ISSN: 2347-467X, Vol. 13, No. (1) 2025, Pg. 162-171

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Determination of Physicochemical Properties, Oxidative and Storage Stability of Fish And Flaxseed Oil

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Abstract

Fish and flaxseed oil being a rich source of omega-3 helps to ameliorate disease and illness; however, unsaturation and oxidation process can affect their nutritional properties and health benefits. The present study therefore conducted to evaluate the oxidative stability and thermal behavior of fish oil (FO) and flaxseed oil (FsO)at different storage conditions. In this regard, the change in peroxide (POV), p-anisidine (PAV) and thiobarbituric acid (TBA) levels were analyzed to evaluate oxidative stability, while differential scanning calorimetry (DSC) was examined to report the changes of thermal behavior at 4°C and 25°C.The results showed a change in POV of FOs from 1.11±0.15 to 1.37±0.37 meq O2/kg, while PAV change from 0.83±0.74 to 1.44±0.64 meq O2/kg at 4°C to 25oC respectively. The changes in TBA were reported from 2.49±1.74 to 3.01±2.08 at different storage conditions. Regarding POV and PAV, the values of FsO changed from 1.95±0.62 to 1.53±0.49 meq O2/kg and 0.52±0.33 to 0.92±0.27 meq O2/kg. Also, low melting temperatures were found for FO while compared to FsO. The study concluded that TBA, PAV and POV values of fish and flaxseed oil were

Article History
Received: 28 November 2024
Accepted: 14 February 2025

Keywords
Fish Oil;
Flax Seed Oil;
Physicochemical Properties;
Oxidative Stability;
Storage Conditions.

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Doi: https://dx.doi.org/10.12944/CRNFSJ.13.1.10

changed according to various storage conditions and intervals. The present study can help to improve various processing techniques, packaging, quality and storage stability of fish and flaxseed oil.

Introduction

Omega-3 (Ω-3) polyunsaturated fatty acids (PUFA) are best known for its beneficial effects to ameliorate diseases and illness.[1] Ω-3 PUFA has anti-inflammatory and anti-arrhythmic properties that help to reduce blood pressure, triglycerides level and appears to improve arterial and endothelial function.[2] Considering that, different epidemiologic studies have provided evidence for a positive role of Ω-3 FA on heart diseases, ischemic stroke, and inflammation.[3] Chemical composition of Ω-3 PUFA includes α-linolenic acid (ALA), stearidonic acid, docosapentaenoic acid, docosa hexanoic acid and eicosa pentanoic acid fatty acids (FA).[4] All mentioned FA are largely found in marine organisms and mainly produce in the liver of the fish; therefore, it is recommended that two servings of FO/week can provide approximately 0.3–0.45 g of eicosa pentanoic acid (EPA) and docosa hexanoic acid (DHA) per day.[5] Dietary supplements of FO containing 3–4 g/day of EPA or a combined EPA and DHA reduce triglycerides level by 20–50% among people with hypertriglyceridemia.[6] It is suggested that consumption of fish and seafood lowers the risk of mortality in men with prostate cancer.[7] Similarly, FO-PUFAs stimulates several signaling pathways and inflammatory cytokines needed to prevent insulin resistance and arrhythmia.[8]

In addition to FO, other vegetable seeds such as flaxseed oils, as a valuable source of Ω-3 FA are of special research interest in health promotion and disease risk reduction.[9] Flaxseed (*Linum Usitatissimmum*) is also identified as linseed, Jawas, alsi and aksebija belongs to Linaceae family. It has unique nutritional properties comprising mainly fat, fiber and protein.[10] Regarding fat content, flaxseed contains a larger amount of total fatty acids and appears to prove the richest source of ALA. Moreover, it is an excellent plant-based source for Ω-3 providing more than fifty percent of ALA.[11] Recent studies showed that ALA is associated with a 10% reduction in total cardiovascular disease and a 20% reduced risk of coronary heart disease.[12]

Since fish and flaxseed oils, being an excellent source of Ω-3 FA helps to ameliorate disease and illness. However, the unsaturation and oxidation process can affect the nutritional properties and beneficial effects of both oils. This oxidation process can result in drastic loss of nutrition and can also affect its sensory properties.[13] Oxidation process of oils results in the development of undesirable organoleptic properties and texture, lower nutritional value, shelf-life and even in the formation of toxic compounds.[14] Changes in storage conditions appear to alter the chemical properties, physical and biological characteristics of fish and flaxseed oil. The present study therefore conducted to analyze the effect of different storage conditions (4°C & 25°C) with and without the availability of oxygen on oxidative stability of fish and flaxseed oil. The present study will help to improve various processing techniques, packaging, quality and storage stability of fish and flaxseed oil.

Materials and Methods

Oil Procurement

The oils including fish and flaxseed oil was procured (VWR international), United States and analyzed for various quality parameters such as peroxide value (POV) and p-anisidine (PAV) value. Further, Thiobarbituric acid (TBA) values were analyzed to assess oxidative stability, while DSC (melting curves) analyzed the degree of unsaturation and thermal behavior of both oils. All analyses were conducted at the Department of Food Science, Purdue University, USA.

Stability Analysis

The stability assessment was performed through analysis of POV, PAV and TBA levels. In addition, saponification, iodine, and FA levels were measured for both fish and flaxseed oil. All analyses were conducted under 2 storage conditions, i) room (25 °C) and ii) refrigeration condition (4 °C) to assess the effect of temperature on the oil's stability. In concomitant, analyses were performed in the presence as well as in the absence of oxygen to

assess the influence of air exposure. The oils were stored for 21 days of time interval. Furthermore, i) a flask containing oils covered with aluminum foil was used for free oxygen availability whereas ii) glass bottles containing oils were completely covered, packed, and darkly stored to provide seized oxygen environment. A standard protocol of AOCS (2015) was adopted to conduct all tests and analyses.[15] All treatment protocols are presented in Table 1.

Table 1: Storage conditions and temperature for fish and flaxseed oils

Treatment	Temperature	Storage condition
T_1	4°C	Airtight glass bottle
T_2		Flask covered with aluminum foil
T_3	25°C	Airtight glass bottle
T_4		Flask covered with aluminum foil

POV Analysis

The POV is indicated as an amount of hydroperoxides and is taken as an indicator of oils oxidation process. For this purpose, 3 grams of oil was diluted with the chemicals i.e., 30 milli liter glacial-acetic acid+ 20 milli liter chloroform + 1 milli liter of saturated KI solution and placed in dark place for 1 minute. Subsequently, water (50 milli liter) was poured into the mixture. Afterwards, the titration process with 0.01 N sodium thiosulphate ($Na_2S_2O_3$) was conducted to complete the process. The POV was taken as meq oxygen/kg sample through applying the following formula:

$$\text{POV} = \frac{(\text{volume of } Na_2S_2O_3 \times \text{normality of } Na_2S_2O_3)}{\text{Oil sample}}$$

PAV Analysis

PAV assessment helps to evaluate the subsequent oxidized product present in oil thus to evaluate the intensity of oxidation process. The analysis was performed through adding the para-anisidine into glacial-acetic acid and preparing a solution of 0.25 g/100 milli liter. Further, 0.5 gram of the sample dissolved with isooctane was taken in a flask (25 milli liter). The isooctane was taken as a blank reagent, and absorbance was estimated at 350 nm. Later, 5 milli liter of the solution and 5 milli liter of isooctane were taken in 2 separate test tubes. Finally, 1 milli liter of para-anisidine was dissolved and mixed and absorbance was measured for both sample and blank reagent after 10 minutes. The final calculation was reported after applying the following formula.

$$\text{PAV value} = 25 \times 1{:}2 \ \frac{\text{sample absorption- blank absorption}}{\text{Weight of sample}}$$

TBA Analysis

TBA assessment was performed following Menoyo and colleagues' study method 16. For this purpose, 2 grams of oil was added to 18 milli liter of perchloric-acid i.e. 3.86%. Later it is homogenized and blended with brinkman polytron 7 for fifteen seconds and then filtered through filter paper (Whatman #1). The filtered compound was then added to 2 milliliters of TBA (20 mM) and incubated for 30 minutes in a water bath while 1 milli liter of TBA (20mM) was considered as blank. The absorbance of both filtered sample and blank was taken at 531 nm and presented as mg/kg of oil.

Saponification, Iodine, and Free Fatty Acid (FFA) Analysis

For saponification, 2 grams of oil was dissolved to 30 milliliters of ethanolic KOH, attached to condenser (30 minutes) to ensure full dissolution of sample. Later, 1 milliliter of phenolphthalein was included and titration process with 0.5 M HCL started until we got pink color (endpoint) and represented as milliequivalents of oxygen per kg (meq O_2/kg) of oil. Moreover, iodine and FFA analysis was performed using the Cd 1d-92 and CA 5a-40 method respectively stated in American Oil Chemists Society guidelines.[15]

Differential Scanning Calorimetry (DSC)

DSC is used to represent the thermal properties of oil. It was performed by taking 5 milligrams of oil in DSC pan, while reference sample was processed using an empty DSC pan. The analysis was operated using discovery DSC (Model Q10, USA) at -74 to 100 °C at 5 °C/minute (heating rate).

Statistical Analysis

Montgomery (2008) method was used to compare means and significance level.[17] Statistix 8.1. software was used for data analysis. ANOVA test with 2 factors factorial under completely randomized model was applied for some oil parameters comprising storage effects whereas, one way analysis of variance was performed for other parameters followed by Tukey's test for mean comparison.

Results

Pov Evaluation

The results showed the POV of 1.11±0.15 meq O_2/kg and 1.95±0.62 meq O_2/kg for FOs and FsO respectively at 4°C without oxygen (Table 2). However, the POV of FO increased to 1.37±0.37 meq O_2/kg and 1.53±0.49 meq O_2/kg for FsO at room temperature at 25°C (Table 2). In comparison, POV for treatment 4(T4) was 2.66±0.94 and 2.30±0.52 meq O_2/kg for FO and FsO individually in the presence of air and this difference might be due to the acceleration of oxidation process when sample exposed to air. Later after 3 weeks of storage, POV reported at 2.19±0.78 meq O_2/kg for FO and 2.41±0.31 meq O_2/kg for FsOs at 21st day of treatment after 3 weeks of storage (Table 2).

Table 2: Storage intervals and conditions on peroxide value, p-anisidine and thiobarbituric acid value of fish and flaxseed oil

Oils	Storage Intervals	Peroxide value (POV) meq O_2/kg	P-anisidine value (PAV) meq O_2/kg	Thiobarbituric acid value (TBA) mg MA/kg*
Fish oil	1st	1.01±0.09^{c}	0.74±0.36^{d}	0.96±0.15^{d}
	7th	1.87±0.65^{b}	0.91±0.35^{c}	1.32±0.12^{c}
	14th	1.99±0.91^{b}	1.1±0.29^{b}	3.53±0.58^{b}
	21st	2.19±0.78^{a}	2.43±0.25^{a}	5.74±0.54^{a}
Flaxseed oil	1st	1.01±0.24^{d}	0.32±0.50^{d}	0.43±0.21^{d}
	7th	2.47±0.33^{a}	0.73±0.18^{c}	1.25±0.80^{c}
	14th	2.01±0.55^{c}	0.95±0.29^{b}	2.30±0.55^{b}
	21st	2.41±0.31^{b}	1.3±0.28^{a}	3.54±1.43^{a}
Fish oil	**Storage Conditions**	**Peroxide value (POV)**	**P-anisidine value (PAV)**	**Thiobarbituric acid value (TBA)**
	(T_1)	1.11±0.15^{c}	0.83±0.74^{d}	2.49±1.74^{d}
	(T_2)	1.93±0.49^{b}	1.21±0.65^{c}	2.68±1.71^{c}
	(T^3)	1.37±0.37^{c}	1.44±0.64^{b}	3.01±2.08^{b}
	(T_4)	2.66±0.94^{a}	1.69±0.63^{a}	3.35±2.16^{a}
Flaxseed oil	(T_1)	1.95±0.62^{c}	0.52±0.33^{d}	1.06±0.87^{d}
	(T_2)	2.37±0.62^{a}	0.78±0.27^{c}	1.31±0.84^{c}
	(T_3)	1.53±0.49^{d}	0.92±0.27^{b}	2.22±1.25^{b}
	(T_4)	2.30±0.52^{b}	1.25±0.33^{a}	2.92±1.80^{a}

* mg malonaldehyde/kg oil

PAV Evaluation

The PAV levels from the study found at 0.83±0.74 for FO at 4°C without air to 1.21±0.65 in the presence of air, while FsO levels reported as 0.52±0.33 to 0.78±0.27 (Table 2). Furthermore, PAV levels at 4°C were found low as compared to 25 °C i. e. 1.44±0.64 to 1.69±0.63 for FO and 0.92±0.27 to 1.25±0.33 for FsO respectively.

TBA Evaluation

Regarding TBA value the study observed a rise in value from 2.49±1.74 (without air) to 2.68±1.71 mg

MA/kg at 4°C with access to oxygen while, minor change at higher temperature (25°C) from 3.01±2.08 (without air) to 3.35±2.16 mg MA/kg in the presence of air was observed for FO (Table 2). Similar results were seen by FsO and value was found in range 1.06±0.87 (without air) to 2.92±1.80 mg MA/kg in the presence of air. During storage intervals, TBA was raised from 0.96±0.15 to 5.74±0.54 on the 21st day for FO, while FsO showed a change of TBA 0.43±0.21 (initially) to 3.54±1.43 at end of the experiment.

Saponification, Iodine and FFA Evaluation

Mean values for saponification, iodine and FFA were found 178±3.26 mg KOH/g oil, 200±1.63 mg/100g and 0.5±0.02% for FO respectively. Whereas for FsO levels were found 218±2.44 mg KOH/g oil and 180±4.08 mg/100g and 2.0±0.07% respectively (Table 3). Furthermore, statistical interpretation revealed a significant effect of FO and FsO on all parameters as manifested by their respective P-values (0.0000 for saponification and iodine value and 0.01 in case of acid value) of both oils.

Table 3: Chemical properties of fish and flaxseed oils

Parameters	Fish oil	Flaxseed oil
Saponification (mg KOH/g oil)	178±3.26	218±2.44
Iodine (mg/100g)	200±3.27	180±4.08
Free FA (%)	0.5±0.02	2.0±0.07
DSC (Melting curves)	66.68±1.52	-49.82±2.68
Onset temperature (°C)		
Peak temperature (°C)	-53.80±2.24	-34.18±1.83
Enthalpy (J/g)	5.69±1.61	108.03±4.41

Thermal Properties of FO and FsO

Melting DSC curves are used to describe thermal properties of oils (Fig 1 and 2). FO and FsO both showed a significant variation might be due to the presence of unsaturated FA present in each oil. The thermogram of FOs showed baseline range from -64.58 to -68.13°C to -53.72 and -56.59°Cat highest temperature, while FsO reported (-35.02°C to -39.77°C) at onset and (-32.5 to -37.16°C) at peak (Table 3).

The results showed more stability of FsO than FO at room temperature by exhibiting less variation of peroxide and anisidine value for FsO. DSC analysis also confirmed this similar behavior by manifesting less peak temperate for FO (-53.80±2.24) than FsO (-34.18±1.83).

Discussion

The results found a change in POV, PAV and TBA values of FO and FsO according to different storage conditions, intervals, oxygen availability and with change in temperature. Lipids may start auto-oxidation through atmospheric oxygen reactions, thermal oxidation at different temperatures, enzymatic oxidation, free radical production leading to deterioration of oils.[18] POV in this regard is considered as the production of peroxides during autooxidation of oils. It is used to evaluate the intensity of oxidative processes in oil.[19] The results showed the remarkable effect of various treatments (storage conditions) and time duration on POV of both oils. In agreement to our findings, a previous study reported the increase in FsO-POV values to 2.8 meq O_2/kg after 12 weeks of storage.[20] Likewise, another study on FsO found an increase in POV increase from 1.56 to 2.34 meq O_2/kg of oil.[21] Additionally, previous studies reported that the oxidation process of FO can be minimized through storage in dark, dry, and cold conditions.[22] Studies on FO showed an increase of POV from 0.92 to 1.61 meq O_2/kg with temperature.[23]

Regarding PAV evaluation, the oxidation process leading to production of primary peroxides and its derivatives (secondary peroxides) can be used as an indicator of lipid oxidation. These peroxide derivatives mainly consist of ketones, aldehydes, and hydrocarbons. Whereas PAV are considered to unsaturated aldehydes.[24] In our study, different storage conditions and duration tend to change the PAV levels of FO and FsO. Previously, a study

conducted on hull FsO found the change in PAV levels before heating at 1.10 and increased to 8.50 after heating conditions.[25] Another study estimated PAV of FsO ranging from 0.45 to 0.52 with the change in temperature.[21]

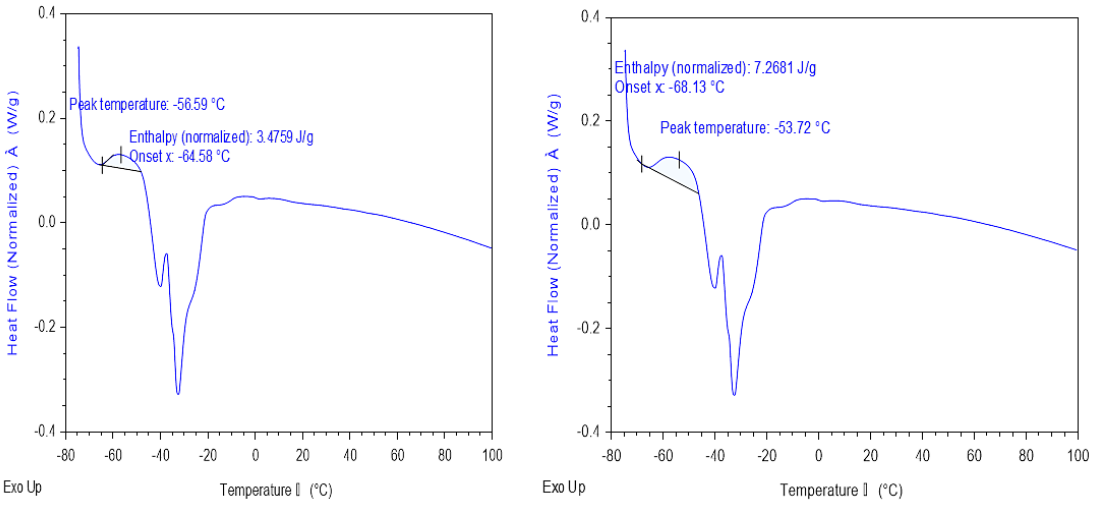

Fig 1. (a) and (b) Melting curves of fish oil

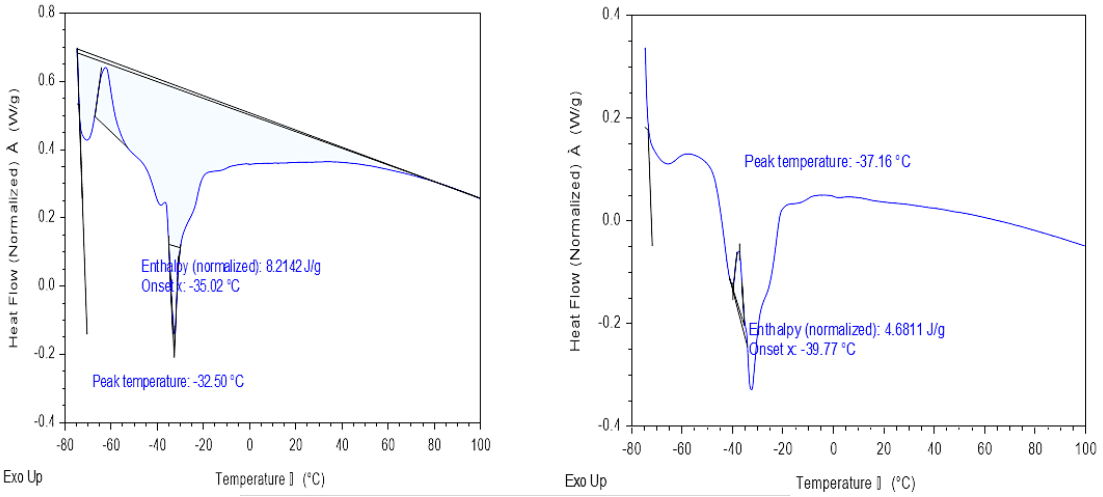

Fig 2. (a) and (b) Melting curves of flaxseed oil

Adding that, the results reported a significant effect of all treatments and conditions on TBA levels of FO and FsO due to acceleration of oxidation process in presence of light, oxygen, and storage condition. TBA is a reliable predictor to evaluate the autooxidation of oils. During the process, TBA produces red colored (proportional to oil rancidity) due to the reaction of secondary oxidative substances such as unsaturated aldehydes and malonaldehydes. A previous study on FsO showed the TBA levels at 0.30 (baseline) reach 0.624 due to environment exposure leading to oxidation.[26] Another study on fortified sausage with Ω-3 FA found high TBA levels in fortified Ω-3 (3.82) than control group (0.60).[27] Babalola and Apata conducted research on vegetable oils, FOs and animal fats and found that FO is more stable for longer storage than other oils (TBA = 0.016 mg of malonaldehyde/ kg)..[28] Equally, Jaswir and co-investigators reported a slight increase in TBA levels in FO till three weeks,

however, it increased from 4.20-6.21 mg MA/kg at 4°C and 2.55-3.80 at -27 °C after three weeks.[29]

The results found high values of saponification, iodine and FFA in FsO as compared to FO. Saponification shows the average molecular mass of FA, while iodine concentrations present the level of unsaturation in oils to evaluate oxidative capacity or oils stability.[30] The results found only the minute difference as compared to previous studies. This difference can be explained in the change of genetic makeup, environmental conditions, and geographical setting. In agreement to our findings, a study reported FFA and iodine for FsO 0.89 to 0.96% and 192.3 to 196.0 mg/100g respectively.[31] Furthermore, Tech and Birch study stated FFA content as 0.75% for FsO.[32] Jaswir and colleagues studied FO of different species of Malaysia at and showed a change in iodine value (178.71 to 180.65 mg/100g) at -4°C and 178.71 to 180.65 mg/100g at -27°C 29.

Melting DSC curves are used to describe thermal properties of oils and showed a less peak temperature for FO as compared to FsO. DSC method is more efficient to work on small samples, easy to use, less preparation steps involved as compared to traditional chemical methods.[33] The less variation in FO can be explained by the high percentage of unsaturated FA in FO. Similarly, the reason for high peaks for FsO (-37.26°C) can be ascribed due to the presence of triacylglycerol (high melting points). The enthalpies of FO and FsO were 5.69±1.61 J/g and 8.9904±3.35 J/g respectively, similar to Dave Oomah and colleagues, who reported enthalpies range 59.52 to 60.68 J/g for nigella seed oils while 75 J/g for raspberry seed oils.[34] Another study evaluates the thermal behavior of FO by DSC and found the time dependence between heat of fusion and melting temperatures on FO. It was reported that 18-C atoms showed melting point of its β-crystals at 73°C while 14-C lowered melting point to 58°C 35. The results were also similar to the past study which explored the phase transition of different fish species and 2 endo and exothermic peaks for salmon and trout fish.[35]

Conclusion

The study concluded that different storage conditions and intervals change the POV, PAV and TBA values of both FO and FsO. This paper provides comprehensive information about the quality and stability analysis of two polyunsaturated oils. The oils stored in the absence or limited access to direct light and oxygen are best for use up to 2-3 months. Moreover, in terms of comparison FsO is more stable to oxidation than FO might be due to more stability of vegetable fat constituent than animal fat. The characteristics of FsO and FO regarding POV, PAV, TBA value and iodine, saponification value can be helpful material for quality oils, however, influence of various processing techniques like packaging, processing and age of oil is lacking and necessitate for future research studies. Future studies focusing on different packaging materials or antioxidant treatments in relation to improving oxidative stability of both oils are recommended. Additionally, the most suitable environmental conditions and impact of long-term storage can be explored to improve the shelf life of these oils.

Acknowledgement

The authors would like to deeply acknowledge the Higher Education Commission, Pakistan as well as the National Institute of Food Science & Technology (NIFSAT), University of Agriculture, Faisalabad for providing with the opportunities to conduct this important research.

Funding Sources

The research was supported financially by Higher Education Commission of Pakistan and National Institute of Food Science and Technology, University of Agriculture, Faisalabad. (Funding No: 1-8/HEC/HRD/2017/7283).

Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

The complete data is available upon request from the corresponding author.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to Reproduce Material from other Sources

Not Applicable.

Author Contributions

- **Sehar Iqbal:** Initial Manuscript Drafting
- **Sajeela Akram:** Conceptualization and Data Collection
- **Saira Zafar:** Final Manuscript Improvement and Revision
- **Syed Hassan Bin Usman Shah:** Final Manuscript Improvement and Revision
- **Rida Fatima Saeed:** Final Manuscript Improvement and Revision
- **Masood Sadiq Butt:** Supervision and Data Analysis
- **Juweria Abid:** Final Manuscript Editing
- **Umar Farooq:** Data Validation and Proofreading
- **Abdul Momin Rizwan Ahmad:** Initial Manuscript Drafting

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