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Comparison of flaxseed oil characteristics of three Pakistani varieties obtained by supercritical CO_2 and two conventional extraction methods

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Abstract

Omega-3 (ω-3) fatty acids have gained importance for having health effects. The present work was designed to analyse the differences in three Pakistani flaxseed varieties and the effect of extraction methods on flaxseed oil quality. The oil was extracted using green solvent *i.e.* supercritical CO_2 (SC-CO_2) and compared with traditional methods namely Soxhlet extraction and mechanical extraction. The oils were significantly different in terms of free fatty acids, iodine values, and peroxide values, while oils were insignificantly different in terms of specific gravity, saponification values, *ρ*-anisidine values, and tocopherol contents. GC-FID results showed that α-linolenic acid (ω-3) was the major fatty acid in oil, ranging from 52.09 to 58.06%. The variation in the different parameters of oils observed in the present work might be correlated with the principle of each type of oil extraction method. Supercritical fluid extraction of flaxseed oil should be employed as it yields superior quality oil by extracting tocopherols and protecting the polyunsaturated oil from oxidation.

Introduction

Omega-3 (ω-3 / n-3 / Ω-3) fatty acids have gained importance among researchers and the general public because of their reported health benefits specifically related to cardiovascular diseases (CVDs). Omega-3 fatty acids, mostly present in sea foods and some vegetable oils are the essential fatty acids, essential in human development and growth. Dietary ω-3 fatty acids are majorly confined to marine-food consuming population. Therefore, both non-marine consumers and vegetarian populations are at risk of not getting sufficient levels of ω-3 fatty acids in their general diet (Goyal *et al.*, 2014).

The anti-inflammatory properties of ω-3 fatty acids can be beneficial in protecting the renal tissues from degeneration among adults. Diet rich in long chain poly-unsaturated fatty acids (PUFAs) have been reported to reduce kidney diseases in efficacy trials on animals (Gopinath *et al.*, 2011). Similarly, Cicero *et al.* (2010) showed that long-term supplementation of ω-3 fatty acids decreases the intensity of hypertension. Various clinical trials, epidemiological and animal studies have proved that ω-3 fatty acids are critically required in brain and nervous system development in infants (Guesnet and Alessandri, 2011), eye health (Chiu *et al.*, 2009), reducing the risk of hypertension, hypercholesterol, cancer including prostate, breast and colon, inflammatory bowel diseases, coronary heart diseases, diabetes, and neurodegenerative disorders (Goyal *et al.*, 2014).

In the past years, several studies have aimed at enriching food products with ω-3 fatty acids, due to their nutritional benefits as well as their low intake in the industrialised world. Flax (*Linum usitatissimum* L.) is a versatile and economically supreme oil seed crop. Flaxseed contains almost 40% oil content. It has approximately 57% α-linolenic acid (ALA) of its total fatty acid content, hence regarded as a rich natural source of ALA which is a ω-3 fatty acid. This feature advocates it's nutritional and health benefits (Singh *et al.*, 2011). Flaxseed oil has been previously used in free form and microencapsulated powder in cookies (Jeyakumari *et al.*, 2016), milk (Goyal *et*

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al., 2015), therapeutic fat spread (El-Waseif *et al.*, 2013), soup powder (Rubilar *et al.*, 2012) and bread (Gökmen *et al.*, 2011). However, there is a risk of consumer rejection for ω-3 fatty acids enriched foods. Many researchers have reported the problems regarding consumer acceptability of ω-3 fatty acids enriched foods (Cortinas *et al.*, 2003; Volker *et al.*, 2005).

Commercially, vegetable oils are produced by mechanical screw pressing; but the oil extraction efficiency is quite low (< 70%) due to the presently available equipment and processes, hence, considered inadequate (Willems *et al.*, 2008). In the past years, the commercial advantage of high yield by solvent extraction (> 99%) has been highlighted. The only drawbacks of solvent extraction are the end product's inferior quality and the co-extraction of unwanted compounds (Venter *et al.*, 2007). The supercritical fluid extraction (SFE) technique has been studied extensively as an alternative to traditional methods of oil extraction (Pradhan *et al.*, 2010). Only SFE offers both reduced processing energy inputs and an alternative solvent approach. Other benefits of this technology include rapid extraction, small amount of organic solvent or no solvent at all, no solvent residue, the preservation of thermally labile compounds, tuneable solvent (SCF) density, selective extraction (small number of compounds extracted), and inexpensive to operate/run (Khaw *et al.*, 2017). The percentage yield recovery of flaxseed oil obtained by supercritical CO_2 is lower in comparison to solvent extraction but higher than that of mechanical extraction (ME). On the contrary, SFE is capable of extracting more amounts of ω-3 fatty acids (ALA) (Bozan and Temelli, 2002). Khattab and Zeitoun (2013) reported that supercritical fluid CO2-extracted flaxseed oil has high contents of PUFAs, which is more important from the nutritional point of view. They also concluded that SFE improves the oxidative stability, thus enhancing the shelf life of flaxseed oil.

The present work was therefore designed to investigate the quality parameters of flaxseed oil of three Pakistani varieties as affected by the extraction methods *i.e.* ME, SE and SFE. The novelty of the present work lies in the exploration of a Pakistani flaxseed variety having high amount of ω-3 fatty acids. Furthermore, the basic goal was to find out the best extraction method and flaxseed variety for obtaining oil with high amount of ω-3 fatty acids instead of just focusing on the amount of oil extracted. The findings of the present work will be useful for selection of economically and nutritionally important flaxseed varieties, especially, as an ingredient for functional foods and nutraceuticals in Pakistan.

Materials and methods

Flaxseed varieties (LS-113, LS-89 and LS-120) used in the present work were procured from Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. The flaxseed varieties were of different species, grown in the same harvesting area and the same harvesting time. All the chemical reagents used were from Sigma-Aldrich (Switzerland) or Merck (Germany).

Mechanical oil extraction

The flaxseed oil was mechanically extracted using a laboratory scale screw press (Carver Mini Screw press). The seeds with moisture content of 8.0% (d.b.) were poured in the hopper of screw press and allowed to fall under gravity. The oil was directly collected in amber coloured glass bottles, flushed with nitrogen and stored in refrigerator until further analysis.

Soxhlet oil extraction

In order to perform Soxhlet oil extractions, samples were crushed in a laboratory grinder and sieved through 16 and 32 mesh sizes, thereby yielding particle sizes of 500 to 1,000 µm. The flaxseed oil was extracted using two different solvents (*n*-hexane and ethanol) at three different times (6, 8 and 10 h). The flaxseed powder was placed in thimble of Soxhlet apparatus and washed with solvent for a specific time according to the treatment plan stated above. The oil solvent mixture was filtered, followed by solvent evaporation in rotary evaporator. The oil was then collected in amber coloured glass bottles, flushed with nitrogen and stored in refrigerator until further analysis.

Supercritical fluid oil extraction

The supercritical fluid extraction was performed with a supercritical fluid extractor (SC-CO_2) (model SFT-150 supercritical fluid extractor incorporation, USA). Ground seeds (100 g) were loaded into a 400 mL vessel covered by glass wool and extracted with CO_2 at a flow rate of 40 mL/min. The temperature was kept 50°C and the extractions were performed at three different pressures (*i.e.* 30, 35 and 40 MPa). The oil was collected in amber coloured glass bottles, flushed with nitrogen and stored in refrigerator until further analysis.

Physicochemical analysis of flaxseed oil

The extracted oils were analysed for specific gravity, free fatty acids, iodine value (IV) and saponification value following the standard methods as described in AOAC (2006).

Oxidative stability

The stability of flaxseed oil was determined through peroxide value (PV) and *ρ*-anisidine value (*ρ*-AnV). The acetic acid-chloroform method AOCS Cd 8-53 (AOCS, 1998) was followed to determine the PV (meqO_2/kg). The p-anisidine values were estimated according to the standard method as described in AOAC (2006).

Tocopherol content by liquid chromatography

Tocopherols were measured following the method of Parry *et al.* (2005). Briefly, 1 mL flaxseed oil was mixed in 160 mL of methanol:tetrahydrofuran (1:1, v/v) followed by analysing over HPLC to determine the tocopherol profiles. For the separation purpose, a Zorbax SB C_{18} column (Agilent Technologies, Palo Alto, CA) was used at room temperature with 3.5 µm particle size and internal diameter of 30 mm × 1.0 mm. The tocopherols were eluted using a mobile phase of water as solvent A and acetonitrile as solvent B. The gradient procedure was performed as follows: (1) the gradient was linear from 80% to 99% of solvent B and the flow rate was 0.3 mL/min and (2) 99% of solvent B was kept for 10 min. The HPLC column was re-equilibrated for 10 min with 50% of solvent B prior to the next injection. The identification of tocopherols was conducted by comparing the HPLC retention time and selected reactant monitoring (SRM) analysis of the sample peaks with those of the pure corresponding commercial tocopherols. The quantification for each tocopherol was accomplished using the total ion counts with external standards and measurements taken in triplicate.

Fatty acid profile by gas chromatography

Fatty acids were esterified as fatty acid methyl esters (FAMEs) (AOAC, 2006) by reacting with borontrifloride and analysed by gas chromatography (Agilent 6890 N) equipped with a capillary column (DB-23, 60 m × 0.25 µm) and flame ionisation detector (FID). Helium gas was used as a carrier gas at 1.2 mL/min. The temperature of injector and detector was maintained at 250°C. For the first 15 min, temperature was kept at 165°C then increased at a rate of 5°C/min until 200°C, and maintained for next 15 min. Fatty acids were identified by comparison of their retention times with those of authentic commercial standards, and the results were reported as the area percentage of the peaks.

Statistical analysis

Statistical analyses were performed with Statistix 8.1 software. The data were analysed by two-way and three-way analysis of variance (ANOVA), and the means were compared by the least significant difference (LSD) test at a significance level of 0.05. Values were expressed as mean ± SD of three replicates (n = 3).

Results and discussion

Effect of different extraction techniques on oil yield (%)

Mechanical extraction

There was an insignificant ($p > 0.05$) difference for oil yield from three varieties by ME (Table 1). The highest oil yield was recorded by ME in V_1 (26.70%) followed by V_2 (25.90%) and V_3, (25.40%). ME of oil has relatively low initial and operational costs and produces uncontaminated oil (Willems *et al.*, 2008). The less amount of oil extraction from ME can be attributed to the low efficiency of this process. Pradhan *et al.* (2010) reported as 25.5% oil recovery from flaxseeds by ME.

Table 1. Oil yield (v/w %) of flaxseed varieties extracted by mechanical extraction method.

Varieties	Oil yield (v/w %)
V_1	26.71 ± 1.14^{a}
V_2	25.90 ± 0.19^{ab}
V_3	25.43 ± 1.12^{b}

V_1 = LS-113; V_2 = LS-120; V_3 = LS-89. Values are means ± standard deviation of three determination (n = 3). Values followed by similar superscript letters in the same column are not significantly different ($p > 0.05$).

Soxhlet extraction

The statistical results for SE showed that the type of solvent used during oil extraction significantly ($p < 0.05$) influenced the oil yield (Table 2). After 6 h extraction, the oil yield for V_1 was 39.85% after using *n*-hexane while it was reduced to 38.23% after ethanol was used (Table 2). The lower yield of extraction by ethanol could be attributed partly to its high polarity and oils are generally less soluble in polar solvents. Non-polar solvents like *n*-hexane are not charged and their dipole moment is zero, which extracts more oil, whereas, in ethanol, as an organic polar solvent, the hydroxyl groups would interfere with the extraction process. Gutte *et al.* (2015) studied the effect of different solvents on the extraction of flaxseed oil, and stated that hexane extracted 14.53% oil followed by dichloromethane (13.37%), petroleum ether (13.09%), ethanol (12.78%), acetone (11.00%) and methanol (9.68%). The oil yield was quite low as they used these solvents to extract flaxseed oil by just mixing the powdered flaxseeds with these solvents.

Table 2. Oil yield (v/w %) of flaxseed varieties extracted by Soxhlet extraction

Varieties	Ethanol			n-hexane		
	6 h	8 h	10 h	6 h	8 h	10 h
V_1	38.23 ± 1.07^{bcd}	38.47 ± 1.01^{abcd}	38.75 ± 0.07^{abcd}	39.85 ± 1.33^{abc}	41.20 ± 1.09^{a}	41.01 ± 1.11^{ab}
V_2	37.31 ± 0.53^{cd}	37.52 ± 1.03^{cd}	37.66 ± 0.16^{cd}	38.20 ± 1.07^{bcd}	38.54 ± 1.21^{abcd}	38.83 ± 1.00^{abcd}
V_3	37.01 ± 1.70^{d}	37.13 ± 1.20^{cd}	37.29 ± 1.03^{cd}	38.15 ± 1.04^{cd}	38.27 ± 1.07^{abcd}	$s38.39 \pm 1.17^{bcd}$

V_1 = LS-113; V_2 = LS-120; V_3 = LS-89. Values are means ± standard deviation of three determination (n = 3). Values followed by similar superscript letters in the same column are not significantly different ($p > 0.05$).

During solvent extraction, it is important to choose an appropriate extraction time, since it is helpful in calculating the optimum holding time needed for maximum extraction (Saxena *et al.*, 2011). The statistical data also showed that the effect of extraction time during SE was insignificant ($p > 0.05$). The oil yield for V_1 was recorded as 38.23% and 39.85% at 6 h with ethanol and n-hexane, respectively. The values increased from 38.23% to 38.75% for V1 with ethanol while the oil yield increased from 39.85% to 41.20% with *n*-hexane after 10 h. The reason for low oil extraction after 6 h could be due to the low density of the solvent retained in sample after 6 h. However, the oil extraction was very efficient initially from 1 h until 6 h since the diffusivities of the oil and solvent increased. Therefore, it can be concluded that the maximum oil yield could be achieved even at shorter residence time. Ghazali and Yasin (2016) concluded that after 6 h further increase in extraction time did not result an increase in oil extraction from *Moringa oleifera* seeds.

Table 3. Oil yield (v/w %) of flaxseed varieties extracted by supercritical fluid extraction method

Varieties	30 MPa	35 MPa	40 MPa
V_1	34.24 ± 1.33^{bc}	37.37 ± 1.87^{a}	38.45 ± 2.07^{a}
V_2	33.26 ± 1.07^{c}	36.30 ± 1.32^{ab}	37.38 ± 1.04^{a}
V_3	33.20 ± 1.05^{c}	36.18 ± 1.14^{ab}	37.25 ± 1.00^{a}

V_1 = LS-113; V_2 = LS-120; V_3 = LS-89. Values are means ± standard deviation of three determination (n = 3). Values followed by similar superscript letters in the same column are not significantly different

Supercritical fluid extraction

The SFE technique has been extensively studied as an alternative to conventional methods of oil extraction (Herrero *et al.*, 2010). The statistical results regarding the oil yield of different flaxseed varieties by SFE showed that pressure significantly ($p < 0.05$) affected the oil yield, while the oil yield was insignificant ($p > 0.05$) between different varieties (Table 3). Highest oil yield was obtained from V_1 (38.45%) followed by V_2 (37.38%) and V_3 (37.25%). The values shown in Table 3 indicate that the oil yield of flaxseed varieties increased with increasing pressure. The oil yield at 30 MPa was significantly ($p < 0.05$) less as compared to 35 and 40 MPa, while the increase in oil yield from 35 MPa to 40 MPa was insignificant ($p > 0.05$). The increased oil yield with an increased pressure in SFE extraction could be due to an increase in the density of CO_2 at higher pressures. The increased pressure increased both the solvation power and the intermolecular interaction strength. The vapour pressure also increased with increased density at higher pressures, hence more flaxseed oil was obtained at higher pressure. Jiao *et al.* (2008) extracted flaxseed oil by SC-CO_2 and reported that after 41 MPa pressure, the increased repulsive solute-solvent interactions resulting from the highly compressed CO_2 caused a little decrease in the yield of flaxseed oil.

At this stage, nine treatments were selected in total, and their physico-chemical analysis was performed. From SE and SFE, for each variety, those treatments were selected which gave maximum yield (*i.e.* SE oil extracted with n-hexane at 6 h and SFE oil extracted at 35 MPa of all the three varieties), while all the three oils of ME were also selected.

Physico-chemical analysis

The statistical results showed that specific gravity was insignificantly ($p > 0.05$) different among the nine treatments. The specific gravity of different flaxseed oils ranged from 0.928 (ME oil of V_3) to 0.933 (SFE oil of V_1) (Table 4).

Rancidity in foods can be assessed through the free fatty acid production. Statistical data showed that the extracted flaxseed oils were insignificantly ($p > 0.05$) different for FFAs content with respect to varieties, but they were significantly ($p < 0.05$) different with respect to extraction methods (Table 4). The lowest FFAs content was observed in oil extracted by SFE of V_2 (0.93%) while the highest was observed in SE oil of V3 (1.05%). The FFAs content of oil extracted by ME of V_1 and V_2 was recorded as 0.98%. The lower amount of FFA in SFE could probably due to the fact that the polar lipids are less soluble in supercritical CO_2 and hence free fatty acids having a negative charge at the carboxyl end group were not extracted

Table 4. Physico-chemical properties, oxidative stability and tocopherol content of flaxseed oil of various varieties with different extraction methods.

Parameters	ME			SE			SFE		
	V_1	V_2	V_3	V_1	V_2	V_3	V_1	V_2	V_3
Specific gravity	0.929 ± 0.11^{a}	0.929 ± 0.14^{a}	0.928 ± 0.09^{a}	0.931 ± 0.18^{a}	0.930 ± 0.16^{a}	0.931 ± 0.12^{a}	0.933 ± 0.15^{a}	0.932 ± 0.13^{a}	0.932 ± 0.14^{a}
Free fatty acids (%)	0.98 ± 0.03^{abc}	0.98 ± 0.05^{abc}	0.97 ± 0.04^{bc}	1.03 ± 0.01^{ab}	1.02 ± 0.02^{ab}	1.05 ± 0.06^{a}	0.94 ± 0.03^{c}	0.93 ± 0.01^{c}	0.94 ± 0.02^{c}
Iodine Value	193 ± 3.96^{ab}	180 ± 4.26^{bc}	185 ± 3.88^{abc}	191 ± 3.24^{ab}	176 ± 3.68^{c}	182 ± 3.85^{bc}	197 ± 4.39^{a}	190 ± 4.96^{abc}	193 ± 3.25^{ab}
Saponification Value	191.6 ± 2.78^{a}	191.7 ± 4.71^{a}	191.4 ± 3.25^{a}	191.7 ± 3.94^{a}	191.7 ± 3.70^{a}	191.5 ± 3.72^{a}	191.5 ± 3.46^{a}	191.5 ± 4.58^{a}	191.5 ± 2.89^{a}
Peroxide Value (meq O_2/kg oil)	2.0 ± 0.08^{cd}	1.9 ± 0.09^{de}	2.1 ± 0.09^{bc}	2.2 ± 0.11^{ab}	2.1 ± 0.09^{bc}	2.3 ± 0.07^{a}	1.8 ± 0.08^{e}	1.8 ± 0.08^{e}	1.9 ± 0.09^{de}
ρ-Anisidine Value	0.98 ± 0.01^{ab}	0.98 ± 0.03^{ab}	1.00 ± 0.04^{ab}	1.02 ± 0.01^{a}	1.00 ± 0.02^{ab}	1.03 ± 0.07^{a}	0.96 ± 0.03^{ab}	0.94 ± 0.01^{b}	0.97 ± 0.05^{ab}
Tocopherols (mg/100 g)	38.13 ± 1.53^{a}	38.29 ± 1.52^{a}	37.80 ± 1.13^{a}	37.90 ± 1.86^{a}	38.14 ± 1.64^{a}	37.62 ± 1.54^{a}	38.26 ± 1.76^{a}	38.39 ± 1.69^{a}	37.97 ± 1.86^{a}

ME = Mechanical extraction; SE = Solvent extraction; SFE = Supercritical Fluid extraction; V_1 = LS-113; V_2 = LS-120; V_3 = LS-89. Values are means ± standard deviation of three determination (n = 3). Values followed by similar superscript letters in the same column are not significantly different ($p > 0.05$).

by SFE extraction. It has been well documented that SC-CO_2 selectively extracts the desired neutral lipids, whereas free fatty acids are charged; so FFAs are less in oils extracted by SC-CO_2 (Taniguchi *et al.*, 1985). Pradhan *et al.* (2010) extracted flaxseed oil by SC-CO_2 and compared it with ME and SE. They found that the free fatty acid value of flaxseed oil extracted by SE was higher (0.55%) as compared to SC-CO_2 (0.40%) and ME (0.35%).

The IV was significantly ($p < 0.05$) different for varieties as well as for extraction methods (Table 4). SFE oil of V_1 (194) showed the highest IV, while the lowest IV was measured in SE oil of V_2 (176). When the extraction methods were compared for IV, SFE showed highest IV for each variety followed by ME and SE. The higher value of IV for SFE oil might be due to the fact that this method of extraction is able to extract high amounts of non-triglyceride lipids (*i.e.* partial esters, phospholipids, sterols, chlorophylls, fat-soluble vitamins and pigments) and non-lipid components like polyphenols and vitamins. For this reason, the SFE extracted oil is of superior quality and more stable. The results of the present work are in agreement with the findings of Khattab and Zeitoun (2013), who while studying the quality of flaxseed oil extracted by different techniques reported that the SFE oil showed significantly higher numbers for both the calculated (193) and determined (196) IV.

Saponification value was insignificantly ($p > 0.05$) affected by varieties and extraction methods. The saponification values of flaxseed oil obtained by different extraction methods are presented in Table 4. The ME oil of V_1 showed a saponification value of 191.6 ± 2.78 mgKOH/g while it was 191.7 ± 4.71 mgKOH/g and 191.4 ± 3.25 mgKOH/g for V_2 and V_3, respectively. Dong *et al.* (2014) studied the effect of oil extraction methods on the characteristics of okra seed fatty oil and reported that saponification values were insignificantly affected by extraction methods with values of 185.67 mgKOH/g, 187.29 mgKOH/g and 188.06 mgKOH/g for SC-CO_2, SE and Screw Press Extraction (SPE), respectively. Hence, it is further supported that extraction method has an insignificant effect on the saponification value.

Oxidative stability

The statistical data showed that peroxide value (PV) of flaxseed oils was significantly ($p < 0.05$) different regarding varieties and extraction methods. The highest PV was recorded for SE oil of V_3 (2.3 ± 0.07 meqO_2/kg oil) while lowest PV was recorded for SFE oil of V_1 and V_2 (1.8 ± 0.08 meqO_2/kg oil) as shown in Table 4. The significantly lower PV of the SFE oil may be advocated by the fact that there are lesser chances of the oxidative reactions while working with CO_2 in a closed system in the absence of oxygen as well as to the lower processing temperatures in comparison to both ME and SE. Khattab and Zeitoun (2013) reported that SFE flaxseed oil showed a significantly lower PV (5.42 meqO_2/kg oil) as compared to the flaxseed oil obtained from SE (6.96 meqO_2/kg oil) since SFE oil has a less exposure to environmental oxygen as well as the higher amount of phenolic acids and lignans in SFE flaxseed oil (naturally-occurring antioxidants).

There was an insignificant ($p > 0.05$) difference

Table 5. Fatty acid profile of flaxseed oil of various varieties with different extraction methods.

Varieties	V_1			V_2			V3		
Extraction method	ME	SE	SFE	ME	SE	SFE	ME	SE	SFE
Saturated									
Palmitic acid $C_{16:0}$	5.25 ± 0.00	5.72 ± 0.00	4.38 ± 0.02	7.13 ± 0.04	7.67 ± 0.08	6.24 ± 0.04	5.29 ± 0.01	5.61 ± 0.01	4.45 ± 0.04
Stearic acid $C_{18:0}$	4.23 ± 0.01	4.82 ± 0.00	3.46 ± 0.01	3.63 ± 0.03	4.17 ± 0.08	3.62 ± 0.10	4.89 ± 0.09	5.21 ± 0.10	4.17 ± 0.04
Arachidic acid $C_{20:0}$	0.17 ± 0.00	0.20 ± 0.00	0.15 ± 0.00	0.16 ± 0.00	0.18 ± 0.05	0.14 ± 0.03	0.17 ± 0.03	0.19 ± 0.11	0.16 ± 0.01
Behenic acid $C_{22:0}$	0.13 ± 0.00	0.15 ± 0.00	0.11 ± 0.01	0.12 ± 0.02	0.15 ± 0.01	0.09 ± 0.02	0.15 ± 0.01	0.17 ± 0.05	0.13 ± 0.02
Linoceric acid $C_{24:0}$	0.11 ± 0.01	0.13 ± 0.01	0.09 ± 0.01	0.10 ± 0.02	0.11 ± 0.02	0.09 ± 0.08	0.13 ± 0.02	0.14 ± 0.02	0.10 ± 0.03
Total	9.89 ± 0.07^{b}	11.02 ± 0.10^{a}	8.19 ± 0.05^{c}	11.08 ± 0.03^{b}	12.12 ± 0.01^{a}	10.12 ± 0.03^{c}	10.63 ± 0.06^{b}	11.32 ± 0.09^{a}	9.01 ± 0.05^{c}
Monounsaturated									
Palmitoleic acid $C_{16:1}$	0.07 ± 0.03	0.00	0.11 ± 0.00	0.07 ± 0.01	0.01 ± 0.03	0.08 ± 0.02	0.09 ± 0.00	0.05 ± 0.01	0.13 ± 0.05
Oleic acid $C_{18:1n-9}$	17.9 ± 0.01	18.30 ± 0.04	18.50 ± 0.09	14.7 ± 0.5	16.1 ± 0.8	17.5 ± 0.6	16.35 ± 0.17	16.74 ± 0.11	17.89 ± 0.10
Gondoic acid $C_{20:1}$	0.09 ± 0.00	0.00	0.21 ± 0.00	0.11 ± 0.03	0.03 ± 0.00	0.25 ± 0.04	0.13 ± 0.03	0.05 ± 0.01	0.23 ± 0.02
Total	18.06 ± 0.10^{c}	18.30 ± 0.04^{b}	18.82 ± 0.13^{a}	14.88 ± 0.08^{c}	16.14 ± 0.05^{b}	17.83 ± 0.01^{a}	16.57 ± 1.04^{b}	16.84 ± 0.12^{b}	18.25 ± 0.09^{a}
Polyunsaturated									
Linoleic acid $C_{18:2n-6}$	15.46 ± 0.01	15.15 ± 0.02	15.88 ± 0.08	15.63 ± 0.04	14.41 ± 0.07	16.27 ± 0.05	14.43 ± 0.20	14.13 ± 0.06	14.79 ± 0.11
α-Linolenic acid $C_{18:3n-3}$	57.08 ± 0.05	56.28 ± 0.03	58.06 ± 0.04	53.81 ± 0.8	52.09 ± 1.20	55.06 ± 0.81	56.03 ± 1.28	54.67 ± 0.12	57.84 ± 1.01
Total	72.54 ± 0.12^{ab}	71.43 ± 0.06^{b}	73.94 ± 0.13^{a}	69.44 ± 0.11^{b}	66.50 ± 0.06^{c}	71.33 ± 0.02^{a}	70.46 ± 0.03^{b}	68.8 ± 0.05^{c}	72.27 ± 0.04^{a}
Total Unsaturation	90.60 ± 0.08^{b}	89.46 ± 0.01^{b}	92.76 ± 0.03^{a}	84.32 ± 0.01^{b}	82.64 ± 0.08^{c}	89.16 ± 0.07^{a}	87.03 ± 0.8^{b}	85.64 ± 0.12^{c}	90.52 ± 0.10^{a}
Unsat/Sat (%)	9.16 ± 0.06^{b}	8.12 ± 0.02^{c}	11.32 ± 0.05^{a}	7.61 ± 0.05^{b}	6.82 ± 0.00^{c}	8.81 ± 0.08^{a}	8.19 ± 0.07^{b}	7.56 ± 0.05^{c}	10.04 ± 0.03^{a}
$C_{18:2}/C_{18:3}$	0.27 ± 0.05^{a}	0.27 ± 0.01^{a}	0.27 ± 0.03^{a}	0.29 ± 0.04^{a}	0.28 ± 0.01^{a}	0.29 ± 0.05^{a}	0.26 ± 0.05^{a}	0.26 ± 0.06^{a}	0.26 ± 0.04^{a}

ME = Mechanical extraction; SE = Solvent extraction; SFE = Supercritical Fluid extraction; V_1 = LS-113; V_2 = LS-120; V_3 = LS-89. Values are means ± standard deviation of three determination (n = 3). Values followed by similar superscript letters in the same column are not significantly different ($p > 0.05$).

in the values of ρ-AnV of flaxseed oils between different varieties, and a significant ($p < 0.05$) difference between extraction methods. The highest ρ-AnV was observed in the SE oil of V_3 (1.03 ± 0.07); this was expected as this oil also yielded the highest FFAs, since higher FFAs means higher chances of oxidation. The values showed that the ρ-AnV of V_1 for SE oil was 1.02 ± 0.01 which was significantly higher than ME oil (0.98 ± 0.01) and SFE oil (0.96 ± 0.03) of the same variety. The higher ρ-AnV is a result of the higher content of free fatty acids which are more vulnerable to oxidation and decomposition to further secondary products that are measured by the ρ-AnV. Khattab and Zeitoun (2013) reported their findings regarding the comparison of extraction techniques [SC-CO_2, SE and Accelerated Solvent Extraction (ASE)]. Their results reflect that the ρ-AnV was higher in the flaxseed oil of SC-CO_2. There is no established limit of ρ-AnV in oils; so it is difficult to assess the health safety/quality of oils in terms of ρ-AnV.

Tocopherol contents

The tocopherol contents were insignificantly ($p > 0.05$) affected by varieties and extraction methods. The mean values for tocopherols in flaxseed oil of various varieties extracted by different methods are shown in Table 4. Among all treatments, the total tocopherol contents ranged from 37.62 ± 1.54 to 38.39 ± 1.69 mg/100 g. The highest total tocopherols were recorded in SFE oil of V_2 (38.39 ± 1.69 mg/100 g), while the lowest tocopherols were recorded in SE oil of V_3 (37.62 ± 1.54). This could be due to the fact that tocopherols are soluble in oil which allows them to be extracted with the oil, hence extracted equally by all extraction methods. Khattab and Zeitoun (2013) reported that tocopherols content in flaxseed oil extracted by different techniques to be in the range of 34.47 - 34.82 mg/100 g. Similarly, Ciftci *et al.* (2012) analysed the lipid components of flax, perilla and chia seeds. Their findings delineated that tocopherol contents were 74.7, 73.4 and 44.6 mg/100 g seed lipid. They further explicated that γ-tocopherol was the dominating isomer in all the seed lipids.

Fatty acid profile of flaxseed oil

The statistical data showed that fatty acid profile was significantly ($p < 0.05$) affected by the extraction methods, but insignificantly ($p > 0.05$) affected by the varieties. The mean values for fatty acid profile of the obtained flaxseed oils are shown in Table 5. The mean values for total saturated fatty acids indicated that for each variety the highest value was observed in the SE extracted flaxseed oil. The total saturated fatty acids were 11.02% in SE, 9.89% in ME and 8.19% in SFE oil of V_1. SFE extracted flaxseed oil had lower amount of total saturated fatty acids than SE and ME in each variety. The saturated fatty acids were insignificantly ($p > 0.05$) different with respect to varieties. The highest total saturated fatty acids were recorded in SE extracted flaxseed oil of V_2 (12.12%), while the lowest in SFE extracted flaxseed oil of V1 (8.19%). The mean values for total unsaturated fatty acids depicted that the maximum values were obtained in SFE extracted flaxseed oil of each variety. The amount of total unsaturated fatty acids was 92.76% in SFE, 90.60% in SE and 89.46% in ME extracted flaxseed oil, respectively. The unsaturation:saturation ratio was also significantly ($p < 0.05$) affected by the extraction methods. The SFE flaxseed oil showed a higher ratio. The highest amount of ALA was recorded in SFE extracted flaxseed oil of V_1 (58.06%).

Conclusion

The oil yield (%) was observed to vary among the Pakistani flaxseed varieties assessed in present work, and the extraction methods had a significant impact on the oil yield. Similarly, there were significant differences between the physico-chemical parameters of the extracted flaxseed oils. The sources of these variations could be the different genotype of varieties as well as the different extraction conditions. Overall, the flaxseed variety V_1 (LS-113) had relatively higher oil yield. Among extraction methods, SFE yielded superior quality of flaxseed oil having higher amounts of ω-3 along with low amounts of free fatty acids, PV and *p*-AnV. In a nutshell, scientists can use these characteristics for selecting economically and nutritionally best flaxseed varieties and the best extraction method to get highest quality flaxseed oil for the targeted end health benefits.

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