Mean ± SD	Treatment Group Mean ± SD
Number of Patients (N)	33	33
Age	35 ± 2.98	34 ± 3.05
Height (cm)	159.45 ± 3.34	163.45 ± 4.19
Weight (kg)	78 ± 3.98	75.98 ± 2.67
Gender (N)	M=18, F=15	M=16, F=17
BMI (Kgm-2)	31.34 ± 1.56	30.98 ± 1.90
(FEV1 %)	37 ± 1.9	41 ± 2.14
MDA (μmol/L)	2.70 ± 0.15	2.40 ± 0.1
Nitrogen Dioxide (NO2) (ppb)	6.50 ± 0.33	5.90 ± 0.30
Thiol	0.45 ± 0.02	0.50 ± 0.03
Superoxide Dismutase (SOD) (U/ml)	154 ± 1.7	157 ± 1.85

The change was noticed in the parameters of individuals post intervention of Avishan-e-Shirazi root extract 5gm daily. Improvement was seen in FEV1 oxidative stress which is explained in table 3.

Table 3: Characteristics of Individuals Pre and Post-Avishan-e-Shirazi Root Extract Treatment

Variables	Control Group Mean ± SD			Treatment Group Mean ± SD			p-value
	Pre-Intervention	Post Intervention	% Change	Pre-Intervention	Post Intervention	% Change	
(FEV1 %)	37.25 ± 1.9	39.2 ± 2.81	5.26%	41.18 ± 2.13	46.5 ± 4.30	11.4%	0.30
MDA (μmol/L)	2.70 ± 0.14	2.45 ± 0.12	16%	2.49 ± 0.19	1.80 ± 0.12	28.5	0.07
Nitrogen Dioxide (No2) (ppb)	6.50 ± 0.33	6.20 ± 0.31	4.72%	5.90 ± 0.34	4.60 ± 0.23	24.7%	0.04
Thiol	0.45 ± 0.02	0.49 ± 0.02	8.5%	0.50 ± 0.03	0.58 ± 0.05	14.8%	0.00
Superoxide Dism-utase (SOD) (U/ml)	154.52 ± 1.73	161.70 ± 2.05	4.4%	157.18 ± 1.85	169.16 ± 2.45	7.36%	0.007
TNF- α (Pg/ml)	16.58 ± 1.33	17.80 ± 2.76	-	18.41 ± 1.61	15.15 ± 2.14	-	0.00

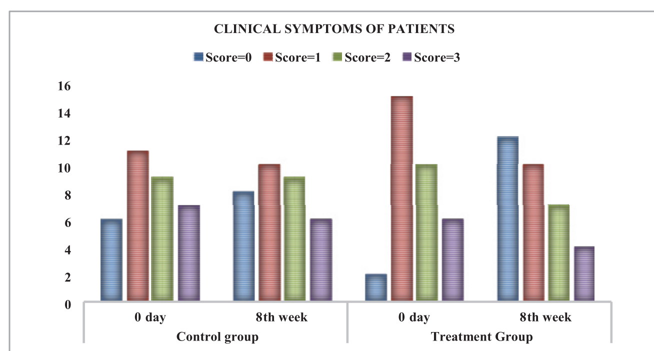

Figure 1: Clinical Symptoms of Patients in a Different Groups

Score 0= no wheezing and cough, Score 1= good sleep with slight wheezing and cough, Score2= wake up once a night, score 3= wake up more than once at night

DISCUSSION

Avishan-e-Shirazi (*Zataria multiflora*) and its components have been shown to have spasmolytic and anti-tussive characteristics that can be used to treat respiratory tract problems. A clinical trial has demonstrated the Avishan-e-Shirazi efficacy in treating acute cough both on its own and in combination with other plants significantly improved [18]. A study conducted in Iran evaluated that *Z. multiflora* is

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widely used in Iranian traditional medicine for the treatment of cough, chest problems, oral cavity infection, dyspepsia, and other problems of the respiratory system [19]. In our study, the mean FEV1 of patients in G_0 was 37 ± 1.9 as compared to 41 ± 2.1 among individuals in G_1 pre the treatment, whereas the mean FEV1 of group G_0 and G_1 post treatment of Avishan-e-Shirazi was 42 ± 2.8 and 46 ± 4.3 respectively. A study conducted to determine the effect of *Z. multiflora* on wheezing and FEV1 along with plasma levels of nitrite included 40 Asthmatic patients who were randomly divided to determine the dosage effect of *Z. multiflora* on respiratory health. they were given different dosages of multiflora in a double-blind manner to increase the efficiency and durability of the test. Forced expiratory volume in 1 second, NO_2 and wheezing during exercise bout or throughout the day were measured at the advent of treatment, which was the baseline measurement (pre-treatment), one month after the treatment and at the end of treatment was recorded to assess the efficacy of the treatment provided in different dosages. After the study, it was elucidated that FEV1 % was significantly improved in the study participants with the intervention of *Z. multiflora* as compared to a control group with p-values ranging from < 0.01 to <0.001, indicating that the results were statistically significant. FEV1 was 7.9 ± 7.16 in the treatment group of low *multiflora* dosage, which is 5.0mg/ day at baseline, which improved to 8.91 ± 6.36 after treatment which showed a difference of 1.01 ± 3.17 indicating improvement in FEV1 with the p-value was <0.05 showing significant results. The second group with a 10mg/day dosage of multiflora had FEV1 9.84 ± 19.68 in the advent which altered to 12.28 ± 9.88 after the intervention. It showed an improvement of 3.72 ± 11.7 with p-value <0.001 among the study participants, indicating the potential beneficial impact of the root as all the other factors were kept constant in the study [20]. The mean NO_2 of patients in G_0 was 6.50 ± 0.33 as compared to 5.90 ± 0.30 among individuals in G_1 pre the treatment, whereas the mean NO_2 of group G_0 and G_1 post treatment was 6.20 ± 0.31 and 4.6 ± 0.23 respectively. A study conducted to determine the effect of *Z. multiflora* on wheezing and FEV1, along with plasma levels of nitrite, included 40 Asthmatic patients. The mean age of participants was 46.6 ± 1.23 years, with Asthma severity of 4.5 ± 3.06. The participant was randomly divided into four groups in a double-blind manner to validate the study results. The aim was to determine the dosage base and overall effect of *Z. multiflora* on respiratory health. After the study, it was concluded that NO2 was significantly improved in the study participants with the intervention of *Z. multiflora* compared to a control group with p value ranging from < 0.01 to <0.001, indicating that the results were statistically significant. NO_2 was -4.33 ± 37.79 in the treatment group of low multiflora dosage, which is 5.0mg/ day at baseline, which decreased to -5.59 ± 37.03 after treatment, which showed the difference of 1.33 ± 11.59 indicating improvement in NO_2 with the p-value was <0.05 showing significant results. The second group with 10mg/ day dosage of *Z. multiflora* had -4.23 ± 8.91 in the advent, which altered to -16.77 ± 12.85 after the intervention. it showed an improvement of -13.15 ± 9.61 with p-value <0.01 among the study participants, indicating the potential beneficial impact of the root as all the other factors were kept constant in the study [20].

CONCLUSIONS

The current study concluded that Avishan-e-Shirazi root extract effectively improves Asthma symptoms among patients. This study manifested significant decrease in inflammatory markers. There was no significant total difference in BMI and weight changes between study participants. Avishan-e-Sherazi root extract showed emerging results with natural treatment. However, the mechanism of action of this extract in Asthma symptoms is still unknown. Further research is needed to understand its therapeutic mechanisms and to develop new treatments based on its extracts.

Authors Contribution

Conceptualization: MA
Methodology: MI
Formal analysis: SM
Writing-review and editing: FK, NF, NAF, AH, AA

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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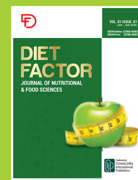

Original Article

Determination of Aflatoxin in Various Spices Samples and its Detoxification using Black Seed Oil: A Biological Approach

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ABSTRACT

Aflatoxins are poisonous compounds generated by specific fungal species that are naturally occurring everywhere and are essentially inevitable. They can seriously endanger human health by contaminating food crops. Aflatoxin contamination of spices is a serious worldwide problem that affects trade and they are cited as the first significant risk in border rejection. **Objectives:** The objectives of this study were to ascertain the aflatoxin content of different spices samples and to use varying concentrations of black seed oil to detoxify positive samples. **Methods:** Thin Layer Chromatography (TLC) was used to determine the aflatoxins in various spices and contaminated sample were detoxified by black seed oil. **Results:** From this study aflatoxins were detected in 70% and 30% spices samples have no aflatoxin. Among contaminated samples 43% had aflatoxins beyond the permissible limits whereas 57% had the aflatoxins below the permissible limits. The positive samples were alleviated by biological method i.e. black seed oil (1-10%) which detoxified aflatoxin in fennel 49.52 ± 1.50 – 92.50 ± 1.94% and detoxification level was found in cumin seed 55.37 ± 1.52 – 87.32 ± 1.83 while in fenugreek it was ranging from 50.20 ± 1.50 – 82.37 ± 1.75. **Conclusions:** This study showed that black seed lowered aflatoxin levels in some spices.

INTRODUCTION

Some types of mould in grains, nuts, spices, and dried fruits contain mycotoxins, which are harmful fungi metabolites. About 300 distinct fungus species, including *Claviceps*, *Fusarium*, *Aspergillus*, *Alternaria*, and *Penicillium*, are known to produce mycotoxins on various substrates due to inappropriate moisture content and temperature. Animals that eat feed tainted with mycotoxin may develop problems with growth, reproduction, or possibly pass away. The majority of these mycotoxins, which are chemically stable and dangerous to humans and animals, are aflatoxins, ochratoxins, zearalenone, nivalenol, deoxynivalenol, citrinin, fumonisins, and patulin, among other strong toxic mycotoxins[1-3]. Mycotoxins have harmful properties such as being immunosuppressive, mutagenic, carcinogenic, teratogenic, and toxic [4]. These genotoxic substances affect the kidneys, liver and immune systems, among other organs. Liver necrosis, anorexia, vomiting, diarrhea and fatty liver are some of their symptoms. Their effects on the reproductive system include a reduction in the percentage of viable sperm, delayed testicular growth, and a drop in testosterone plasma concentration and reduced resistance to bacterial, fungal, parasitic

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andencephalopathy and interstitial fibrosis are two additional associated symptoms [5, 6]. Furthermore, because mycotoxins are frequently combined in food, humans are exposed to many mycotoxins at once [7]. They have the potential to contaminate a broad variety of agricultural and food items, such as grains, oilseeds, spices, tree nuts, and dairy products [8]. Fungal species including Aspergillus flavus, A. parasiticus, and A. specific create aflatoxin, a low-molecular-weight secondary metabolite that is extremely poisonous, carcinogenic, and mutagenic. These fungi are most commonly found in hot, humid climates and are known to produce aflatoxin in food and feed. They are present in soil, plants, animals, and many types of nuts. They are especially prone to infect grains, including wheat, soybeans, rice, corn, peanuts, spices, and oil seeds [9]. Spices have long been known to have therapeutic properties, and research on their capacity to transfer biological activity is gradually making a comeback in the field of human health [10]. Due primarily to their phenolic content, these spices have already been employed as raw materials for a wide range of industries (such as the food, pharmaceutical and cosmetic sectors) as flavorings, food colorings, essential oils, sweeteners, and even for their nutraceutical properties. Spices are becoming more and more popular for their creative application as a source of these naturally occurring bioactive chemicals, along with the growing trend towards the consumption of more natural and ecologically friendly meals [11]. The optimal parameters for the development of fungi, and ultimately the production of aflatoxin, are 25–33 °C, 16–30% moisture content, and 80–100% relative humidity [12]. If the spices are subjected to a fungal contamination during the production process, marketing, post-harvest procedures, farming and processing they are hazards and regrettably, a lot of spices are prone to aflatoxins contamination and are highly vulnerable to toxic fungal strains [13]. A number of techniques have been used to stop the synthesis of aflatoxins or to eliminate, inactivate or reduce their bioavailability in contaminated foods. Aflatoxin detoxification can be achieved by physical (UV light, heat, or ionizing radiation), chemical (adding hydrolytic, chlorinating, or oxidizing agents), or biological techniques [14, 15]. However, due to drawbacks of physical and chemical techniques including the loss of nutritional value, the need for costly equipment as well as their detrimental impacts on humans is rising [16, 17]. This is claimed to be safe, non-pathogenic for human and to maintain the nutritional value of food [18, 19].Biological control is an emerging approach for the degradation of toxins with no threats to health or food material and can significantly reduce 20-90% of infections [20]. *Nigella* sativa, also known as black seed or kalonji, is a member of the *Ranunculaceae* family and is considered lucky to have several biologically active compounds that have been shown to have a range of medical benefits, including antibacterial, immune-modulating, antioxidant, and anti-inflammatory effects [21]. Black seed oil's antifungal properties show considerable potential for *A. flavus* prevention andits oil's minimum inhibitory concentration value is significantly lower than that of other essential oils, making it economically viable for the suppression of food-borne fungi and the biological treatment [22].

The main objectives of this study was to determine the aflatoxin in various spices samples that were collected from the local market and to detoxify highly positive sample using a biological approach i.e. varying concentrations of black seed oil.

METHODS

Collection of Spice Samples

This study was done in Food and Biotechnology Research Centre, PCSIR Laboratories Complex's in Lahore. A total ten spice samples (red pepper, turmeric clove fennel cumin seed fenugreek black pepper cinnamon garlic and ginger) were collected from local market, Lahore. After identification, the entire spices were ground using an electric grinder and stored in a polythene bag for further study.

Aflatoxins Extraction and Analysis

Aflatoxin extraction from spices was done by using the AOAC (2023) technique [23]. Briefly 50g sample of each spices were put in a conical flask and mix it with 200 ml of solvent (aceto-nitrile: water, 9:1). Shake it in an orbital shaker for half an hour at room temperature. Whatman filter paper No. 4 was used to filter the extract. Then the filtrate was evaporated in a rotary evaporator and residue was kept for further analysis. This residue was redisolved in known concentration of chloroform for TLC.

Thin Layer Chromatography (TLC)

Using a micro syringe, samples 5, 10, 15, 20, and 25μl were spotted on a TLC plate at a distance of almost 1.5 cm from the base. In a similar manner, standard spots of 1, 2.5, and 5μl were completed. Following spotting, the plate was placed in the tank which containing a mobile phase anhydrous diethyl ether and was developed, dried and then added to the second tank, which had acetone-chloroform (1:9, v/v) as a second mobile phase. The plate was examined at 365 nm UV light to decide whether aflatoxins were present or not. The sample color and Rf values correspond to the aflatoxin standard [24].

Calculations:

The following formula was used to calculate the aflatoxin concentration:

Contents of aflatoxins (μg/kg) = $S \times Y \times V / W \times Z$

Where:

S = Aflatoxin standard volume in μl

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Y= Aflatoxin concentration in mg/ml of the reference standard
Z = The volume of sample extract (µl) needed to produce the desired level of fluorescence to that of S = ml of aflatoxins standard which was determined under UV.
W = Effective Weight, in gram, of original sample contained in final extract
V = Volume, in ml, of solvents (chloroform), needed to dilute final extract

Detoxification by Biological Method

To detoxify a sample contaminated with aflatoxin, black seed oil was utilized which was extracted from black seed by soxhlet apparatus. Fifteen gram of contaminated fennel sample were combined with 100 ml of 1%, 2%, 3%, 5%, and 10% black seed oil in a fume hood and left for six hours at 25 ºC. After being shook for three minutes, it was filtered and then redissolved in aceto-nitrile to be spotted on a TLC plate for aflatoxin analysis.

Statistical Analysis

The trials were conducted in triplicate (n=3), and the data was presented as mean ± SD. ANOVA techniques were used for analysis of variance.

RESULTS

Ten samples were chosen at random from Lahore's surrounding market. Aflatoxin B1 was quantitatively examined utilizing the Thin Layer Chromatography (TLC) method. Three of the ten local spices samples were found to be free of aflatoxin contamination, whereas the remaining seven samples were found to be contaminated with aflatoxin B1. Four samples had aflatoxin contamination levels below acceptable limits and three samples had contamination levels above acceptable limits as determined by the European Commission's allowed limit of aflatoxin contamination (10 ppb) for spices and (50 ppb) for red pepper. Aflatoxin B2, G1 and G2 were not detected in any spices sample. The following table 1 displays the aflatoxins' TLC results.

Table 1: Detection of Aflatoxin in Various Spices Samples for by TLC

Sample ID	Samples Names	Aflatoxins (ppb)				Permissible (EU limits)	Status
		B1	B2	G1	G2		
1	Red pepper	40.82 ± 1.38	ND	ND	ND	50 ppb	Fit
2	Turmeric	6.27 ± 0.08	ND	ND	ND	10 ppb	Fit
3	Clove	ND	ND	ND	ND	10 ppb	Fit
4	Fennel	70.50 ± 1.6	ND	ND	ND	10 ppb	Unfit
5	Cumin Seed	9.86 ± 0.12	ND	ND	ND	10 ppb	Unfit
6	Fenugreek	20.42 ± 0.42	ND	ND	ND	10 ppb	Unfit
7	Black Pepper	ND	ND	ND	ND	10 ppb	Fit
8	Cinnamon	ND	ND	ND	ND	10 ppb	Fit
9	Garlic	2.17 ± 0.06	ND	ND	ND	10 ppb	Fit
10	Ginger	8.68 ± 0.10	ND	ND	ND	10 ppb	Fit

*ND means Not Detected

In this study aflatoxin contaminated samples were decontaminated using a biological approach i.e. different concentrations of black seed oil (1–10%) and the outcomes demonstrated that every concentration in the harmful sample of fennel, ranging from 49.52 ± 1.50 – 92.50 ± 1.94%, eliminated aflatoxin. These findings showed that 10% black seed oil was the most successful treatment, reducing AFB1 by up to 92.50 ± 1.94% (Table 2) in fennel sample.

Table 2: Detoxification of Aflatoxins in Fennel Sample by Black Seed Oil

Concentration of Black Seed Oil for Detoxification of AF	Initial Levels (ppb)	Levels after Detoxification (ppb)	Reduction (%)
Black Seed Oil (1%)	70.50 ± 1.60	35.61 ± 1.32	49.52 ± 1.50
Black Seed Oil (2%)	70.50 ± 1.60	28.21 ± 1.02	60.03 ± 1.55
Black Seed Oil (3%)	70.50 ± 1.60	20.45 ± 0.40	71.01 ± 1.62
Black Seed Oil (5%)	70.50 ± 1.60	7.92 ± 0.08	88.76 ± 1.84
Black Seed Oil (10%)	70.50 ± 1.60	5.29 ± 0.06	92.50 ± 1.94

This treatment was also done with the other contaminated samples (cumin seed and fenugreek) and detoxification level was found in cumin seed 55.37 ± 1.52 – 87.32 ± 1.83% (Table 3) while in fenugreek it was ranging from 50.20 ± 1.50 to 82.37 ± 1.75% (Table 4).

Table 3: Detoxification of Aflatoxins in Cumin Seed by Black Seed Oil

Concentration of Black Seed Oil for Detoxification of AF	Initial Levels (ppb)	Levels after Detoxification (ppb)	Reduction (%)
Black Seed Oil (1%)	9.86 ± 0.12	4.40 ± 0.086	55.37 ± 1.52
Black Seed Oil (2%)	9.86 ± 0.12	3.82 ± 0.06	61.25 ± 1.56
Black Seed Oil (3%)	9.86 ± 0.12	3.04 ± 0.04	69.17 ± 1.60
Black Seed Oil (5%)	9.86 ± 0.12	2. 18 ± 0.02	77.89 ± 1.75
Black Seed Oil (10%)	9.86 ± 0.12	1.25 ± 0.01	87.32 ± 1.83

Table 4: Detoxification of Aflatoxins in Fenugreek by Black Seed Oil

Concentration of Black Seed Oil for Detoxification of AF	Initial Levels (ppb)	Levels after Detoxification (ppb)	Reduction (%)
Black Seed Oil (1%)	20.42 ± 0.42	10.15 ± 1.32	50.20 ± 1.50
Black Seed Oil (2%)	20.42 ± 0.42	8.50 ± 1.02	58.37 ± 1.53
Black Seed Oil (3%)	20.42 ± 0.42	6.75 ± 0.40	66.94 ± 1.58
Black Seed Oil (5%)	20.42 ± 0.42	5. 02 ± 0.08	75.41 ± 1.67
Black Seed Oil (10%)	20.42 ± 0.42	3.60 ± 0.06	82.37 ± 1.75

DISCUSSION

Thin layer chromatography is still used for both qualitative and quantitative mycotoxin analysis. The primary reasons for this are the low operating costs, the large sample throughput, and the simplicity of target compound identification utilizing UV-Vis spectral analysis [25, 26]. TLC techniques were employed in a number of investigations to quantify the quantities of aflatoxins (B1, B2, G1 and G2) in spice samples. Hussain *et al.*, in 2023 employed the TLC to determine the amount of aflatoxins in feeds [27]. This TLC method is widely used and highly

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beneficial for determining the aflatoxin levels in spices [28]. Mycotoxins are dangerous and thermo-stable secondary metabolites of fungi that can penetrate food and feed and withstand a range of food microbiological stabilization techniques, such as heating [29]. Consequently, contaminated food and feed exposes humans and animals to negative effects. These can appear on a wide range of foods, such as grains, crops, nuts, fruits, and dried fruits, cheese, and spices, at any point during storage, harvesting and production [30]. Among mycotoxins, aflatoxins are the most dangerous. Currently, a variety of methods (physical, chemical, and biological) are used to detoxify and decontaminate aflatoxins from food and feed [31]. The industry does not use physical or chemical methods due to their high cost, negative effects on texture and taste, and reduction in nutritional value [32]. The majority of researchers have determined that biological methods are the most effective means of decontaminating aflatoxins [33]. Additionally, it was reported that biological methods have been deemed the most effective due to their high efficiency, low cost, eco-friendliness and ability to maintain nutritional quality, when compared to physical and chemical, methods used to prevent the production, reduction, elimination and deactivation of aflatoxin in contaminated food [34]. By using biological techniques for the detoxification of aflatoxins it may not alter the organolaptic properties of food items [35]. Mycotoxins can be absorbed by living or dead microorganisms and stored in their bodies or on their cell walls. As a result of degradation, extracellular or intracellular enzymes can carry out enzymatic degradation. Enzymatic alterations have the ability to alter, diminish, or eliminate toxicity in this manner [36]. It is also believed that moulds that produce aflatoxins break them down through the action of the peroxidase enzyme in the mould mycelia. Aflatoxin reaction with free radicals occurred due to the breakdown of hydrogen peroxides, which is catalyzed by peroxidase. In the presence of hydrogen peroxide and chloride ions, certain peroxidases, such myeloperoxidase, generate hypochlorite and singlet oxygen which efficiently eliminates aflatoxins [37]. In 2021, Nazir *et al.*, also utilize the black seed oil for detoxification of aflatoxin in rice and feed samples and detoxification level was found to 63–100% [38]. In another investigation, black seed oil was applied to contaminated wheat samples which decreased the aflatoxin level up to 81-87% [39]. Black seed oil was used to treat tainted spices in order to detoxify aflatoxin B1. It was discovered that this oil was incredibly effective, reducing contamination levels by 92%. In the black seed oil numerous bioactive compounds and antioxidants found when it was treated with aflatoxin contaminated samples, these bioactive compound showed the inhibitory effects that may decrease the aflatoxin levels. Moreover this oil is well-known for its antifungal properties against a wide variety of fungus. For instance, reported that black seed oil was effective at 0.15%, completely inhibiting *F. moniliforme* and *A. alternata* at doses of 0.1% and 0.15% [40-42].

CONCLUSIONS

This study reveals that the biological method using 10% black seed oil for detoxification of aflatoxin in spices is very effective.

Authors Contribution

Conceptualization: MKS
Methodology: SA
Formal analysis: NZ, ZH, AM, KR
Writing-review and editing: MKS, IS

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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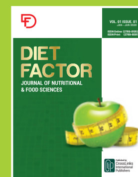

Original Article

Shelf Life Extension of Fresh Cut Carrot by the Application of Cinnamon Extract Infused Edible Coating

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ABSTRACT

The consumer demand for fresh cut fruits and vegetables is increasing rapidly owing to fast paced life style changes. The main problem with fresh cut fruits and vegetables is deterioration in term of color, taste, firmness etc. To solve these issues, researcher and processors have been using edible coatings to maintain quality of fresh cut commodities. **Objective:** To assess the potential effects of cinnamon extract infused edible coating formulated for shelf life extension of fresh cut carrots. **Methods:** Cinnamon extract was added as an antioxidant, aimed to aid in shelf life extension of fresh cut carrots. Cinnamon extract was obtained in an aqueous medium. The treatments were stored at 5oC for 21 days for shelf life study. The treatments were assessed for weight loss%, %acidity, TSS, firmness, color and ascorbic acid content at 7 days. **Results:** Coated treatments had better quality after storage period in term of wt. loss %, firmness and color, as compared to non-coated control treatments. **Conclusions:** Result suggests that edible coating enhanced with antioxidants have the potential to extend shelf life of fresh cut fruits and vegetables.

INTRODUCTION

Elevated household income, bustling urban lifestyle, and the expansion of cold chain facilities have collectively contributed to a surge in the demand for convenience foods, such as minimally processed or fresh-cut fruits and vegetables. Fresh-cut products are defined as items that have been prepared and are ready for cooking or consumption while still retaining the fresh attributes of raw produce. The vulnerability of fresh-cut vegetables to deterioration arises from factors such as browning, dehydration, microbial invasion, and the decline in nutritional value, primarily stemming from tissue damage during preparation. Furthermore, rapid respiration, increased enzyme activities, and the consequent microbial growth worsen the deterioration of fresh-cut vegetables [1]. Carrot, scientifically known as *Daucas carota L.*, is a widely consumed taproot vegetable either in its raw state or when cooked. The vegetable is notably abundant in β-carotene, a precursor of vitamin A, as well as various minerals and phenolic antioxidants [2]. The quality of fresh-cut carrots deteriorates primarily due to alterations in color, texture, smell, and biochemical attributes [3]. Research conducted by highlighted the utilization of hygroscopic salts like calcium lactate or calcium chloride to maintain the freshness of fresh-cut carrots [4].

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Additionally, some studies, such as those by have proposed the use of edible coatings. However, concerns have been raised regarding the safety of chemical additives, prompting consumers and researchers to seek natural alternatives for the preservation and enhancement of food quality [5-7]. The utilization of edible coatings has been documented as a means to mitigate the adverse impacts of minimal processing. Edible coating has emerged as a promising technique for the preservation of food and is characterized as "thin layers of materials that envelop food surfaces, can be consumed, and are regarded as integral components of the overall food item" [8]. The fundamental purpose of these coatings is to establish a partially permeable shield against oxygen, carbon dioxide, and moisture losses, thereby eliciting a comparable effect to storage in a modified atmosphere. Additionally, these edible coatings furnish supplementary nutrients, enhance quality, and are acknowledged as sensory enhancers. Food-grade solvents, fillers, and binding agents are the main ingredients of edible coatings. The incorporation of polysaccharide, protein, lipids, bioactive, and composite-based elements into an edible coating matrix helps to improve the minimally processed food's quality and mitigate significant post-harvest losses of highly perishable commodities[9].

Consequently, this study was conducted to establish a cost-effective and straightforward pretreatment method to enhance the shelf life of fresh-cut carrots, as well as to assess the variations in physical, biochemical, and sensory characteristics throughout the storage period of the coated fresh cut carrots.

METHODS

In this research experiment, carrot samples were evaluated to study the effect of designed treatments throughout the storage period. The tests were carried out in laboratories of Post-Harvest Research Centre, Ayub Agricultural Research Institute, Faisalabad. For this purpose, carrots were collected from field at the time of maturity and immediately transported to the laboratory. The carrots were washed and cut into uniform pieces having approximately 8 ± 2 mm thickness and 40 ± 10 mm length. Cinnamon aqueous extract was prepared using method described by with some modifications [10]. 250-gram cinnamon was added in 1 litre distilled water and heated at 70oC for 40 minutes. Afterwards it was filtered and stored in glass bottle for further use. Then the samples were dipped in coating solutions for 5 minutes prepared according to the treatment plan. Coating solutions were prepared with varying concentrations of cinnamon extract i.e. 0.5%, 1.0%, 1.5%, 2.0% while concentrations of pectin (2%) and glycerol (1%) remained unchanged in all treatments. After dipping, carrots were air dried at room temperature for 10 hours and then sealed in air tight zipper bags and stored at 5° C for 21 days. The tests for firmness, acidity, Total Soluble Solids (TSS), colour and ascorbic acid (mg/100ml) were done after 7 days' interval. All the tests were performed in triplicates. The firmness of carrot pieces was measured using a penetrometer with plunger size of 3mm. After placing the carrot piece on a hard surface, force was applied which caused the plunger to penetrate through the carrot's surface. This force was measured as carrot firmness in kg/cm2 [10]. The acidity was determined by following titration method of [11]. Carrot juice was prepared and 10ml of the sample juice was mixed with 100ml distilled water. This solution was titrated against 0.1M NaOH with 1% phenolphthalein as an indicator. Light pink colour appeared indicating the end point which lasted for almost 10 seconds. Carrot juice was prepared and total soluble solids in the juice were determined through digital refractometer (HI 96801). TSS values were measured in Brixo [12]. The ascorbic acid content in fresh-cut carrot slices was determined by using 2,6-dichlorophenol indophenol dye following the method used in the study [13, 14]. In short, a 4% oxalic acid solution was used to extract ascorbic acid from carrot juice which was then titrated against the dye until a light pink colour was achieved. The volume of dye used was noted down. The ascorbic acid content was calculated in mg per 100g of the sample by using the following formula:

$$\text{Dye Factor} = \frac{0.05}{\text{Titre}}$$

$$\text{Vitamin C (mg/100g)} = \frac{\text{dye factor} \times \text{titre} \times \text{volume of sample made}}{\text{Weight of sample} \times \text{volume of extract taken}} \times 100$$

RESULTS

Mean acidity values of fresh-cut carrot were statistically significant ($p < 0.05$) as indicated in table 1. An increase in acidity was observed in all treatments throughout the storage. The lowest acidity was reported at 0 day (0.71%) whereas the highest acidity was observed at 21 days of storage (1.76%) in T1 (Table 1).

Table 1: Acidity of Fresh Cut Carrot

Treatments	Days of Storage (Mean ± SD)			
	0	7	14	21
T_o	0.71 ± 0.01^l	0.98 ± 0.01^j	1.43 ± 0.01^d	1.70 ± 0.02^b
T_1	0.71 ± 0.01^l	0.88 ± 0.01^k	1.09 ± 0.01^i	1.76 ± 0.01^a
T_2	0.71 ± 0.02^l	1.12 ± 0.02^h	1.15 ± 0.01^g	1.36 ± 0.02^e
T_3	0.71 ± 0.02^l	1.35 ± 0.01^e	1.41 ± 0.01^d	1.54 ± 0.01^c
T_4	0.71 ± 0.01^l	1.14 ± 0.01^{gh}	1.24 ± 0.01^f	1.52 ± 0.02^c

T_o=0.5% Cinnamon extract, T_1= 1% Cinnamon extract, T_2= 1.5% Cinnamon Extract, T_3= 2% Cinnamon Extract, T_4= 2.5% Cinnamon Extract

Table 2 showed the changes in TSS of fresh cut carrot during storage. TSS increased in all treatments with minimum value of 7.33 at 0 day and maximum value of 11.03 at 21 days in To (Control) (Table 2).

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Table 2: TSS of Fresh Cut Carrot

Treatments	Days of Storage (Mean ± SD)			
	0	7	14	21
T_o	7.33 ± 0.32[h]	9.9 ± 0.06[b]	10.8 ± 0.06[a]	11.03 ± 0.15[a]
T_1	7.33 ± 0.20[h]	9.77 ± 0.10[bc]	9.93 ± 0.10[b]	10.73 ± 0.32[a]
T_2	7.33 ± 0.17[h]	9.13 ± 0.06[e]	9.43 ± 0.32[cde]	9.8 ± 0.10[b]
T_3	7.33 ± 0.21[h]	8.77 ± 0.32[f]	9.33 ± 0.12[de]	9.67 ± 0.23[bcd]
T_4	7.33 ± 0.32[h]	8.1 ± 0.06[g]	9.23 ± 0.12[e]	9.4 ± 0.35[de]

T_o=0.5% Cinnamon extract, T_1= 1% Cinnamon extract, T_2= 1.5% Cinnamon Extract, T_3= 2% Cinnamon Extract, T_4= 2.5% Cinnamon Extract

Color values in terms of L*, a* and b* of fresh cut carrots are given in table 3, 4 and 5. As evident from the table, L* values increased in all treatments for up to 14 days followed by a decrease towards the end of storage. a* and b* values were non-significant for all treatments during storage. Minimum L* value was 34.27 observed at 0 day whereas the maximum L* value was observed in T3 (44.69) at 14 days of storage. In case of a* values, the maximum value was observed at 0 day (32.59) while the minimum value of 19.28 was observed at 7 days in To (Table 3).

Table 3: Color (L*) of Fresh Cut Carrot

Treatments	Days of Storage (Mean ± SD)			
	0	7	14	21
T_o	34.27 ± 1.91[f]	43.02 ± 1.91[cde]	50.46 ± 0.95[a]	48.81 ± 2.95[ab]
T_1	34.27 ± 1.87[f]	42.02 ± 1.42[cde]	44.06 ± 3.12[bcd]	42.02 ± 6.74[cde]
T_2	34.27 ± 2.06[f]	38.47 ± 2.91[def]	39.90 ± 3.08[cde]	41.63 ± 4.87[cde]
T_3	34.27 ± 1.12[f]	37.29 ± 1.91[ef]	44.69 ± 6.1[bc]	39.76 ± 5.06[cdef]
T_4	34.27 ± 1.91[f]	38.85 ± 1.12[def]	40.47 ± 1.91[cde]	38.59 ± 2.84[def]

T_o=0.5% Cinnamon extract, T_1= 1% Cinnamon extract, T_2= 1.5% Cinnamon Extract, T_3= 2% Cinnamon Extract, T_4= 2.5% Cinnamon Extract

Table 4 showed the color (a*) of fresh cut carrots treated with various cinnamon extract concentrations over different storage times. Initially, all treatments had the same a* value of 32.59. Over 21 days, T0 (0.5% extract) showed a notable decline in color, dropping to 27.38. T1 (1% extract) maintained relatively stable color with a slight decrease to 26.21. T2 (1.5% extract) and T3 (2% extract) had moderate reductions, ending at 28.56 and 29.51, respectively. T4 (2.5% extract) experienced the most significant color loss, falling to 24.41. This indicates that higher cinnamon extract concentrations may not be as effective in preserving color stability over time (Table 4).

Table 4: Color (a*) of Fresh Cut Carrot

Treatments	Days of Storage (Mean ± SD)			
	0	7	14	21
T_o	32.59 ± 1.75[a]	19.28 ± 4.62[f]	21.7 ± 4.29[ef]	27.38 ± 1.75[abcd]
T_1	32.59 ± 3.26[a]	32.15 ± 5.42[ab]	30.8 ± 1.64[abc]	26.21 ± 0.5[cde]
T_2	32.59 ± 3.77[a]	24.79 ± 1.75[def]	29.68 ± 1.75[abcd]	28.56 ± 2.55[abcd]
T_3	32.59 ± 2.85[a]	27.22 ± 3.02[abcde]	31.36 ± 7.04[abc]	29.51 ± 2.12[abcd]
T_4	32.59 ± 1.75[a]	26.88 ± 0.73[bcde]	28.13 ± 5.47[abcd]	24.41 ± 2.89[def]

T_o=0.5% Cinnamon extract, T_1= 1% Cinnamon extract, T_2= 1.5% Cinnamon Extract, T_3= 2% Cinnamon Extract, T_4= 2.5% Cinnamon Extract

Table 5 presented the color (b*) values of fresh cut carrots treated with different concentrations of cinnamon extract over a 21-day storage period. At day 0, all treatments had the same b* value of 20.89. Over time, T1 (1% extract) initially showed an increase in b* value to 24.27, but decreased to 21.10 by day 21. T2 (1.5% extract) decreased to 18.58 on day 7 but recovered slightly to 22.60 by day 21. T3 (2% extract) increased to 24.01 by day 14 and remained relatively high at 23.60 on day 21. T4 (2.5% extract) initially rose to 23.14 but dropped to 19.32 by day 21. This suggests that cinnamon extract concentration affects the b* value, with higher concentrations initially showing better color retention but eventually declining (Table 5).

Table 5: Color (b*) of Fresh Cut Carrot

Treatments	Days of Storage (Mean ± SD)			
	0	7	14	21
T_o	20.89 ± 0.93[bcde]	20.61 ± 3.72[cde]	21.39 ± 2.00[abcde]	22.66 ± 3.13[abc]
T_1	20.89 ± 2.24[bcde]	24.27 ± 1.85[a]	23.72 ± 1.90[abc]	21.10 ± 0.93[abcde]
T_2	20.89 ± 2.60[bcde]	18.58 ± 0.87[e]	22.28 ± 0.93[abcd]	22.60 ± 0.71[abc]
T_3	20.89 ± 2.39[bcde]	21.36 ± 0.93[abcde]	24.01 ± 0.47[ab]	23.60 ± 3.49[abc]
T_4	20.89 ± 0.93[bcde]	23.14 ± 1.62[abc]	20.83 ± 1.75[bcde]	19.32 ± 1.66[de]

T_o=0.5% Cinnamon extract, T_1= 1% Cinnamon extract, T_2= 1.5% Cinnamon Extract, T_3= 2% Cinnamon Extract, T_4= 2.5% Cinnamon Extract

Table 6 showed the changes in firmness of fresh cut carrot during storage. A decrease in firmness was observed in all treatments with maximum value at 0 day (1.77) in T4 (2.5% Cinnamon extract) whereas minimum value (1.19) was observed at 21 days of storage in To (control) (Table 6).

Table 6: Firmness of Fresh Cut Carrot

Treatments	Days of Storage (Mean ± SD)			
	0	7	14	21
T_o	1.74 ± 0.03[ab]	1.61 ± 0.02[d]	1.33 ± 0.04[f]	1.19 ± 0.08[g]
T_1	1.76 ± 0.02[a]	1.68 ± 0.02[c]	1.49 ± 0.03[e]	1.30 ± 0.03[f]
T_2	1.76 ± 0.03[a]	1.68 ± 0.03[bc]	1.48 ± 0.04[e]	1.27 ± 0.03[f]
T_3	1.75 ± 0.02[a]	1.67 ± 0.06[cd]	1.45 ± 0.01[e]	1.28 ± 0.05[f]
T_4	1.77 ± 0.02[a]	1.68 ± 0.02[c]	1.45 ± 0.06[e]	1.29 ± 0.08[f]

T_o=0.5% Cinnamon extract, T_1= 1% Cinnamon extract, T_2= 1.5% Cinnamon Extract, T_3= 2% Cinnamon Extract, T_4= 2.5% Cinnamon Extract

The vitamin C content of fresh cut carrot during storage is given in table 7. As the table indicates, a decrease in vitamin C content was observed in all treatments of fresh cut carrot. The highest (14.40%) and the lowest (7.84%) vitamin C content was observed in To (Control) at 0 and 21 days of storage respectively (Table 7).

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Table 7: Vitamin C Content of Fresh Cut Carrot

Treatments	Days of Storage (Mean ± SD)			
	0	7	14	21
T_o	14.40 ± 0.04[a]	12.69 ± 0.12[e]	11.99 ± 0.05[f]	7.84 ± 0.22[i]
T_1	14.23 ± 0.35[ab]	13.87 ± 0.09[bcd]	11.1 ± 0.6[g]	9.71 ± 0.1[h]
T_2	14.14 ± 0.11[abc]	13.75 ± 0.35[cd]	11.04 ± 0.14[g]	10.04 ± 0.44[h]
T_3	14.14 ± 0.45[abc]	13.58 ± 0.12[d]	11.02 ± 0.46[g]	9.99 ± 0.04[h]
T_4	14.13 ± 0.18[abc]	13.58 ± 0.12[d]	11.03 ± 0.02[g]	10.85 ± 0.08[g]

T_o=0.5% Cinnamon extract, T_1= 1% Cinnamon extract, T_2= 1.5% Cinnamon Extract, T_3= 2% Cinnamon Extract, T_4= 2.5% Cinnamon Extract

Table 8 indicated the weight loss % of fresh cut storage during storage for 21 days. An Increase in weight loss % of all samples was observed as indicated in the table 8.

Table 8: Weight Loss % of Fresh Cut Carrot

Treatments	Days of Storage (Mean ± SD)			
	0	7	14	21
T_o	0.00 ± 0.00[j]	19.77 ± 0.21[d]	28.36 ± 0.04[b]	32.00 ± 0.02[a]
T_1	0.00 ± 0.00[j]	15.31 ± 0.72[ef]	24.08 ± 0.02[c]	27.39 ± 0.00[b]
T_2	0.00 ± 0.00[j]	13.88 ± 0.32[f]	16.27 ± 0.00[e]	18.40 ± 0.07[d]
T_3	0.00 ± 0.00[j]	4.76 ± 0.00[i]	6.56 ± 0.02[h]	8.93 ± 0.22[g]
T_4	0.00 ± 0.00[j]	0.80 ± 0.03[j]	3.39 ± 0.02[i]	4.43 ± 0.03[i]

T_o=0.5% Cinnamon extract, T_1= 1% Cinnamon extract, T_2= 1.5% Cinnamon Extract, T_3= 2% Cinnamon Extract, T_4= 2.5% Cinnamon Extract

DISCUSSION

TSS increased in all treatments with minimum value of 7.33 at 0 day and maximum value of 11.03 at 21 days in To (Control). In case of b* values, the maximum value (24.27) was observed at 7 days in T_1 whereas the minimum value (18.58) was observed at 7 days in T_2. Carrot color pigment variations are caused by minimal processing methods and storage duration, which affect carotenoid pigment [15, 16]. Decrease in firmness maybe attributed to degradation of insoluble protopectin to more soluble pectin and pectic acids in fruits and vegetables [17]. A decrease in vitamin C content was observed in all treatments of fresh cut carrot. The highest (14.40%) and the lowest (7.84%) vitamin C content was observed in T_o (Control) at 0 and 21 days of storage respectively. Similar decrease in vitamin C content was also reported by in fresh-cut carrot disks during 8 days of storage at 4°C [18]. Maximum weight loss was observed in T_o at 21 days (32.00%) whereas minimum weight loss was observed in T_4 (0.80%) at 7 days of storage. Similarly, an increase in weight loss % of fresh-cut carrots was also reported by Wang after treatment with carrot puree edible films followed by storage at 5°C for 12 days. The weight loss serves as an indicator for the transpiration-induced dehydration of vegetables and involves the transfer of water from the cell to the surrounding atmosphere. As such, it may be used to assess the effectiveness of coating treatments for the preservation of fresh-cut carrots [19]. In this research study, fresh-cut carrots coated with varying percentages of cinnamon extract were analyzed for several quality parameters over 0, 7, 14, and 21 days of storage. Acidity levels showed a gradual increase over time in all samples, with higher cinnamon extract concentrations resulting in a slower rate of increase indicating better preservation [14]. The oxidation of reducing sugars may cause an increase in acidity during storage, and the breakdown of polysaccharides and pectic substances [16]. Total Soluble Solids (TSS) remained relatively stable in carrots coated with higher percentages of cinnamon extract, suggesting that the extract helps maintain the carrots' natural sugars. Moisture loss and rising soluble solid concentrations are the primary causes of the rise in TSS. Furthermore, it is linked to the respiration and fruit-ripening processes that break down complex carbohydrates into soluble solids. The enzymes amylases, starch phosphorylase, and 1, 6-glucosidase catalyze the fast breakdown of starch into sugars including sucrose, glucose, and fructose [15]. Weight loss was significantly lower in samples with higher cinnamon extract percentages, likely due to the antimicrobial and moisture-retentive properties of cinnamon. These results are in agreement with the findings of Kowalczyk and colleagues who also reported an increase in weight loss % of different carrot cultivars treated with different chemicals during 12 days of storage at room temperature. Firmness was better retained in coated samples, especially those with higher cinnamon concentrations, suggesting the structural advantages offered by the extract [20]. The decrease in firmness of fresh-cut carrots was also reported by [17] during storage at 5°C in perforated polyethylene packaging [17]. Non-significant differences were observed in a* and b* values and an increase in L* values of fresh-cut carrots was seen when treated with edible coatings and stored for 21 days at 5°C. In a separate study, reported an increase in the whiteness index of fresh-cut carrots stored for 10 days at 5°C after exposure to abiotic stresses such as heat shock and UV-C irradiation [21]. An efficient preservative method for extending the shelf life of thinly sliced carrots is alginate-based coating enhanced with α-tocopherol acetate, an antioxidant [22].

CONCLUSIONS

This research experiment investigated the potential of edible coating enriched with cinnamon extract to enhance the shelf life of fresh-cut carrots. The results showed the effectiveness of the coating in alleviating quality deterioration during storage period of 21 days at. Fresh-cut carrots coated with pectin and cinnamon extract exhibited significantly lower weight loss, better retention of firmness and better color retention compared to non-coated treatment group. The results indicate that the edible coating acted as a physical barrier, reducing moisture loss and maintaining firmness and other quality parameters. The presence of cinnamon extract, a natural antioxidant, may have contributed to these positive effects by delaying oxidative degradation processes. The findings of this study

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align with previous studies, highlighting the potential of edible coatings enhanced with antioxidants in extending the shelf life of fresh-cut commodities.

Authors Contribution

Conceptualization: HN, MHR, ZY
Methodology: HN, MHR, SM
Formal analysis: HN, HUN, MHR, SM, ZI
Writing, review and editing: HN, RZ, SR, AM, BS

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

Assessment of Moringa Seed Oil: Fatty Acid Profile, Oxidative Stability and Antioxidant Activity

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ABSTRACT

Moringa oleiferais called the "miracle tree" and it has more vitamins than even some fruits and vegetables like oranges, carrots etc. **Objective:** To assess functional groups and compare oxidative stability, fatty acid profile, free fatty acid concentration, and antioxidant activity to commercial vegetable oil. **Methods:** The moringa seed oil was extracted by cold press extraction and solvent extraction by n-hexane and petroleum ether. Functional group, lipid peroxidation, fatty acid profile, antioxidant activity, and FFAs % were analyzed by FTIR, TBARS, GC-MS, DPPH, and titration respectively. **Results:** The FTIR spectra of prepared samples showed common functional groups of triglycerides, including a sharp peak at 2984 cm-1 for aliphatic C-H stretching. The maximum TBARS value was 0.234 ± 0.03% in T0 at 30 days of storage, whereas the lowest was 0.167 ± 0.04% in T1 at 0 day. The GC-MS analysis of screw press moringa oil showed a high percentage of monounsaturated fatty acids, with 71.38 ± 0.01% oleic acid, 7.01 ± 0.01% palmitic acid, and 1.92 ± 0.01% linoleic acid. At 15 days of storage, FFAs were 2.28 ± 0.06%, showing low hydrolytic rancidity. The Antioxidant Activity in DPPH analysis was 44.46 ± 0.02%, showing high antioxidant properties. **Conclusions:** The characteristics of moringa seed oil indicate that it could be an effective edible oil and suitable for the production of food items and other edible products in the food and nutraceutical sectors.

INTRODUCTION

The Moringaceae family includes the plant known as *Moringa oleifera* (*M. oleifera*). It is a perennial that is commonly found in Southern and Eastern Asia, and it is likely the most cultivated species in the northwest of the Indian subcontinent. Tropical and subtropical regions are suitable for *M. oleifera* cultivation [1]. It is considered as the "miracle tree" because of its many beneficial features, such ascontaining seven times more vitamin C than oranges and ten times more vitamins than carrots. It is extensively grown for the many uses of its tender seed pods and verdant leaves as food and medicine.Its high protein content makes it an excellent supplement as well [2]. Moringa seed oil have high amount of oleic acid (78%) and low amount of essential fatty acids such as linolenic acid 2.2% and linoleic acid 0.77% similar to olive oil [3]. It is also a good source of behenic acid wich is use to stabilize and solidify the semi-solid and solid fat foods without hydrogenation of oil [4]. According to, unsaturated fatty

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acids have potent antioxidant, anticancer, and antihyperlipidemic properties that are beneficial to human health [5]. Furthermore, α-and δ-tocopherol are abundant in moringa seed oil, with levels of 45–80 mg/100 g and 0.21–0.53 mg/100 g, respectively [6]. Tocopherols are important nutrients as well as antioxidants that protect cells from damage [7]. Moringa oil has been found to have excellent antioxidant property, as it has significant quantity of tocopherols. Moreover, low concentration of polyunsaturated fatty acids increases stability and oxidative resistance [8]. Commercial vegetable oil is an important constituent in different food composition and vital element of our daily diet. [9]. According to vegetable oil contains triesters (a byproduct formed by the interaction of fatty acids and glycerol), triglycerides (98g/100g) and other substances are also present in trace amount [10]. Vitamins, polyphenols, tocopherols, phytosterols and diglycerides shouldn't be removed during processing because these elements provide health benefits for people [11]. Although processing and refining of vegetable oil increases its shelf life but still there are some disadvantages. One of the disadvantages is the loss of compounds such as phytosterols, squalene, tocopherols, phospholipids and polyphenols that provide medicinal and technologicalbenefits. The formation of 3-MCPD-esters, trans fatty acids, glycidyl ester and polymeric triacylglycerolsare unwanted compounds during processing which is another drawback of processing [12]. These compounds affect the safety of oil at different levels [11]. There is a substantial correlation between high use of commercial edible oil and a high risk of coronary heart disease. The onset of Coronary Heart Disease (CHD) was caused by the buildup of atheromatous plaque in the arteries, which deprived the functioning heart of blood and oxygen. Numerous risk factors, such as elevated BMI, elevated triglyceride levels, diabetes, physical activity, and infection, contribute to the development of plaque in the arteries [13]. Due to their significant impact on cholesterol metabolism, dietary fatty acids may be linked to Cardiovascular Diseases (CVD). Non-Communicable Diseases (NCDs) will be responsible for over 75% of all deaths globally by 2030, according to health statistics published in the World Health Organization 2015 Gazette [14]. Even though Pakistan is an agricultural nation, the country nevertheless spends a lot of money importing edible oil [15]. Pakistan is only producing conventional oil-seed crops, which accounts for 18 % of the total need. To meet the needs, a significant sum of foreign currency is being used for the import of edible oil. A household's food expenses are significantly impacted by the import of edible oil [16]. Pakistan produces about 0.7 million tons of edible oil, compared to imports of between 1.8 and 8.0 million tons. Pakistan produces approximately 3 million tons of edible oil annually, of which 6.6 million tons are exported to meet the country's growing demands [17]. Oil extraction techniques have the potential to modify tiny components that have functional qualities and support stability during oxidation. Because solvent extraction is affordable and easy to use, it has gained a lot of popularity. It can result in high oil recovery efficiency and enable many extractions to be conducted simultaneously [18]. Petroleum ether and n-hexane were used to extract moringa oil, and the results showed a 29.98 and 33.47 weight percent oil yield, respectively [19]. The high tocopherol content of *M. oleifera* oil helps to preserve it from oxidative damage during processing and storage. Of all the tocopherols, α tocopherol stands out as being the most significant since it supports a number of bodily biochemical processes, models the expression of proteins involved in cholesterol metabolism, and inhibits and promotes cell growth [20]. Moringa oil has a higher tocopherol content than other oils. Moringa oil can be used as a vegetable oil source for human consumption in diets [21].

METHODS

Seeds of moringa oleifera (50 kg) were bought from a local market of Multan in the processed form of dried seeds after removal of its shell, were stocked for further use. All the reagents of analytical grade were purchased from Hale Marketing International. The purchased seeds of Moringa oleifera were sun dried and reduce into small particle sizes through the use of a grinding machine and were stored at 25oC for further use [22]. Moringa seed oil was extracted using two different methods. These methods include solvent extraction using n-hexane (T1) and petroleum ether (T2) and mechanical extraction (T3). For solvent extraction, 10g of moringa seed powderwas placed in a thimble and then fit in a Soxhlet extractor and used n-hexane as a solvent. The extraction was performed for 2-3 h at 50-60°C till 2-3 back siphon. For extraction with petroleum ether 10 g of moringa seed powder were treated with 210ml solvent for about 6 hours. Then, solvent was evaporated using rota-vapor and hot air oven. The extracted oil was then recovered by removing the hexane using an oven. For mechanical extraction, whole moringa seeds were used, and the oil was extracted using a mechanical extractor. The yield was calculated by dividing grams of extract over original powder respectively. Each Treatment (group) was denoted by T0, T1, T2 and T3, and three replicates were taken for each treatment. Each group contained approx. 12.5 kg seed before extraction. After the extraction of Moringa oil different analytical methods were performed to determine the different valuable properties of moringa seeds oil. Moringa seed oil free fatty acid composition was analyzed using method No. Ca 5a-40(2). 5g oil was mixed

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with 50ml ethanol and phenolphthalein indicator 2-3 drops were added, then titrated against 0.1 KOH (reagent grade, 90%, flakes) until a pink color endpoint [23]. Moringa seed oil peroxide value was determined using the approved Method No. Cd 8b-90. 5g oil was mixed with 30ml POV solution glacial acetic acid (99% ACS Grade) /chloroform (≥99% ACS Reagent Grade) in 3:2 and 30ml distilled water, then titrated with 0.01N sodium thiosulphate (Na2S2O4) (reagent grade 98, pellets anhydrous 1310-73-2) until a colorless endpoint was reached, using starch solution as indicator [23]. Moringa seed oil saponification value was determined according to, method no. Cd 3-25. 2g oil was refluxed with 0.5N ethanolic potassium hydroxide (0.1N Reagent Grade, c(KOH) = 0.1 mol/l 0.1N) for 45 min on reflux condenser, then cooled and titrated with 0.5N H2SO4 (96% ACS Reagent Grade) using indicator phenolphthalein [23]. A blank sample was run simultaneously, and the saponification value was calculated using formula. Moringa seed oil acidity value was determined according to method no. Cd 3d-63 [23]. A 10g oil sample was titrated with 0.1N NaOH (reagent grade, ≥98%, pellets anhydrous) until a light pink endpoint was reached, using 2-3 drops of phenolphthalein as indicator. The acidity value was then calculated using formula. Moringa seed oil fatty acid profile was analyzed using GC-MS. Methyl esters were prepared using methanol. The sample was injected through Agilent 7693A Auto sampler into the Agilent 5977B GC/MSD and Agilent 8890 GC system with helium as the carrier gas, and a temperature program was fixed between 70-280°C. The injection and detector temperatures were 240-250°C. Peaks were identified by comparison to standards, and fatty acid content were calculated as percentages (%) [24]. Moringa seed oil lipid peroxidation was assessed using thiobarbituric acid (reagent grade, ≥98%). 10ml oil was mixed with 50ml deionized water and 2.5ml of 4M HCl (ACS reagent, 37 7647-01-0), then heated to obtain total volume of 50ml. After boiling, 5ml was combined with 5ml TBA reagent and heated to 100°C for 35min. Absorbance was measured at 538nm using a Agilent Cary 60 UV-visible spectrophotometer that has a wavelength range from 190-1100 nm that can scanned in under 3 seconds, with deionized water as a blank [25].

$$\text{TBARS} = \frac{\text{Absorbance} \times \text{Volume Factor}}{\text{Slope of curve}} \times \text{Dilution Factor}$$

The free radical scavenging ability of Moringa seed oil was determined using DPPH. A 0.5 mL DPPH solution (0.15 mM) was added to 1g of the extract oil (in methanol). After mixing and 30 minutes at room temperature, the absorbance was measured at 517 nm using an Epoch Eliza reader (Bio-Tek Instruments, Inc., Winooski, VT, USA [26].

The radical scavenging activity of DPPH was measured by using the formula:

$$\text{Total antioxidant (\% Inhibition)} = \frac{\text{A Blank} - \text{A sample}}{\text{A Blank}} \times 100$$

According to the method of with some modifications, total phenolic content of moringa seed oil were determined by using Folin-Ciocalteu (FC) reagent [27]. A 300µLof oil sample were mixed with 600µL of 10% FC reagent and mixed with vortex. Then 2400µL of 700mM Na2CO3 (Powder, ≥99.5%, ACS reagent) solutionwere added. After this, mixed solution was placed in the dark at room temperature for 30 minutes. Epoch Eliza reader (Bio-Tek Instruments, Inc., Winooski, VT, USA) were used to obtain the absorbance at 765 nm. These results were expressed as mg GAE (gallic acid equivalent) per 100g-1. Commercial oil and moringa seed oil samples were analyzed by using FTIR spectroscopy. Protocol explained by was followed with slight modifications [29]. A drop of each sample was placed on the IR crystal port and compared with conventional sample analysis. The spectra were obtained using an Agilent Cary 360 FTIR spectrometer, operating at a resolution of 4 cm-1 and scanning 10 times over a range of 4000-650 cm-1. The resulting spectra were analyzed using ORIGINPRO 8.5 software, measuring peak height and area. 100ml packaging of moringa seed oil was stored for 30 days at room temperature (25° C) in the dark place. Triplicate analyses were performed to ensure data reliability. Significant differences were assessed using ANOVA, following procedures [28]. The Completely Randomized Design (CRD) was computed using STATISTIX 8.1 software.

RESULTS

Moringa seed oil was extracted with solvent extraction (n-hexane and petroleum ether) and mechanical extraction. The yield of oil by solvent extraction (n-hexane and petroleum ether) and mechanical extraction were 24.77 ± 0.16%, 23.44 ± 0.20% and 22.61 ± 0.08% respectively as shown in (Figure 1). There was a significant difference ($p<0.05$) between the treatments for the analysis of oil extraction yield.

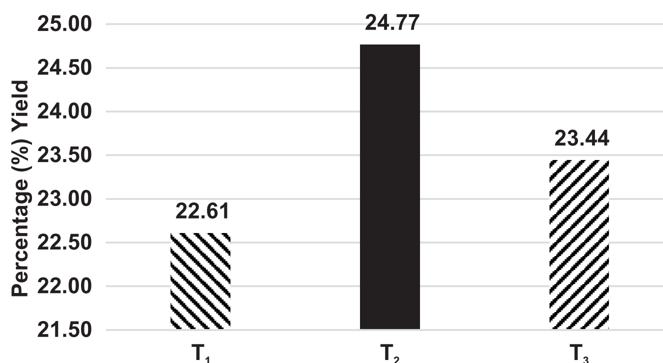

Figure 1: Percentage Oil Yield

Free fatty acid value was used to assess the hydrolytic rancidity and overall quality of an oil, indicating the quantity of free fatty acids present. The graph presents the values

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of free fatty acids in commercial edible oil and moringa seed oil extracted using solvent extraction (petroleum ether and n-hexane) and the cold press method at 0 day, 15 days, and 30 days of storage. The results showed that at 0 day, T0 had the highest content 4.47 ± 0.01% of FFA at day 15 and T1 had lowest content of 2.28 ± 0.06% FFA at day 1 as shown in (Table 1). Triglycerides' hydrolysis was the primary cause of an increase as it produces free fatty acids and other oxidation harms. Significant difference ($p<0.05$) was shown in between the treatments when analyzing free fatty acids in moringa seed oil.

Table 1: Influence of Storage and Treatment on FFAs

Days	T_0	T_1	T_2	T_3	Mean ± SD
0 Day	4.28 ± 0.01^B	2.28 ± 0.06^E	3.61 ± 0.05^D	3.78 ± 0.01^C	3.48 ± 0.03^B
15th Day	4.47 ± 0.01^A	2.31 ± 0.05^E	3.57 ± 0.03^D	3.80 ± 0.06^C	3.53 ± 0.03^A
30th Day	4.37 ± 0.02^A	2.41 ± 0.09^E	3.58 ± 0.04^D	3.82 ± 0.03^C	B3.54 ± 0.04^A
Mean ± SD	B4.37 ± 0.01^A	2.33 ± 0.06^D	3.58 ± 0.04^C	3.80 ± 0.03^B	-

T_0: (Commercial edible oil)
T_1: (Screw press extracted moringa seed oil)
T_2: (n-hexane extracted moringa seed oil)
T_3: (Petroleum ether extracted moringa seed oil)

Peroxide value was a measurement of the amount of lipid oxidation in an oil, which provides a measure of its stability and freshness. According to graph, highest peroxide value was 2.86 ± 0.01meq O_2/kg found in T0 at day 30 and lowest peroxide value was 0.85 ± 0.04meq O_2/kg found in T1 at 0 day of storages shown in (Table 2). Which means it can be rancid and off flavor rapidly than the moringa seed oil extracted by cold press method. Significant differences ($p<0.05$) were assessed in between the treatments when analyzing free fatty acids in moringa seed oil.

Table 2: Influence of Storage and Treatment on Peroxide Value

Days	T_0	T_1	T_2	T_3	Mean ± SD
0 Day	2.80 ± 0.08^A	0.85 ± 0.04^E	1.35 ± 0.01^D	1.86 ± 0.02^B	1.71 ± 0.03^B
15th Day	2.85 ± 0.01^A	0.88 ± 0.012^E	1.44 ± 0.01^C	1.93 ± 0.01^B	1.77 ± 0.01^A
30th Day	2.86 ± 0.01^A	0.89 ± 0.02^E	1.46 ± 0.01^C	1.94 ± 0.01^B	1.78 ± 0.01^A
Mean ± SD	2.83 ± 0.03^A	0.87 ± 0.02^D	1.41 ± 0.01^C	1.91 ± 0.01^B	-

T_0: (Commercial edible oil)
T_1: (Screw press extracted moringa seed oil)
T_2: (n-hexane extracted moringa seed oil)
T_3: (Petroleum ether extracted moringa seed oil)

Saponification value can be obtained by determining the quantity of alkali required to saponify a specific volume of oil, thereby indicating the average molecular weight (or chain length) of the fatty acids present. The highest saponification value 189.37 ± 0.06mgKOH/g was observed in T0 and T3 had the lowest saponification value of 181.3 ± 0.05mgKOH/gas shown in (Figure 2). Saponification value between the treatments showed significant results ($p<0.05$) in moringa seed oil.

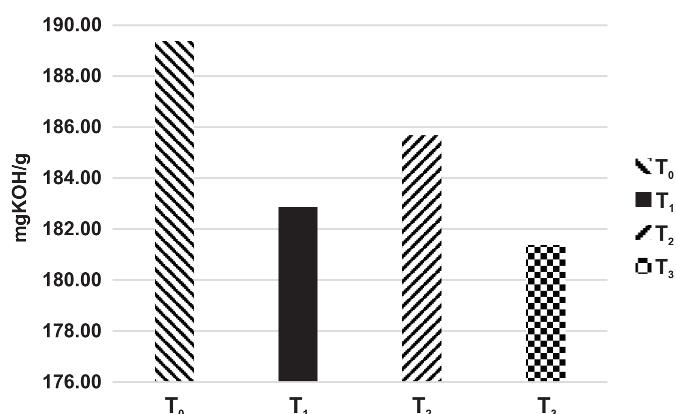

Figure 2: Influence of Treatment on Saponification Value

Overall, Moringa seed oil has relatively low acidity, which was reflected in its low free fatty acid level. It was also chemically stable and meets cosmetic and food industry standards. This indicates that the oil does not have high acidity, allowing the oil's benefits to be retained and increasing the storage time. The highest Acidity value was 0.577 ± 0.001% which observed in T2, and lowest value was 0.507 ± 0.002% observed in T1 as shown in (Figure 3). Acidity between the treatments showed significant results ($p<0.05$) in moringa seed oil.

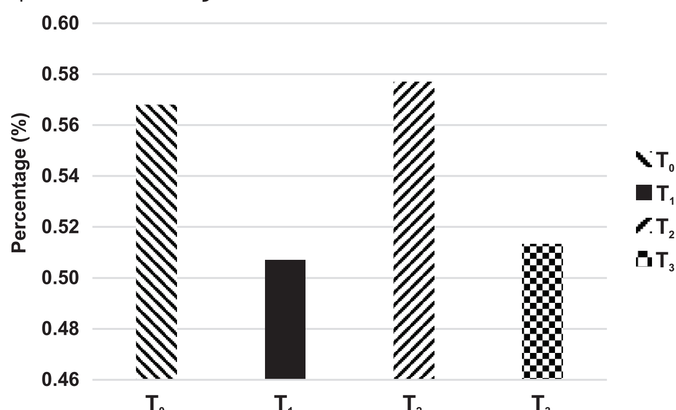

Figure 3: Influence of Treatment on Acidity (%)

Total phenolic contents were based on the F.C (Folin-Ciocalteu) reagent's response with the test sample. Due to the reduction of phosphotungstic and phospomolybdic acid in an alkaline media in the presence of phenolic substances, a blue chromophore was produced. According to Table 3, highest TPC value 46.35 ± 0.02mg GAE/g in T1 was found at 15 and 30 days and the lowest TPC value 38.34 ± 0.36 was observed in T0 at 0 day. Significantly differentiated ($p<0.05$) results were indicated in treatments when total phenolic content was analyzed.

Table 3: Influence of Storage and Treatment on Total Phenolic Content (TPC)

Days	T_0	T_1	T_2	T_3	Mean ± SD
0 Day	38.34 ± 0.36^G	45.67 ± 0.26^B	43.78 ± 0.30^D	42.56 ± 0.25^E	42.58 ± 0.29^C
15th Day	39.15 ± 0.02^F	46.35 ± 0.02^A	44.36 ± 0.01^C	44.37 ± 0.03^C	43.55 ± 0.02^B

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30th Day	39.25 ± 0.02^F	46.35 ± 0.02^A	45.25 ± 0.02^B	45.23 ± 0.02^B	44.02 ± 0.02^A
Mean ± SD	38.91 ± 0.13^D	46.12 ± 0.1^A	44.46 ± 0.11^B	44.05 ± 0.1^C	-

T_0: (Commercial edible oil)
T_1: (Screw press extracted moringa seed oil)
T_2: (n-hexane extracted moringa seed oil)
T_3: (Petroleum ether extracted moringa seed oil)

DPPH has a dark red color and was a very stable radical. It has a 1, 1-diphenyl-2-picrylhydrazyl chemical structure. Food's color changes from dark red to yellow when antioxidants present in it absorb free radicals. According to Table 4, highest DPPH value 46.44 ± 0.02% was found at 0-day storage in T2 and the lowest DPPH value 31.22 ± 0.02% was observed at 30 days' storage in T0. Significant differences ($p<0.05$) were assessed in between the treatments when analyzing DPPH value of moringa seed oil.

Table 4: Influence of Storage and Treatment on DPPH Value

Days	T_0	T_1	T_2	T_3	Mean ± SD
0 Day	31.46 ± 0.02^J	35.45 ± 0.02^G	46.44 ± 0.02^A	45.65 ± 0.02^D	39.75 ± 0.02^A
15th Day	31.34 ± 0.02^K	35.35 ± 0.02^H	46.36 ± 0.01^B	45.37 ± 0.03^E	39.60 ± 0.02^B
30th Day	31.22 ± 0.02^L	35.25 ± 0.04^I	46.25 ± 0.02^C	45.23 ± 0.02^F	39.60 ± 0.02^C
Mean ± SD	31.34 ± 0.02^D	35.35 ± 0.02^C	46.35 ± 0.01^A	45.41 ± 0.02^B	-

T_0: (Commercial edible oil)
T_1: (Screw press extracted moringa seed oil)
T_2: (n-hexane extracted moringa seed oil)
T_3: (Petroleum ether extracted moringa seed oil)

FTIR spectroscopy was an analytical technique used to determine the chemical composition and functional groups of substances by analyzing their infrared absorption spectra. It was widely used for quality control and characterization in several industries, including pharmaceuticals and food. FTIR analysis of commercial edible oil and moringa seed oil obtained from solvent extraction (n-hexane and petroleum ether) and cold press technique, showed a peak at 2905 cm-1 for O-H stretching and 2984 cm-1 for C-H stretching. The wavelength of 1691 and 2290 cm-1 for the stretching vibration of the -C=O and O-C=O functional groups respectively. The peak at 1390 cm-1 representing the bending vibration of -C-C- bond. The absorption peak at 1155 cm-1 was related to the bending motion of C-O-C chemical bond. Moreover, the absorption peak showed at 1028-1302 cm-1 and 959-971 cm-1 were related to the stretching of C-O bonds and bending of C-H bonds. All the oils showed similar characteristics bands that can further be used for qualitative measurementas shown in (Figure 4-7). Theoretically, oils need conversion to react with alkali. However, FTIR spectra of moringa seed oil showed no significant alteration in the absorption band of molecules. Significantly differentiated ($p<0.05$) results were observed of FTIR analysis between treatments of moringa seed oil (Figure 4).

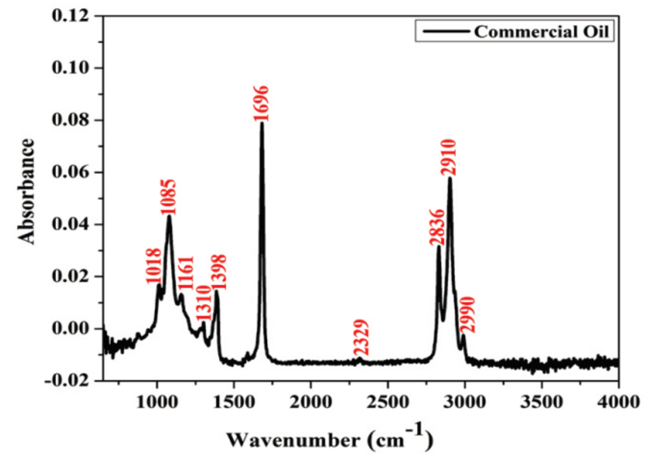

Figure 4: FTIR Analysis of Commercial Edible Oil

Figure 5 displayed the FTIR analysis of cold-pressed moringa seed oil, revealing prominent peaks for functional groups such as triglycerides, with a characteristic aliphatic C-H stretching observed at 2984 cm^{-1}.

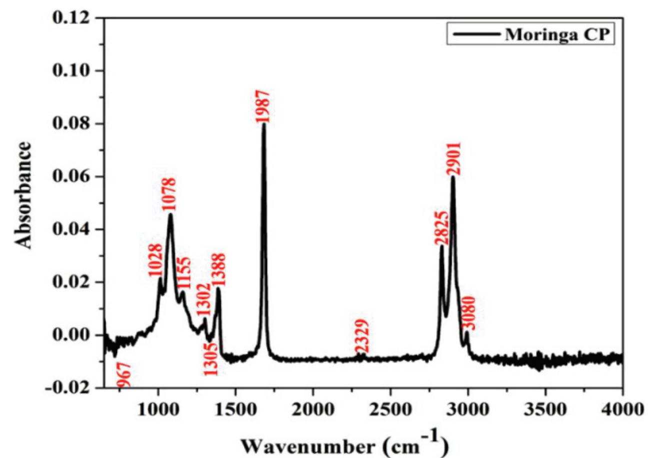

Figure 5: FTIR Analysis of Moringa Seed Oil Extracted with Cold Press

Figure 6 displayed the FTIR analysis of moringa seed oil extracted with n-hexane, revealing characteristic peaks for triglycerides, including a distinct aliphatic C-H stretching band at 2984 cm^{-1}.

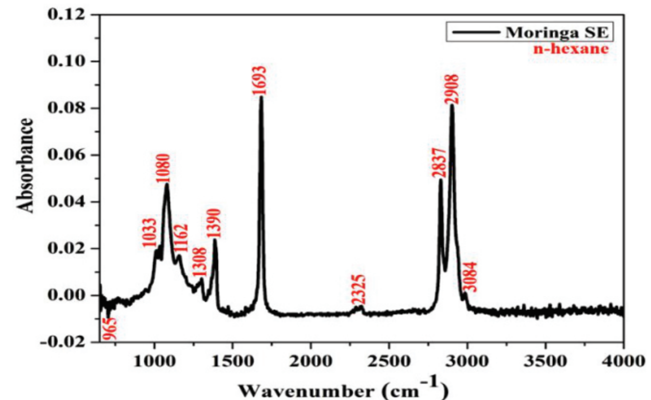

Figure 6: FTIR Analysis of Moringa Seed Oil Extracted with n-hexane

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Figure 7 displayed the FTIR analysis of moringa seed oil extracted with petroleum ether, revealing characteristic peaks for triglycerides, with a distinct aliphatic C-H stretching observed at 2984 cm^{-1}.

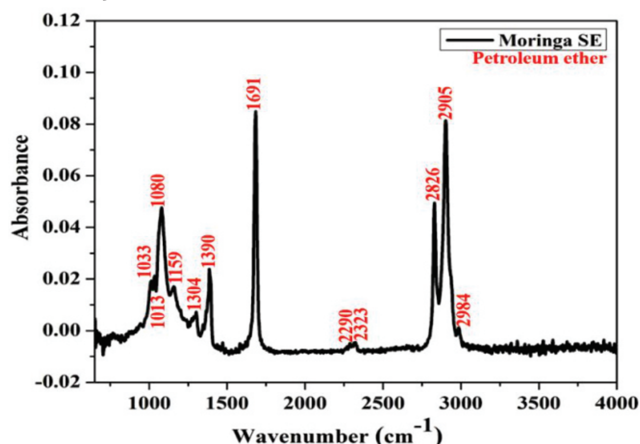

Figure 7: FTIR Analysis of Moringa Seed Oil Extracted with Petroleum Ether

The TBARS analysis Measures Malondialdehyde (MDA) and other secondary oxidation-related compounds that result from lipid peroxidation. As the result shows in Table 5, the highest TBARS content of 0.234 ± 0.03 mg MDA/kg was found in T0 at 30 days of storage and lowest content 0.167 ± 0.04 mg MDA/kg was observed in T1 at 0-day storage. Significant differences ($p<0.05$) were shown in between the treatments when analyzing TBARS value in moringa seed oil.

Table 5: Influence of Storage and Treatment on TBARS

Days	T_0	T_1	T_2	T_3	Mean ± SD
0 Day	0.23 ± 0.03^A	0.16 ± 0.04^F	0.17 ± 0.03^D	0.18 ± 0.05^B	0.185 ± 0.03^C
15th Day	0.23 ± 0.03^A	0.17 ± 0.02^E	0.17 ± 0.03^D	0.18 ± 0.05^B	0.187 ± 0.03^B
30th Day	0.23 ± 0.03^A	0.17 ± 0.02^E	0.18 ± 0.02^C	0.18 ± 0.05^B	0.19 ± 0.03^A
Mean ± SD	0.23 ± 0.03^A	0.16 ± 0.02^D	0.17 ± 0.02^C	0.18 ± 0.05^B	-

T_0: (Commercial edible oil)
T_1: (Screw press extracted moringa seed oil)
T_2: (n-hexane extracted moringa seed oil)
T_3: (Petroleum ether extracted moringa seed oil)

Gas Chromatography-Mass Spectrometry (GC-MS) was a technique that combines gas chromatography with mass spectrometry to separate and identify compounds in a sample. Widely employed in environmental investigation, forensics, and food safety. GC-MS analyses were carried out for oil samples using an Agilent 5977B GC/MSD instrument. Results showed that unsaturated fatty acids in the and moringa seed oil obtained by mechanical extraction and solvent extraction (petroleum ether and n-hexane) were higher than the amount of saturated fatty acids which was best suited having healthful attributes. GC-MS analysis performed for the moringa oil indicated that fatty acids profile of commercial edible oil and moringa oil by solvent extraction contained 63.79 ± 0.01%, 67.77 ± 0.01% and 68.2 ± 0.1% oleic acid respectively. Fatty acids profile of moringa oil by mechanical extraction contained 71.38 ± 0.01% oleic acid. Other fatty acids like linolenic acid, linoleic acid and palmitic acid were present in amount 1.92 ± 0.01%, 0.976 ± 0.001%, and 7.01 ± 0.01% respectively as shown in (Figure 1). Significantly differentiated ($p<0.05$) were shown in fatty acid profiling between the treatments were analyzed in moringa seed oil (Figure 8).

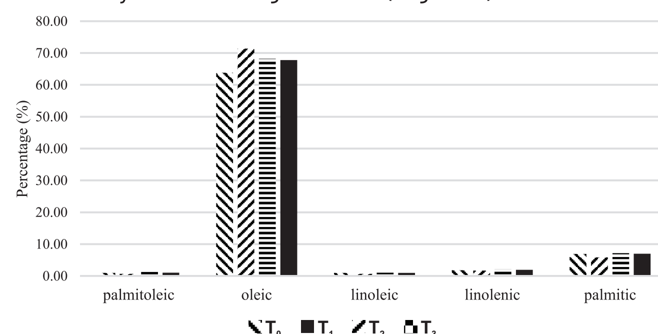

Figure 8: Influence of Treatment on Fatty Acid Composition (%)

DISCUSSION

Moringa seed oil was extracted with solvent extraction (n-hexane and petroleum ether) and mechanical extraction. Triglycerides' hydrolysis was the primary cause of an increase as it produces free fatty acids and other oxidation sources. The percentage of moringa oil indicated by was 40%, 33.6% and 36.7% respectively in accordance to the results of this study as shown in figure 1 [30, 31]. As compared to the investigating the free fatty acid concentration of moringa seed oil and table 1obtained different results [32, 33]. Found the same outcomes when analyzing the free fatty acids of moringa seed oil extracted through cold pressing. The results were identical as used a cold pressed oil to determine the free fatty acids of moringa seed oil [34]. The findings of the investigation showed that the concentration of the utilized extract influenced the levels of free fatty acids. When comparing results of v with table 2in which they compared the peroxide value of moringa seed oil, they give contrasting outcomes [35]. When examining the peroxide value of moringa seed oil by solvent extraction, determined the same conclusions [36]. They concluded that the essential oil derived from the moringa seeds has the lower value of peroxide value, of 1.5±meq O_2/kg compared to other sections. Similar outcomes were observed by who assessed the peroxide value of moringa seed oil by cold press as the results came in figure 3 [37]. The results of the analysis showed that the concentration of the extract employed affected peroxide value. In contrast, observed different outcomes when comparing the saponification values of sage and fennel essential oils [38, 39]. Investigated the same outcomes during analysis of the saponification value of moringa seed oil according to the results of figure 2. They also identified that among all the oils, the oil extracted from the seeds of moringa had the

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least value of saponification, at 179. 5±0. They were also comparatively lower at 25mgKOH/g compared to the other sections [40]. Established the same research results, using solvent extraction to determine the saponification value of moringa seed oil. Consequently, the analysis clearly showed that the extract's concentrate level impacted the saponification value [38]. Observed different outcomes in contrast to figure 3when comparing the acidity values of sage and fennel essential oils. The identical conclusions were examined by when they analyzed the acidity value of moringa seed oil [39]. In comparison to the other sections, they determined that the oil extracted from moringa seeds has the lowest acidity value of 0.494 ± 0.025%. Similar results with figure 5have been identified in the study conducted by in which the acidity value of moringa seed oil was evaluated extracted with solvent extraction [40]. As demonstrated by the results of the analysis, the acidity value was influenced by the concentration of the extract utilized. Antioxidant activity of olive oil in showed the contrasting results with figure 7 [41]. They used ethanolic extract of olive seeds to measure total phenolic contents and DPPH. Results revealed that total phenolic contents of olive oil were 42.87mg GAE/g at concentration of 25 microgram/ml. When measuring the total phenolic contents of two moringa seed species of Portugal according to the antioxidant activity of table 3 given above, shows the same results and findings [42]. They showed through their findings that both species were excellent sources of phenolic chemicals. In M. stenopetala, extract had a total phenolic concentration of 43.45 ± 0.023mg GAE/g, which was higher than the 42.18 ± 0.024 mg GAE/g found in methanolic extract. However, in the M. ovalifolia species, methanolic extracts (44.56 mg GAE/g) had higher phenolic levels than aqueous extracts (41.12 mg GAE/g). Comparable outcomes were found with the study of as shown in table 3 [43]. When they assessed the phenolic concentration of moringa seed oil by cold press. The phenolic fractions were extracted using cold press machine, and the TPC was then measured. The results of the TPC study showed that the cold press extract had a TPC value of 44.13mg GAE/g, which was similar to our findings. Contrasting results were also measured with this study mentioned in table 4by in which they compared the antioxidant activity of essential oils [44]. They compared three different species of essential oils at 60 degrees Celsius for a month, varying the concentrations from 600 to 1000 g/ml. They found that essential oil demonstrated 100% more radical scavenging activity. From their research, they concluded that essential oil has the greatest DPPH, PV, TBA, and BCB values. When examining the radical-scavenging capabilities of moringa seed oil, determined the same conclusions with table 4 [26]. They concluded that the essential oil derived from the moringa seeds has the maximum degree of free radical elimination activity, at 47.95%, compared to other sections. Similar outcomes of table 4were also observed by who assessed the antibacterial and antioxidant properties of moringa seed crude methanol extract [45]. They used 87.97 g of moringa seed powder to make 38.50 g of extract. After that, the concentrate was dried. They compared conventional ascorbic acid at the same concentrations to extract concentrations of 25, 50, 100, 200, and 400 g/ml in order to determine antioxidant activity. The results of the analysis showed that the concentration of the extract employed affected antioxidant activity. The antioxidant activity decrease with change in extraction method. Contrasting results were also observed by with figure 5 in which they studied the peaks for O-H, C-H, C=O, and O-C=O stretching [3]. Spectra showed distinctive peaks 3005 cm-1 peak for cis-olefinic bonds, indicative of unsaturated fatty acids, and the 1747 cm-1 peak for ester groups, representing total lipids. In contrast, observed different outcomes when comparing the FTIR analysis of moringa oil as observed in figure 6 [46]. Variations in environmental conditions during FTIR measurement, such as temperature or humidity, can introduce minor spectral differences. Similar findings were also determined by as they conducted the TBARS content of moringa seed oil as this study has mentioned in table 5 [47]. Variation is observed in the results due to differences in cultivars and variety. In the study to investigate the effects of lipid peroxidation on cold press moringa seed oil, stated that the cessation oil obtained from the moringa seeds contains a lesser value of TBARS content 0.192±0.09 mg MDA/kg [48]. Similar outcomes were observed by who assessed the lipid peroxidation 0.179±0.06 mg MDA/kg of moringa seed oil extracted by cold press [49]. The results of the analysis showed that the concentration of the extract employed affected TBARS value. The value change with the change in extraction method. Contrasting results of this study were shown with the study when comparing fatty acid profiles [50]. Similar results have been identified in the study conducted by in which fatty acid profile of moringa seed oil was evaluated, studies highlight oleic acid (C18:1) as the predominant fatty acid as with the analysis mentioned in figure 8 [51]. Variations with other studies results were due to Moringa plant sources, such as different species or varieties of Moringa oleifera, or even geographic origin, can lead to differences in the fatty acid composition of the extracted oil. The change in percentage of extracted oil yield was due to the change in environment, variety, difference in cultivar and soil condition or the chemical composition of moringa plant.

CONCLUSIONS

The nature of the oil extracted from *Moringa oleifera* seeds, variety PKM 1 showed that this oil can be used effectively as one of the edible oils for human consumption. The potential beneficial aspects of moringa oil were superior to other ordinary vegetable oils. Its composition, which was very different from most other oils in terms of the range and density of its components comprising essential fatty acids

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and antioxidants, means there was great potential for high nutritional and industrial value from the oil. According to the research, moringa oil contains many valuable nutrients and health-enhancing compounds. It was highly stable when exposed to oxygen, thus making it a valuable ingredient in food supplements and technical applications. It has a great effect in improving people's health and promoting sustainable approaches in different sectors.

Authors Contribution

Conceptualization: MHUH, SA, UF, UM,
Methodology: MHUH, SA, UM, MAK, MI
Formal analysis: MHUH, MS, SA, UF, MI
Writing, review and editing: SA, MAK, UF, HN, UM, AM, TBQ

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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