FATTY ACID PROFILING AND FUNCTIONAL GROUP CHARACTERIZATION OF BARLEY (HORDEUM VULGARE L.) GRAIN OIL USING GAS CHROMATOGRAPHY–MASS SPECTROMETRY AND FT–IR ANALYSIS

F. Umrani[1], S. K. Khanzada[1], A. K. Khanzada[1], G. A. Mugheri[2], B. S. Khilji[1], A. Panhwar[3], D. B. Mashori[4] and A. Kandhro[5]

[1] Institute of Plant Sciences, University of Sindh, Jamshoro, Sindh, Pakistan
[2]Department of Chemistry, Cadet College Larkano, Sindh, Pakistan
[3]Centre of Environmental Studies, PCSIR Laboratories Complex, Karachi, Sindh, Pakistan
[4]Department of Zoology, GC University Hyderabad, Sindh, Pakistan, Pakistan
[5]M. A. Kazi Institute of Chemistry, University of Sindh, Jamshoro, Sindh, Pakistan
Email: Corresponding author: samina.khanzada@usindh.edu.pk

ABSTRACT: This study investigates the fatty acid profile and functional group characteristics of barley (Hordeum vulgare L.) grain oil extracted from five varieties collected in Sindh, Pakistan. Oils were extracted via Soxhlet using hexane, converted to fatty acid methyl esters (FAMEs), and analyzed through Gas Chromatography–Mass Spectrometry (GC–MS). Major fatty acids identified included hexadecanoic acid (C16:0), octadecanoic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3). Total saturated fatty acids (SFA) ranged from 22.0% to 25.1%, while total unsaturated fatty acids (UFA) ranged from 71.9% to 77.9%. Fourier Transform Infrared Spectroscopy (FT–IR) confirmed the presence of typical lipid functional groups such as aliphatic C–H, carbonyl C=O, and C–O ester linkages. These results demonstrate that barley grain oil is rich in nutritionally beneficial unsaturated fatty acids, highlighting its potential for use in food, nutraceutical, and industrial applications.

Key words: Fatty acids; Barley oil; GC–MS; FT–IR; Triglycerides; FAMEs; Hordeum vulgare

(Received 29.10.2025 Accepted 15.12.2025)

INTRODUCTION

Barley, a member of the grass family Poaceae, is a major cereal grain and self-pollinating, diploid species with 14 chromosomes. [1].Barley (Hordeum vulgare L.) is traditionally recognized for its carbohydrate, protein content and dietary fiber content [2]. A small amount of barley is used as a health food because of its continuous and stable sugar release in the gastrointestinal tract [3].However; In addition, barley also contains a small amount of lipids [4]. Lipids (oils and fats) are important constituent of our diet which supply notably for the growth and metabolism of various purposes in body. About, 9.0 kcal/g of energy as well as fatty acids are provided by them and make possible absorption of fat soluble vitamins like vitamins A, D, E and K [5, 6]. Fats and oils are chemically similar, but their existing state is different. Usually, oils are liquids at normal temperature and fats are solids at room temperature [7, 8]. Its lipid fraction, though modest, contains essential fatty acids with established roles in nutrition and health. Unsaturated fatty acids, particularly linoleic and oleic acids, contribute to cardiovascular protection and metabolic regulation [9]. Thus, accurately characterizing the fatty acid profile of barley oil is essential for assessing its nutritional and industrial value [10].Analytical techniques such as Gas Chromatography–Mass Spectrometry (GC–MS) and Fourier Transform Infrared Spectroscopy (FT–IR) provides precise molecular-level information regarding fatty acid composition and functional group characteristics. This study aims to characterize the fatty acid composition and functional groups present in barley grain oil extracted from five varieties cultivated in Sindh, Pakistan, using validated extraction, derivatization, and instrumental procedure

MATERIALS AND METHODS

Sample Collection: Five varieties of barley grain; Sorab–96(SO-96), Rakshan – 10(RA-10), Snobar -96 (SN-96), Awaran- 2992 (AW-2992) and Local Turbat (LT) were collected from diverse regions of Sindh.

Oil Extraction: The Oil from seeds was extracted by following, AOCS Aa 4- 38 (AOCS 2013) method. Oil extraction was carried out using a Soxhlet extractor with hexane, consistent with the methodology.

Preparation of Fatty Acid Methyl Esters (FAMEs): The first step for fatty acid (FA) composition evaluation is converting of fatty acid (FA) into its fatty acids methyl ester (FAME). The standard procedure was followed for the preparation of fatty acids methyl ester (FAME). Through this method 1.0 ml of extracted barley grain oil were taken in 100 ml round bottom flask which

containing 1 g of potassium hydroxide (as a catalyst) and by adding 20 ml of methanol (as a solvent). After the mixture was refluxed for 30 min at 60-70 °C then cooling the mixture and transferred into a separatory funnel for extraction with 10 ml portion of n-hexane for 3 times. The fatty acids methyl ester (FAME) was passed into the n-hexane (upper) layer. By the help of 100 ml beaker the n-hexane (upper) layer was separated in addition solvent was evaporated in order to obtain fatty acids methyl ester (FAME). The ester (sample) was treated by anhydrous sodium sulphate to make sample moisture free finally analyzed by GC-MS.2.3 Gas Chromatography–Mass Spectrometry (GC–MS)

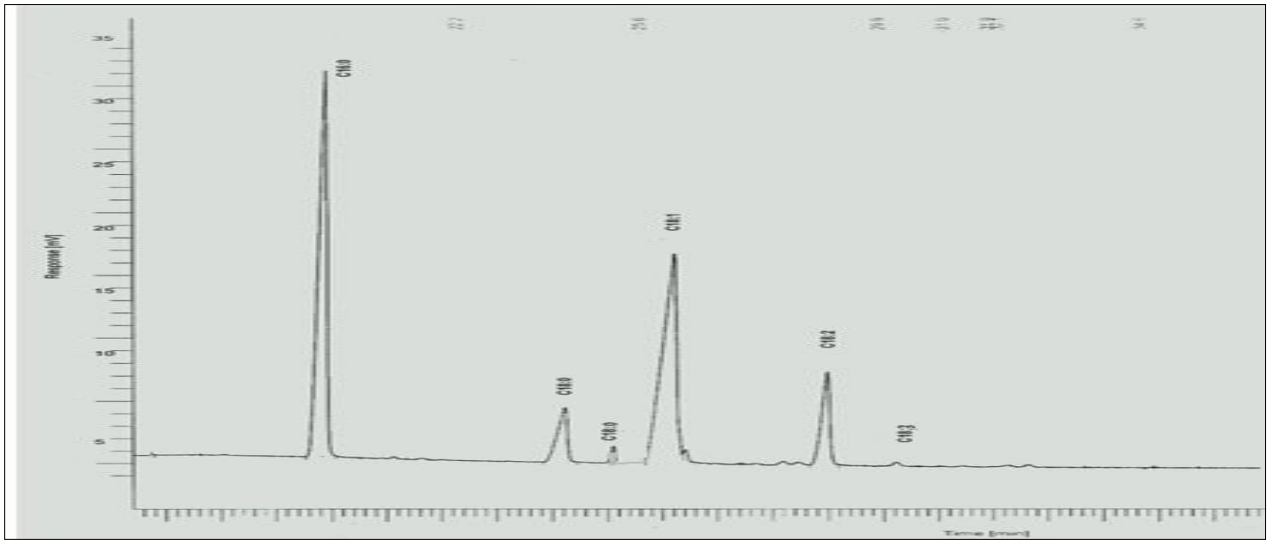
Gas Chromatography–Mass Spectrometry (GC–MS) Analysis: Five different prepared fatty acids methyl ester samples of barley grain oil samples were analyzed and identified by gas chromatography mass spectrometry (GC-MS). The high sensitive instrument (Agilent 6890 N gas chromatography GC) coupled with (mass selective detector) Agilent Technologies (MS-5975 inert XL). Separation of the FA components achieved on a 5% phenyl methylsiloxane containing HP–5MS capillary column. The column measurements were 30 m lenght, 0.25 mm i.d and 0.25 µm thickness of its film (Agilent Technologies, Palo Alto, CA, USA). Sample solution of 1.0 µL methyl ester was injected on a capillary column (HP-5MS) through auto sampler (7683-B). Helium was used as a carrier gas with 1.0 mLmin-1 flow rate; detector and injector temperatures were set in the range of 150 °C and 270 °C, split ratio was 1:35. Initially the temperature of oven was set at 150 °C for 2 minutes than temperature was raised to 270 °C at the rate of 0°C/min (4 min hold). Total analysis time of sample running was 45 minutes. The mass spectrometer instrument was operated in the electron impact (EI) mode at 70 eV in the examiner range between 50-550 m/z respectively.

Fatty acids identification was based on retention times and mass spectral fragmentation patterns and compared against reference spectra.

FT-IR Analysis: FT-IR analysis was by using (Thermo Nicolet iS10) with deuterated triglycinesulfate as a detector (DTGS), the IR spectra of oil samples were recorded. For getting the data of functional groups, OMINIC software (7.2 versions) was used. IR range 4000-650 cm-1, Resolution 4 cm-1, scans 32, SB-ATR accessory with diamond crystal was used as the instrumental programming. For recording the spectra about 50 µl of oil samples was placed on the diamond crystal. To avoid the interference of air / or residues of previous samples a back ground spectrum was recorded before recording the sample spectra of oil. Functional groups were identified by comparing absorption bands with known reference values.

RESULTS AND DISCUSSION

Fatty Acid Composition (GC–MS): In the present study the results revealed that the six major fatty acids were observed such as (lipid, hexadecanoic acid, Octadecanoic acid, oleic acid, linoleic acid, linolenic acid) as shown in Table 1.1,shows Quantitative ranges of fatty acid composition of Barley Verities.. The most abundant Linoleic acid fatty acid was observed 52.4%, 52.6%, 55.7%, 54.6% and 54.2% for sample 1 to 5 respectively. While the low percentage was found in Octadecanoic acid 1.3%, 1.1%, 1%, 1.1% and 0.9% correspondingly. Figure. 1.1 representative chromatogram of barley grain samples. The percentage of saturated fatty acids in barley grain was found 23.8%, 24.8%, 25.1%, 22.5% and 22% whereas, the unsaturated fatty acids results was found 71.9%, 73.5%, 74.3%, 77.9% and 75.8% respectively.



Figure; 1.1 Representative chromatogram of varieties of barley grain samples

Table: 1.1 Quantitative ranges of fatty acid composition of Barley Verities.

Fatty acid Composition	Sample 01	Sample 02	Sample 03	Sample 04	Sample 05
	V#Sorab-96	V#Rakhshan-10	V#Sanober-96	V#Awaran-2992	V#Local Turbat
%Lipid	1.9	2.4	2.1	2.3	2.2
Hexadecanoic acid (C16:0)	22.5	23.7	24.1	21.4	21.1
Octadecanoic acid(18:0)	1.3	1.1	1	1.1	0.9
Oliec acid (C18:1)	14.2	15.5	12.9	16.5	15.3
Linoleic acid (18:2)	52.4	52.6	55.7	54.6	54.2
Linolenic acid (18:3)	5.3	5.4	5.7	6.8	6.3
Total SFA	23.8	24.8	25.1	22.5	22
Total USFA	71.9	73.5	74.3	77.9	75.8

FT–IR Functional Group Characterization: FT-IR is a powerful analytical technique that is used to identify and characterize the chemical composition of a wide range of materials. By measuring the frequencies and intensities of the absorbed or transmitted radiation, the chemical composition and structure of the sample can be determined. Figure.1.2 indicates the FTIR normal spectra of (A) sample1, (B) sample 2 (C) sample 3 (D) sample 4 (E) sample 5 oil in the mid-infrared region. On careful examination of the intensities of various bands present in the barley grain. Table 1.2 shows the FT-IR spectral regions and functional groups found in barley grain samples.

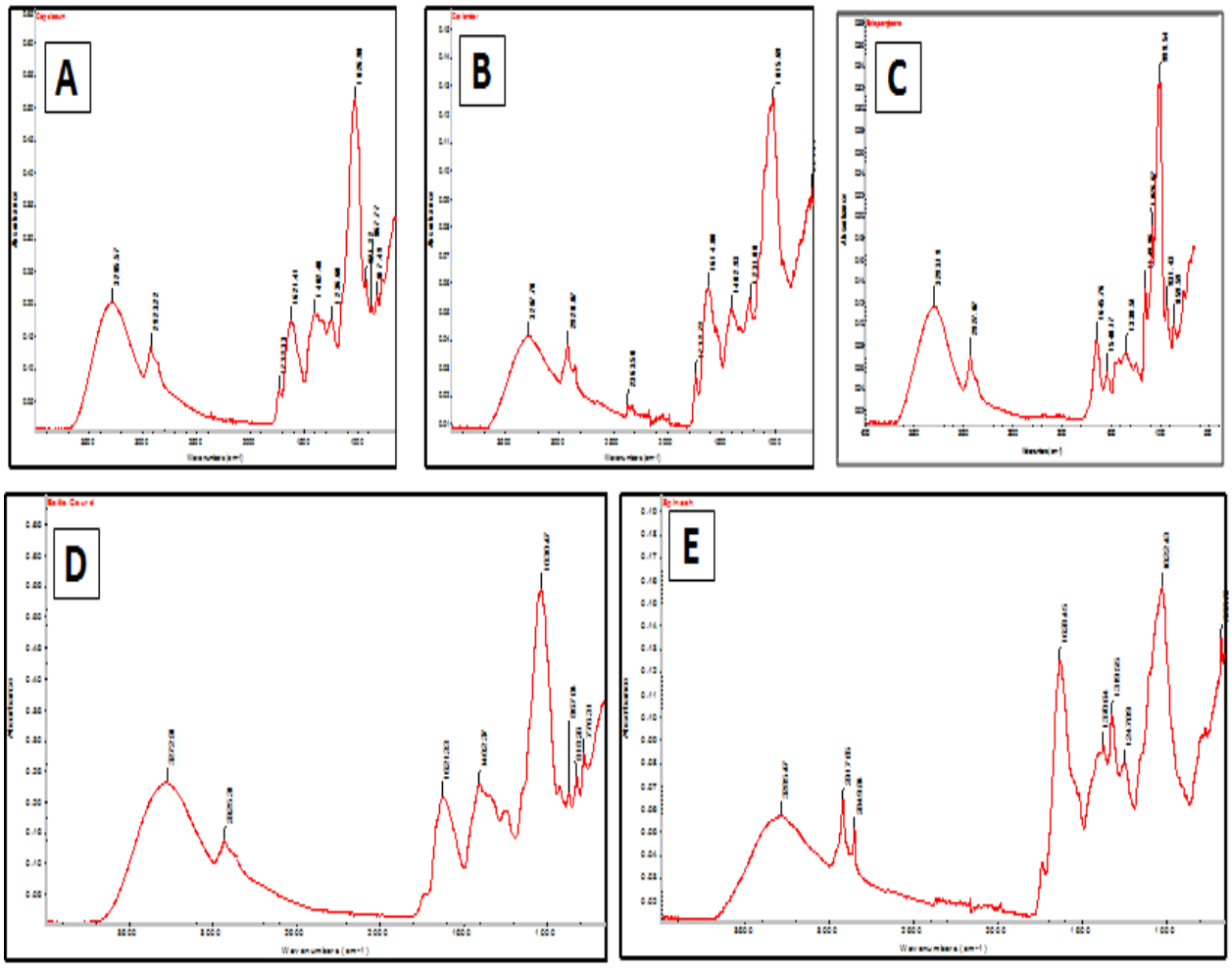


Figure: 1.2 FT-IR spectra's of barley grain

Table 1.2 FT-IR spectral regions and functional groups found in barley grain samples

S.No:	Range (cm^{-1})	Functional group	Mode of vibration
10	3029-2989	=C-H (cis)	Stretching
02	2946-2881	-C-H (CH_2)	Stretching (asy)
03	2881-2782	-C-H (CH_2)	Stretching (sy)
04	1795-1677	-C=O (ester)	Stretching
05	1486-1446	-C-H (CH_2)	Bending (sci)
06	1382-1371	-C-H (CH_3)	Bending (sym)
07	1290-1211	-C-O	Stretching
		-CH2-	Bending
08	1211-1147	-CH2	Stretching
		-CH2-	Bending
09	1128-1106	-C-O	Stretching
10	1106-1072	-C-O	Stretching
11	754-701	-(CH2)n-	Rocking
		-C=CH- (cis)	Bending (out of plane)

Conclusion: The Barley grain oil samples from Sindh demonstrated a high proportion of unsaturated fatty acids, with UFA: SFA ratios favorable for human health. Linoleic acid (C18:2), an essential omega-6 fatty acid, and oleic acid (C18:1), a monounsaturated fatty acid, dominated the unsaturated fraction. The presence of linolenic acid (C18:3) further enhances the nutritional value, given its role as an omega-3 precursor. FT–IR confirmed functional groups characteristic of triglycerides, validating the GC–MS findings. Although barley is not cultivated primarily for its oil content, the quality of its lipid fraction suggests potential applications in nutraceuticals, functional foods, and related industries.

REFERENCES

[1] El Khier, M. K. S., Ishag, K. E. A., & Yagoub, A. A. (2008). Chemical composition and oil characteristics of sesame seed cultivars grown in Sudan. Research Journal of Agriculture and Biological Sciences, 4(6), 761-766.

[2] Melkamu Hordofa, Gashaw Meteke., Wassie Haile. 2019. Effects of Different Blended Fertilizers on Yield and Yield Components of Food Barley (Hordeum vulgare L.) On Nitisols at Hulla District, Southern Ethiopia. Acad. Res. J. Agri. Sci. Res. 7(1): 49-56

[3] Ottman M.J. (2009). Response of wheat and barley varieties to phosphorus fertilizer. University of Arizona, USA.

[4] ATA (Agriculture Transformation Agency). 2013. Transforming the use of fertilizer in Ethiopia: Launching the national fertilizer blending program. Addis Ababa, Ethiopia.

[5] G. Otunola, G. Adebayo, and O. Olufemi, "Evaluation of some physicochemical parameters of selected brands of vegetable oils sold in Ilorin metropolis," International Journal of Physical Sciences, vol. 4, pp. 327-329, 2009.

[6] D. Strayer, M. Belcher, T. Dawson, B. Delaney, J. Fine, B. Flickinger, P. Friedman, C. Heckel, J. Hughes, and F. Kincs, "Food Fats and Oils, Institute of Shortening and Edible Oils," Washington, DC, 2006.

[7] Lipids, fatty acids, structure, composition, biochemistry and function http://lipidlibrary.aocs.org/Lipids/whatlip/index.htm (accessed Feb 25, 2015).

[8] Khor GL. Dietary fat quality: a nutritional epidemiologist's view, Asia Pacific Journal of Clinical Nutrition, 13 (2004) S22.

[9] Khor GL. Dietary fat quality: a nutritional epidemiologist's view, Asia Pacific Journal of Clinical Nutrition, 13 (2004) S22.

[10] Xin, H., Khan, N. A., & Yu, P. (2021). Steam pressure induced changes in carbohydrate molecular structures, chemical profile and in vitro fermentation characteristics of seeds from new Brassica carinata lines. Animal Feed Science and Technology, 276, 114903.