PAPER

VARIATION IN PHYSICO-CHEMICAL/ANALYTICAL CHARACTERISTICS OF OIL AMONG DIFFERENT FLAXSEED (*LINUM USITTATISSIMUM* L.) CULTIVARS

NAZIA YAQOOB[1,2], IJAZ AHMED BHATTI[1], FAROOQ ANWAR[3,4]*, MUHAMMAD MUSHTAQ[5] and WILLIAM E. ARTZ[6]

[1]Department of Chemistry, University of Agriculture Faisalabad, Pakistan
[2]Department of Chemistry, Govt College for Women University, Faisalabad, Pakistan
[3]Department of Chemistry, University of Sargodha, Sargodha, Pakistan
[4]Department of Pharmaceutical Chemistry, College of Pharmacy, Prince Sattam bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia
[5]Department of Chemistry, Govt College University Lahore, Pakistan
[6]Department of Food Science and Human Nutrition, University of Illinois Urbana, Champaign, Illinois, Usa
*Corresponding author: fqanwar@yahoo.com

ABSTRACT

The present study evaluates and compares the proximate parameters of flaxseed, as well as the physicochemical characteristics of the extracted flaxseed oils of locally grown eight cultivars. The oil, protein, fiber and ash content of the seeds ranged from32.56-39.98%, 16.02-18.50%, 23.30-26.88 and 3.20-3.60%, respectively showing considerable variation among cultivars. The quality attributes such as unsaponifiable matter, peroxide value, acid value, *para*-anisidine value, conjugated dienes and trienes as well as tocopherols content of the tested flaxseed oils varied significantly ($p<0.05$) among cultivars. The major tocopherol was γ-tocopherol (173.7 to 257.9 mg/L) followed by relatively low quantities of α-tocopherol (8-12 mg/L), while δ-tocopherol was not detected. α-Linolenic acid was found to be the principal fatty acid in the range of 44.51 to 54.87%, while the second major fatty acid present in the oils was oleic acid (21.05 to 30.96%). The variation in the characteristics of oils among different cultivars observed during present investigation might be attributed to difference in genetic makeup and harvesting regimes of the flax plants.

- Keywords: flaxseed, folch method, tocopherols, phenolic antioxidants, fatty acids, GC-MS -

INTRODUCTION

Flax (*Linum usitatissimum* L.) is a multi-purpose and economically important oilseed crop. Flaxseeds, which contain approximately 36 to 40% oil, have long been used in human diet and animal feed (TOURE and XUEMING, 2010). By virtue of the presence of physiologically active components, which provide health benefits beyond basic nutrition, flaxseed is often grouped as "functional food" (HASLER *et al.,* 2000). Historically, the oil extracted from flaxseeds, has been used as a basic component in the preparation of paints or polymers, linoleum, varnishes, inks and cosmetics (El-BELTAGI *et al.,* 2007; ZHANG *et al.,* 2008; JHALA and HALL, 2010). However, during the past decade, there has been an increasing interest in the use of flaxseed oil to improve human health status due to its high nutraceutical potential (OOMAH, 2001; CHOO *et al.,* 2007). The potential health benefits of flaxseed oil including reduction in serum cholesterol levels and decreased incidence of diabetes, breast and colon cancer can be ascribed to the presence of high-value antioxidants, tocopherols, lignans and essential fatty acids (Muir and Westcott, 2003; HOSSEINIAN *et al.,* 2006; CHOO *et al.,* 2007; TOURE and XUEMING, 2010). Flaxseed oil is one of the richest sources of unsaturated fatty acid, especially, linolenic acid (C18:3) with amount in the range of 50-60% of the total fatty acids present (FLACHOWSKY *et al.,* 1997).

The agronomic conditions, such as the soil characteristics, agro-climatic conditions and the cultivar influence the unsaturated fatty acids composition in flaxseed (DUAN *et al.,* 2003). Moreover, studies indicate that the oil content, fatty acids profile and other physicochemical properties vary in the flaxseed crops grown in different parts of the world (TAYLOR and MORRICE, 1991; WAKJIRA *et al.,* 2004; HALL *et al.,* 2006). Traditionally, flaxseed has been grown in the Asian subcontinent for its oil; however in Pakistan its applications have been limited to industrial uses. The present study mainly focused on the evaluation and comparison of proximate parameters of flaxseed, as well as the physicochemical characteristics (such as the refractive index, density, iodine value, acid value, peroxide value, *para*-anisidine value etc.) and the composition of tocopherols and fatty acids of flaxseed oil from different flax cultivars (*Linum usitatissimum* L.) indigenous to Pakistan to assess their nutritional value.

EXPERIMENTAL

Seeds and chemicals/reagents

Commercially available hybrid varieties (Chandni, LS-108, LS-105, LS-99 and LS-29) of flaxseed used in this study were obtained from the Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan and LS-33, LS-31 and LS-13 were obtained from National Agriculture Research Center (NARC) in Islamabad, Pakistan. Three different seed samples for each of the flaxseed variety were collected (8×3=24). All the reagents (analytical and HPLC grade) used were from Merck (Darmstadt, Germany) or Sigma–Aldrich (Buchs, Switzerland). Pure standards of tocopherols (α-tocopherol, γ-tocopherol, δ-tocopherol), and fatty acid methyl esters were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Extraction of oil

Seeds of different flaxseed cultivars were crushed with a domestic electric grinder. The oil from the seeds was extracted using the Folch method (FOLCH *et al.,* 1957). After oil extraction, the solvent was removed under vacuum in a rotary evaporator (Eyela, Rotary Vacuum Evaporator N.N. Series equipped with an aspirator and a digital water bath SB-651, 33 Japan) at 45°C. The extracted oil was then stored in a refrigerator at 4°C until used for analyses.

Analysis of oil seed residues

Proximate analyses of oilseed residues (meals), left after oil extraction, were completed according to standard methods. Protein contents (N-6.25) were determined according to AOAC method 954.01 (AOAC, 1990), using a Kjeldahl apparatus. The fiber contents were determined employing ISO method 5983 (ISO, 1981). Briefly, 2 g of finely ground defatted sample was taken and boiled with 250 mL of 0.255 *M* H_2SO_4, followed by the filtration and washing of insoluble residues. The residues were then boiled with 250 mL of 0.313 *M* NaOH, filtered, washed, and dried. The dried residues were weighed and burnt at 600°C using a muffle furnace (Eyela, TMF-2100, Tokyo, Japan) and the loss of mass was determined gravimetrically. Ash contents were determined by following ISO method 749 (ISO, 1977). Two grams of meal was carbonized by heating on a gas flame and then ashed in an electric muffle furnace at 600°C, until a constant mass was achieved.

Physicochemical properties of oil

The extracted oils were analyzed for density, refractive index, peroxide value (PV), acid value, iodine value (IV), saponification value, and unsaponifiable matter following AOCS methods Cc10a-25, Cc7-25, Cd1-25, Cd8-53, F-9a-44, Cd3-25, and Ca61-40, respectively (AOCS, 1997). The determinations of conjugated dienes (CD) and conjugated trienes (CT) were made using a Hitachi U-2001 spectrophotometer. The oil samples were diluted with isooctane and ab-

sorbance values recorded at 232 and 268 nm for conjugated dienes (CD) and conjugated trienes (CT), respectively. Specific extinctions were determined following the IUPAC method II D.23 (IUPAC, 1987). The *para*-anisidine value of the flaxseed oil samples was monitored according to IUPAC method II D.26 (IUPAC, 1987). The oil samples diluted with isooctane were reacted with *p*-anisidine solution (0.25% w/v) in acetic acid for 10 min and the absorbance of the resulting colored solution recorded at 350 nm using a spectrophotometer.

Tocopherols

For tocopherol analysis, an HPLC method was adopted from YAQOOB *et al.* (2010), with some modifications. A Waters Alliance 2695 HPLC system equipped with YMC-Pack ODS AM-303, C 18 column (250mm x 4.6mm x 5μm) and Agilent series 1050 diode array detector, (UV 295 nm) was used. The temperature of the column was maintained at 30°C. The chromatographic separation was performed by isocratic elution with a mixture of acetonitirile and isopropanol (40:60 v/v) at a flow rate of 1 mL/min (Pressure 120 bar). Briefly, flaxseed oil (1 g) was accurately weighed into a 5 mL sample vial wrapped in aluminum foil to prevent photo-oxidation. The oil was dissolved in 5 mL acetonitrile before injection. Samples were injected into the column through an injection loop (20 μL). Tocopherols were identified by comparing the retention times of the unknowns with those of pure standards of α-, γ-, and δ-tocopherols. Acquisition of data was made using Agilent Chem-station software. The samples were prepared and analyzed separately in triplicate.

Fatty acids profile

Fatty acid methyl esters (FAMEs) were prepared by IUPAC standard method 2.301 (IUPAC, 1987) which involved the trans-esterification of fatty acids with methanol under base –catalyzed conditions. Briefly, 0.2 g of oil was placed in 10 mL capped vials; 5mL of redistilled methanol was added followed by the addition of a pellet of KOH. The content of the vials were heated at 60°C in a heating mantle until the droplets of fats disappeared. Upon cooling, the reaction mixture was gently transferred to a separating funnel. Small amount of n-hexane was added. Separating funnel was shaken gently. The upper hexane layer was recovered and washed with distilled water. This hexane solution was dried over anhydrous sodium sulfate, filtered and used for gas chromatographic analysis.

FAMEs were separated on an Agilent 5890 series II GC fitted with a 7673B auto sampler and a capillary column (30 m × 0.25 mm × 0.25 μm) with a DB-WAX (film thickness 0.20 μm) stationary phase and a flame ionizing detector (FID). Helium was used as carrier gas at a flow rate of 1.5 mL/min. Other conditions were as follow: injection volume 1μL, split mode (split ratio1:100), injector temperature, 280°C, initial oven temperature, 170°C; hold up 2 min, 170-240°C (ramp rate 2°C/min) hold up 10 min, detector temperature 260 °C. FAMEs were identified by comparing their relative and absolute retention times with those of authentic standards. The FA composition was reported as a relative percentage of the total peak area. The internal standard used was nonadecanoic acid. All of the quantifications were done by Agilent Chem-station software.

Statistical analysis

Three different seed samples for each of the variety were taken and analyzed individually in triplicate and data reported as mean ± SD (n= 3× 3 =9). An analysis of variance (ANOVA) was performed using Minitab 2000 Version 16.1 statistical software (Minitab Inc. State College, PA, USA). Significant differences ($P<0.05$) of means were calculated using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Proximate analysis of seeds

The data obtained for the proximate analysis of flaxseeds of eight different cultivars grown in Pakistan is presented in Table 1. Oil contents varied from 33.25 to 38.38% indicating a significant difference among cultivars selected ($p<0.05$). The variety Chandani had the highest oil yield whereas LS-13 contained the lowest. In another study from Pakistan, Anwar *et al.*, (2013) investigated the oil yield for soxhlet-extracted flaxseed to be 42.80%; such variation in oil yield may be linked to the different extraction method used.

The oil content of Pakistani flaxseeds are comparable to those grown in Canada, North America and Egypt, i.e. 36%, 31.9 to 37.8% and 36-39%, respectively (HETTIARACHCHY *et al.,* 1990; OOMAH and MAZZA 1998; El-BELTAGI *et al.,* 2007). However, the present oil contents from Pakistani flaxseed cultivars were lower than those reported for Polish flaxseed cultivars, *i.e.*, 41.4% (KOZLOWSKA, 1989) but higher than Ethiopian flaxseed cultivars, 29.1-35.9% (WAKJIRA *et al.*, 2004). Such variations in flaxseed oil content with in the countries might be linked to varietal and agro climatic conditions of the regions.

The moisture, crude protein, fiber and ash contents of different cultivars of Pakistani flaxseed ranged from 5.98 to 6.22%, 16.02 to 18.50%, 23.30 to 26.80% and 3.21 to 3.60%, respectively. There was no significant difference

Table 1 - Proximate analysis of Pakistani flaxseed (*Linum usittatissimum.* L) cultivars.

Variety	Parameters				
	Oil content (%)	Moisture content (%)	Protein content (%)	Fiber content (%)	Ash content (%)
Chandni	38.38±1.80^{ab}	6.02±0.11^{a}	18.50±0.56^{ab}	26.80±1.22^{a}	3.60±0.12^{ab}
LS-108	36.38±1.52^{de}	6.12±0.13^{ab}	18.00±0.45^{d}	26.80±0.98^{a}	3.55±0.11^{d}
LS-105	37.01±1.46^{e}	6.22±0.15^{ab}	17.98±0.63^{cd}	26.00±1.06^{ab}	3.50±0.11^{cd}
LS-99	38.02±1.60^{c}	6.10±0.13^{a}	17.86±0.48^{bc}	25.5±1.14^{ab}	3.50±0.13^{bc}
LS-33	35.30±1.35^{f}	5.99±0.12^{ab}	16.02±0.62^{a}	23.50±0.88^{b}	3.21±0.12^{a}
LS-31	34.98±1.47^{a}	5.98±0.10^{ab}	16.62±0.54^{a}	23.3±1.16^{b}	3.26±0.14^{a}
LS-29	38.00±1.59^{d}	6.02±0.14^{a}	17.99±0.39^{e}	25.75±1.06^{ab}	3.58±0.14^{e}
LS-13	33.25±1.31^{bc}	6.00±0.11^{b}	17.00±0.52^{ab}	23.68±1.14^{b}	3.40±0.16^{ab}

Values (mean ± SD) are average of triplicate samples of each cultivar, analyzed individually in triplicate (n = 1 x 3 x 3), (P<0.05). Different letters in superscript indicate significant differences.

Table 2 - Physico-chemical characteristics of oil extracted from Pakistani flaxseed (*Linum usittatissimum.* L.) cultivars.

Parameters	Varieties							
	Chandni	LS-108	LS-105	LS-99	LS-33	LS-31	LS-29	LS-13
Refractive index (40°C)	1.4729±0.009^{a}	1.4728±0.006^{a}	1.4707±0.005^{a}	1.4737±0.007^{a}	1.4732±0.005^{a}	1.4734±0.006^{a}	1.4706±0.009^{a}	1.4728±0.007^{a}
Density g/mL (25°C)	0.928±0.18^{a}	0.929±0.12^{a}	0.9278±0.14^{a}	0.928±0.14^{a}	0.928±0.16^{a}	0.929±0.15^{a}	0.928±0.19^{a}	0.9279±0.21^{a}
Iodine Value g of I/100 g of oil	198±3.96^{a}	199±4.26^{a}	195.9±3.24^{a}	201±4.39^{a}	195±3.85^{a}	196±4.96^{a}	197±3.68^{a}	199±3.88^{a}
Unsap matter (%)	2.20±0.04^{c}	2.60±0.04^{a}	2.20±0.02^{c}	2.00±0.02^{d}	2.4±0.03^{b}	1.96±0.05^{d}	2.0±0.02^{d}	1.80±0.04^{e}
Saponification value mg of KOH/100 g of oil	189±2.78^{a}	187±3.25^{a}	185.9±3.70^{a}	186±3.46^{a}	185±4.58^{a}	185.86±3.72^{a}	187±3.94^{a}	184±4.71^{a}
FFA (% as oleic acid)	1.399±0.03^{d}	1.579±0.04^{c}	1.624±0.02b^{c}	1.399±0.03^{d}	1.725±0.05^{a}	1.721±0.04^{ab}	1.447±0.02^{d}	1.732±0.05^{a}
Peroxide value (meq/kg of oil)	1.00±0.02^{c}	1.20±0.03^{ab}	1.18±0.02^{ab}	1.00±0.04^{c}	1.14±0.03^{b}	1.20±0.02^{a}	1.22±0.03^{ab}	1.14±0.05^{b}
$^{1cm}\mathcal{E}_{1\%}$ (λ 232)	5.07±0.20^{ab}	4.70±0.15^{b}	4.81±0.28^{b}	4.79±0.19^{b}	4.80±0.22^{b}	4.75±0.14^{b}	5.55±0.22^{a}	4.76±0.18^{b}
$^{1cm}\mathcal{E}_{1\%}$ (λ 268)	1.80±0.08^{ab}	2.00±0.09^{a}	1.90±0.08^{a}	1.60±0.04^{cd}	1.50±0.05^{d}	1.80±0.07^{ab}	1.70±0.05^{bc}	1.40±0.06b^{cd}
Para-anisidine value	1.13±0.05^{c}	1.41±0.05^{ab}	1.40±0.06^{ab}	1.29±0.05^{bc}	1.36±0.05^{ab}	1.42±0.06^{ab}	1.48±0.05^{a}	1.32±0.04^{ab}

Values (mean ± SD) are average of triplicate samples of each cultivar, analyzed individually in triplicate (n = 1 x 3 x 3), (p<0.05).
Different letters in superscript indicate significant differences.

in moisture content for flaxseeds between different cultivars (p>0.05). However, crude protein, fiber and ash contents were notably different among the cultivars of flaxseeds (p<0.05). Depending on the cultivar and growing conditions, flaxseed has been reported to contain an average of 23% to 34% protein, 4% ash and 5% fiber (MUIR and WESTCOTT, 2003). Our results are comparable to the previous reports on flaxseed cultivars grown in different regions of the world. Protein contents of Polish and North American cultivars were reported to be greater than 20%, while Canadian cultivars had protein generally less than 20% (OOMAH and MAZZA, 1998; CHOO *et al.,* 2007). Crude fiber content in different flaxseed residues has been reported to be in the range of 7-10% (GUTIERREZ *et al.,* 2010). The difference in oil, crude protein, and fiber and ash contents of flax seed of different cultivars might be attributed to differences in growing conditions and genetic makeup of flax plants (OOMAH and MAZZA 1993; DUAN *et al.,* 2003).

Physico-chemical properties of oil

The physico-chemical parameters determined for oils extracted from eight different cultivars of flaxseeds are presented in Table 2. The results indicated that the refractive index (40°C) and density (25°C) ranged from 1.4706 to 1.4737 and 0.9278 to 0.929 mg/mL, respectively, with non-significant (p>0.05) difference among cultivars Our findings are consistent with the previous reports in which the refractive index of flaxseed oil at 20°C was reported to be 1.475, while the density of flaxseed oil at 25°C was 0.925 to 0.935 (PRZYBYLSKI, 2005). The density of flaxseed oil is greater than most other vegetable oils, and this might be attributed to the greater content of linolenic acid (GREEN and MARSHALL, 1984).

The iodine value, unsaponifiable matter, saponification number and acid value are characteristic for flaxseed oils that contain a large percentage of polyunsaturated fatty acids. The iodine values for the tested oils ranged from 195 to 199 g of I_2/100 g of oil with non-significant

(p>0.05) among cultivars. Iodine value for flaxseed oil has been reported to vary between 180 to 203 g of I_2/100g of oil (PRZYBYLSKI, 2005). LONG *et al.* (2011) reported iodine value of flaxseed oil to be 162 I_2/100g. The saponification value and unsaponifiable matter of the tested flaxseed oils ranged from 184 to 189 mg of KOH/100g of oil and 1.8 to 2.6%, respectively. Saponification values did not differ significantly (p>0.05) whereas the unsaponifiable matter varied significantly within the oils of different cultivars (p<0.05). In previous reports, the percentage of unsaponifiable matter in flaxseed oil was in the range of 0.1 to 1.7% for raw oil, and up to 0.6% for refined flaxseed oil (ESKINETAL, 1996; CHOO *et al.,* 2007). TEH and BIRCH (2013) reported the unsaponifiable value to be 0.4% for cold pressed flaxseed oil.

Free fatty acids (FFA) are produced by the hydrolysis of triglycerides (LAFONTAN and LANGIN, 2009). The FFA content of the tested flaxseed oils ranged from 1.40 to 1.73%, as oleic acid. The FFA content varied significantly within different flaxseed cultivars (p<0.05). In a previous report, FFA value for flaxseed oil was reported to be 0.1 to 2.0% (PRZYBYLSKI, 2005). LONG *et al.* (2011) reported the FFA in the flaxseed oil extracted by enzymatic extraction and solvent extraction to be 1.5 and 1.1%, respectively. FFA value for the cold pressed flaxseed oil was reported to be 0.75% (TEH and BIRCH, 2013). FFA in most of the freshly extracted crude vegetable oils is normally below 1.0%. These hydrolytic products are mainly formed as result of chemical hydrolysis (due to presence of moisture in seeds) or enzymatic hydrolysis. A low value of oil FFA is an indication that the seeds have been preserved under proper storage conditions with good state.

The peroxide value for the flaxseed oils of different cultivars ranged from 1.0 to 1.22 meq/kg of oil that is well below the limit for peroxide value. CHOO *et al.*, (2007) reported the peroxide value ranging from 0.5 to 2.9meq/Kg of cold-pressed flaxseed oil sold in New Zealand. Peroxide value for the enzymatic, solvent extracted flaxseed oils was reported to be 1.2 and 1.0 meq/kg of oil, respectively (LONG *et al.* 2011) while that for cold pressed flaxseed oil was reported to be 2.04 meq/kg of oil (TEH and BIRCH, 2013). Peroxide value is an indicator of primary oxidation products; the extent of these products may range up to 10-15 meq/kg of oil (CHOO *et al.,* 2007).

The *p*-anisidine value of the oils extracted from different cultivars of flaxseed ranged from 1.13 to 1.48(p<0.05). The values are higher than those investigated by CHOO *et al.* (2007) who reported the *p*-anisidine value to be in the range of 0.36 to 0.4. However, the present values were comparable to the values of enzymatically and solvent extracted flaxseed oil reported previously, 1.2 and 1.0, respectively (LONG *et al.,* 2011). Conjugated dienes and trienes produced as a result of secondary oxidation of polyunsaturated fatty acids can be determined by measuring the absorbance at 232 and 272 nm, respectively (Frankel, 2005). The specific extinction at 232 and 272 nm of different flaxseed oils ranged from 4.70 to 5.55 and 1.40 to 2.00, respectively. CHOO *et al.*, (2007) reported absorbencies at 232 and 270 nm of 1.7 to 2.75 and 0.2 to 0.4, respectively for cold pressed flaxseed oil. TEH and BIRCH (2013) reported absorbencies at 232 and 272 nm for the cold pressed flaxseed oil to be 2.02 and 0.02, respectively which is very low as compared to our present results. Our results are comparable to those reported previously (REED *et al.*, 2001).

Tocopherol content

The tocopherol contents of the different flaxseed oils are shown in Table 3. Gamma (γ)-tocopherol was the main tocopherol in flaxseed oils, with contribution of approximately 90% of the total tocopherols. The γ-tocopherol content ranged from 173.7 to 257.9 mg/kg of oil and significantly differed in different cultivars (p<0.05). Alpha (α) tocopherol was the other tocopherol found in the oils (~10%) while delta tocopherol was not detected. The contents of α tocopherol varied from 39 to 18.7 (mg/kg of oil) showing a significant difference among different cultivars (p<0.05). The difference in the contents of tocopherols might be due to the varying ge-

Table 3 - Tocopherol contents (mg/kg) of oil extracted from Pakistani flaxseed (*Linum usittatissimum.* L.) cultivars.

Tocopherol	Cultivars							
	Chandni	LS-108	LS-105	LS-99	LS-33	LS-31	LS-29	LS-13
γ-tocopherol	204.0±5.0^{bc}	217.8±4.8^{b}	179.6±5.8^{de}	173.7±5.5^{e}	201.3±6.2^{c}	192.2±4.9^{cd}	257.9±5.6^{a}	190.8±4.3^{a}
α-tocopherol	24.9±0.2^{e}	20.4±0.2^{f}	30.2±0.2^{c}	18.4±0.3^{g}	27.2±0.3^{d}	20.9±0.2^{f}	38.5±0.3^{a}	32.8±0.3^{b}
δ-tocopherol	ND	ND	ND	ND	ND	ND	ND	ND
Total tocopherols	228.9	238.2	209.8	192.1	228.5	213.1	296.4	223.6

Values (mean ± SD) are average of triplicate samples of each cultivar, analyzed individually in triplicate (n = 1 x 3 x 3), (P<0.05). Different letters in superscript indicate significant differences. ND = not detected.

Table 4 - Fatty acid composition (g/100g of FA) of oil extracted from Pakistani flaxseed (*Linum usittatissimum.* L) cultivars.

Fatty acid (FA)	Varieties							
	Chandni	LS-108	LS-105	LS-99	LS-33	LS-31	LS-29	LS-13
C 16:0	5.95±0.07^{cd}	5.94±0.07^{b}	5.8±0.06^{b}	6.14±0.07^{a}	6.11±0.19^{bc}	6.52±0.01^{bc}	6.27±0.01^{d}	6.21±0.03^{cd}
C 18:0	4.63±0.04^{c}	4.70±0.12^{d}	4.41±0.03^{cd}	4.97±0.08^{bc}	4.83±0.06^{ab}	4.68±0.06^{a}	4.58±0.01^{d}	4.4±0.04^{bc}
C 18:1 (n-9)	30.46±0.63^{ab}	28.86±0.53^{de}	26.25±0.35^{e}	30.96±0.47^{c}	21.05±0.45^{f}	28.33±0.56^{a}	24.09±0.58^{d}	26.75±0.59^{bc}
C 18:1 (n-7)	1.69±0.58^{a}	1.64±0.51^{a}	1.61±0.54^{a}	1.53±0.41^{a}	1.46±0.44^{a}	1.83±0.64^{a}	1.69±0.49^{a}	1.8±0.63^{a}
C 18:2 (n-6)	11.26±0.04^{ab}	11.18±0.01^{d}	7.63±0.74^{cd}	11.88±0.21^{bc}	11.68±0.8^{a}	10.5±0.01^{a}	10.08±0.1^{e}	9.42±0.13^{ab}
C 18:3 (n-3)	46.02±0.10^{f}	47.67±0.11^{d}	54.29±0.44^{c}	44.51±0.12^{e}	54.87±0.01^{a}	48.13±0.14^{g}	53.29±0.02^{b}	51.41±0.08^{e}

Values (mean ± SD) are average of triplicate samples of each cultivar, analyzed individually in triplicate (n = 1 x 3 x 3), (P<0.05). Different letters in superscript indicate significant differences.

netic makeup and growing conditions of different cultivars (OOMAH and MAZZA, 1997). Our results are consistent with previous reports in which gamma tocopherol was reported as the predominant tocopherol in flaxseed oils (GREEN and MARSHALL, 1984; HERCHI *et al.* 2011). The γ- and α-tocopherol content of the Pakistani cultivars is considerably greater than that in the flaxseed oil varieties of American, Canadian, New Zealand and Turkish origin which contain an average127, 93, 140 and 146 (mg/kg of oil) of tocopherols, respectively (BUDINETAL.,1995; OOMAH and MAZZA, 1997; CHOO *et al.,* 2007; BOZAN and TEMELLI, 2008).

The tocopherol contents of Pakistani flaxseed cultivars were comparable to those reported for Egyptian cultivars (210 mg/kg of oil) (EL-BELTAGI *et al.*, 2007). TEH and BIRCH (2013)reported the contents of γ-tocopherol to be 370 (mg/kg of oil) in cold pressed flaxseed oil, which is considerably higher the present finding.

Fatty acid profile

The fatty acid profile showed a significant variation in the contents of fatty acids within the oils of different flaxseed cultivars (p<0.05) as shown in Table 4. The amount of total unsaturated fatty acids in flaxseed oils of the selected cultivars was observed to be in the range of 88.79 to 89.78% while the amount of total saturated fatty acids ranged from 10.21 to 11.20 % with non-significant (p>0.05) varation among cultivars. One distinct feature of flaxseed oil is the presence of high amount of linolenic acid. In the current study the quantities of linolenic acid were observed to be 44.51 to 54.87%, for different cultivars (p<0.05).

The results are comparable to the previous reports for American and Egyptian flaxseed varieties with 45 to 52% and 46 to 50% alpha linolenic (ALA) acid, respectively (DECLERCQ *et al.,* 1992; El-BELTAGI *et al.,* 2007). For Ethiopian flaxseed cultivars the ALA contents were 52% (WAKJIRA *et al.,* 2004). However, the ALA contents of the Pakistani cultivars were less than those reported for the flaxseed cultivars grown in New Zealand and Canada, i.e. 59.65 and 59%, respectively (HETTIARACHCHY *et al.,* 1990; CHOO *et al.,* 2007). Moreover, BOZAN and TAMELLI (2008) reported ALA levels to be 56.5 to 61% for flaxseed from Turkish origin, i.e. greater than our findings.

The trends for FA results in the present study are also in agreement with the reports that with an increase in ALA in flaxseed oil, there is a corresponding decrease in oleic acid (CHOO *et al.* 2007). The flaxseed cultivar LS-33 had the highest contents of ALA (54.87%) and lowest amount of oleic acid (21.05%), while LS-99 contained the lowest amount of alpha linolenic acid (44.51%) and the highest amount of oleic acid (30.96%). Overall, the amount of linolenic acid ranged from 44.51 to 54.87%, while that of oleic acid ranged from 21.06 to 30.96% for different cultivars of flaxseed grown in Pakistan.

CONCLUSIONS

The oil yield considerably varied among the selected flaxseed cultivars. Similarly, the significant differences for most of the physico-chemical/analytical characteristics among the tested oils were recorded. Such variations in oil quality characteristics might be linked to different genetic makeup of the cultivars as well as to their variable harvesting conditions. Overall, the flaxseed cultivar Chandi, LS-99 and LS-29 had relatively higher oil yield; the cultivar NS-29, LS-108, Chandi and LS-33 exhibited greater amount of tocopherols whereas those of LS-33, LS-29 and LS-105 were rich in alpha linolenic acid (ALA) among others. The findings of this comparative study can be useful for selection of economically and nutritionally important flaxseed cultivars, especially, as ingredient for functional foods and nutraceuticals.

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