

Evaluation of canola seeds of different cultivars with special emphasis on the quantification of erucic acid and glucosinolates

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RESUMEN

Evaluación de semillas de canola procedentes de diferentes variedades con especial énfasis en la cuantificación de ácido erúico y glucosinolatos.

Este estudio describe de la caracterización de semillas y de sus aceites de cinco cultivos locales de canola: Zafar-2002, Bulbul (Frontier), Dunkeld, Oscar y Con 11. El contenido de aceite de las semillas de canola varió entre un 34.3 y un 39.3%. Los niveles de proteína, fibra, ceniza y humedad fueron 22.1-41.0, 12.0-14.0, 3.0-3.5 and 4.0-7.5%, respectivamente. El contenido de glucosinolatos (GSL) en las semillas de canola examinadas varió entre un 49.7 y un 78.1 mmol g⁻¹. El aceite de canola extraído tuvo un índice de yodo de 118.2-124.6 g de I/100g de aceite; índice de refracción (40°C), 1.460-1.464; densidad (24°C), 0.914-0.919 mg mL⁻¹; índice de saponificación, 187-195; materia insaponificable, 0.51-1.10%; acidez (% como ácido oleico), 0.40-1.40, y color (cubeta de 1 pulgada), 1.35-1.73 R + 21.0-38.0 Y. El índice de peróxido (meq/ kg de aceite) y la extinción específica a 232 and 270 nm fueron 2.00-7.08, 2.17-3.16 and 0.44-0.91, respectivamente. El aceite de las semillas de los cinco cultivos de canola contenían principalmente ácido oleico (C18:1), ácido linoleico (C18:2) and ácido linolenico (C18:3) a unos niveles de 49.16-62.14, 14.61-23.45 and 6.97-9.10%, respectivamente. Las concentraciones de ácido palmítico (C16:0), ácido esteárico (C18:0), ácido erúico (C22:1) y ácido gadoleico (C20:1) variaron entre 3.47 y 6.00, 1.51 y 2.10, trazas y 13.03, y 1.30 y 10.63%, respectivamente. Una pequeña cantidad de ácido araquídico (20:0) con una contribución por debajo del 1% fue también detectada. El contenido de tocoferoles (α , γ , and δ) en los aceites de canola fue de 77.1-270.3, 191-500, 3.5-15.6 mg kg⁻¹, respectivamente. La presencia de niveles relativamente altos de ácido erúico y GSL en el presente análisis de la canola incide en la necesidad de reducir el contenido de estos dos constituyentes anti-nutricionales en los cultivares investigados.

PALABRAS CLAVE: Ácido erúico – Canola – Caracterización – Estabilidad oxidativa – Glucosinolatos – Propiedades físico-químicas – Tocoferoles.

SUMMARY

Evaluation of canola seeds of different cultivars with special emphasis on the quantification of erucic acid and glucosinolates.

This study reports the characterization of the seeds and seed oils of five locally grown canola cultivars: Zafar-2002, Bulbul (Frontier), Dunkeld, Oscar and Con-11. The oil

contents from canola seeds ranged from 34.3 to 39.3%. The levels of protein, fiber, ash and moisture were found to be 22.1-41.0, 12.0-14.0, 3.0-3.5 and 4.0-7.5%, respectively. The glucosinolate (GSL) contents in the canola seeds examined ranged from 49.7 to 78.1 mmol g⁻¹. The extracted canola seed oils revealed an iodine value of 118.2-124.6 g of I/100g of oil; refractive index (40 °C), 1.460-1.464; density (24 °C), 0.914-0.919 mg mL⁻¹; saponification value, 187-195; unsaponifiable matter, 0.51-1.10%; acidity (% as oleic acid), 0.40-1.40, and color (1-in. cell), 1.35-1.73 R + 21.0-38.0 Y. Peroxide value (meq/ kg of oil) and specific extinctions at 232 and 270 nm were determined to be 2.00-7.08, 2.17-3.16 and 0.44-0.91, respectively. The seed oils of the five canola cultivars mainly consisted of oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids at levels of 49.16-62.14, 14.61-23.45 and 6.97-9.10%, respectively. The concentrations of palmitic (C16:0), stearic (C18:0), erucic (C22:1) and gadoleic (C20:1) acids ranged from 3.47 to 6.00, 1.51 to 2.10, traces to 13.03 and 1.30 to 10.63%, respectively. A small amount of arachidic acid (20:0) with a contribution below 1% was also detected. The contents of tocopherols (α , γ , and δ) in the canola oils accounted for 77.1-270.3, 191-500, 3.5-15.6 mg kg⁻¹, respectively. The presence of rather high levels of erucic acid and GSL in the present analysis of canola emphasized the need to further reduce the contents of these two anti-nutritional constituents in the investigated cultivars.

KEY-WORDS: Canola – Characterization – Erucic acid – Glucosinolates Physicochemical properties – Oxidative stability – Tocopherols.

1. INTRODUCTION

Supra optimal levels of erucic acid in seed oil and glucosinolates in seed meal of regular rapeseed have almost prohibited the utilization of this crop from human and animal consumption. Some earlier studies on animals show that erucic acid (C22:1), when consumed in large quantities may adversely affect heart tissues and thus promotes myocardial lesions (Kramer *et al.*, 1973; Clandinin and Yamashiro 1982). Glucosinolates (GSL) and their decomposition products can cause several health hazards in animals, including enlargement of the thyroid, and negative effects on adrenal glands and kidneys (Zukalova and Vasak 2002; Barillari *et al.*, 2005).

Canola, a “double-low” variety of the rapeseed (*Brassica napus* or *Brassica campestris*), contains

less than 2% erucic acid (C22:1) in the oil and less than 30 micromoles per gram of glucosinolates in the seed meal. Due to these characteristics, it has gained considerable recognition as a potential oil-seed crop worldwide. (Hu *et al.*, 1997; Neff *et al.*, 1997).

Currently, canola oil which is generally available in the market contains only traces of erucic acid, 5 to 8% saturated, 60 to 65% monosaturated, and 30 to 35% polyunsaturated fatty acids. Canola oil is the preferred oil for health-conscious consumers because it is the lowest in saturated fat among the major edible oils (Shahidi and Shukla, 1996). Canola oil contains appreciable amounts of antioxidants such as vitamin E, phenolic acids, flavonoids and insoluble condensed tannins, all of which are beneficial to human health (Zambiasi, 1997). Rapeseed/canola seed meal, constituting 50-58% of the weight of seeds is a potential source of essential amino acids like lysine, methionine, cystine, threonine and tryptophan (Sarwar *et al.*, 1984; Larsen and Sorensen, 1985).

In Pakistan, after cotton, rape seed/mustard and canola are the major oilseed crops grown in an area of about 365,000 ha with an annual production of 350,000 tons contributing about 19% to the overall domestic production of edible oils (PARC, 2007). The oil-seed producers are in continuous efforts to develop true canola cultivars with a reduced content of erucic acid and GSL in order to fulfill international standards. A number of diverse canola cultivars are currently under trials in Pakistan for attaining maximum seed oil under the native environmental conditions, but it is not certain whether the canola cultivars being grown have maintained their desired oil characteristics. In view of this, we sought to characterize such canola cultivars being grown under indigenous environmental regimes. The primary objective of the present study was to conduct a detailed analysis and to investigate the physico-chemical characteristics of canola seed and the seed oils of five commonly grown cultivars with special emphasis on quantifying and comparing the contents of the oil, erucic acid and glucosinolates.

2. MATERIALS AND METHODS

2.1. Collection of canola seeds

The seeds of five, indigenously grown canola cultivars : Zafar-2002, Bulbul (Frontier), Dunkeld, Oscar and Con-II were obtained from the Ayub Agricultural Research Institute (AARI), Jhang Road, Faisalabad, Pakistan. Three seed samples for each cultivar were assayed.

2.2. Reagents and chemicals

All reagents (analytical and (HPLC) and chemicals used for all analytical procedures were from Merck (Darmstadt, Germany) or Sigma Aldrich (Buchs, Switzerland). Pure standards of

tocopherols (DL- α -tocopherol, (+)- δ -tocopherol, - γ -tocopherol) and fatty acid methyl esters (FAMES) were obtained from Sigma Chemical Co. (St. Louis, MO).

2.3. Proximate analysis of seeds

Moisture determination

Moisture content of the seeds was determined at 105°C according to ISO method 666 (ISO 1977) using an electric oven (VOC-300 SD; EYELA, Tokyo, Japan).

Extraction of oil

The seeds of all five canola cultivars were crushed in a domestic electric grinder. The crushed seed material was conditioned (100°C) in an electric oven (VOC-300 SD; EYELA, Tokyo, Japan) for 30 minutes. After conditioning, the ground seed material (~75 g) was fed into a Soxhlet extractor. The extraction was performed with 300 mL of *n*-hexane (B.P. 68°C) on a water bath for 6 h. The oils extracted were stored in a refrigerator at 4°C until further analysis.

Determination of glucosinolates in canola seeds

The glucosinolates were determined after their hydrolysis with myrosinase by milling the whole canola seeds separately with water and acidified 40% methanol to liberate bound and free glucose. The extracts were filtered through activated charcoal paper to remove any interfering phenolics. The total glucosinolate contents were estimated following a glucose oxidase/peroxidase calorimetric method (Smith and Dacombe 1987).

Analysis of oilseed residues

After oil extraction, the canola oilseed residues were analyzed for protein, fiber, and ash contents. Protein content ($N \times 6.25$) was determined according to the AOAC method 954.01 (AOAC, 1990). Fiber content was estimated by an ISO method 5983 (ISO, 1977). Briefly, a fat-free, finely ground sample (2.5 g) of meal was weighed and boiled with sulfuric acid (0.255 mol L⁻¹), followed by separation and washing of the insoluble residue. The residue was further boiled with sodium hydroxide (0.313 mol L⁻¹), followed by separation, washing, and drying. The dried residue was weighed and ashed in a muffle furnace (TMF-2100, EYELA, Tokyo, Japan) at 600°C, and the loss of mass was determined. The determination of ash content was made following the ISO method 749 (ISO, 1977). Two grams of meal were carbonized by heating on a gas flame. The carbonized material was then ashed in an electric muffle furnace (TMF-2100, EYELA, Tokyo, Japan) at 550°C, to achieve a constant mass.

2.4. Analysis of extracted oils

Physical and chemical characteristics

The density, refractive index, iodine value, peroxide value, acidity, saponification value and unsaponifiable matter of the extracted canola oils were determined following the AOCS official methods Cc 10a-25, Cc 7-25, Cd 1-25, Cd 8-53, F 9a-44, Cd 3-25, and Ca 61-40, respectively (AOCS, 1997). The color intensities (Red +Yellow, 1-in. cell) of the oils were determined by a Lovibond tintometer (Tintometer Ltd., Salisbury, Wiltshire, United Kingdom). Specific extinctions at 232 and 270 nm were determined using a spectrophotometer (U-2001, Hitachi Instruments, Inc., Tokyo, Japan). Absorbance of the oils, diluted with iso-octane, was recorded in the UV region at 232 and 270 nm. Specific extinctions ($\epsilon^{1\%1\text{cm}}(\lambda)$) were calculated following the standard IUPAC method (IUPAC, 1987).

Fatty acid composition

Canola oils were transmethyated according to the standard IUPAC method 2.301 (IUPAC, 1987) and analyzed on a SHIMADZU gas chromatograph model 17-A, fitted with an SP-2330 (SUPELCO Inc. Supelco Park Bellefonte, PA 16823-0048, USA) polar capillary column (30 m \times 0.32 mm; 0.20 μm), and a flame ionization detector. Oxygen-free nitrogen was used as a carrier gas at a flow rate of 3.0 mL min^{-1} . The column temperature was programmed from 180 to 220°C at a linear increment of 5°C min^{-1} . The initial and final retention times were 2 and 10 minutes, respectively. Injector and detector temperatures were maintained at 230 and 250°C, respectively. A sample volume of 1.0 μL was injected using split mode (split ratio, 1:65). Fatty acid methyl esters (FAMES) were identified by comparing their relative and absolute retention times to those of authentic standards. A data-handling software ((Data APEX Ltd., Prague, The Czech Republic), Chromatography Station for Windows (CSW32) was used for the quantification.

Tocopherols content

Tocopherols (α , γ and δ) in the oil samples were analyzed by high performance liquid chromatography following Lee *et al.* (2003). An HPLC (Sykam GmbH, Kleinostheim, Germany) equipped with an S-1121 dual piston solvent delivery system and S-3210 UV/Vis diode array detector was used. One gram of oil was made up to a volume of 10 mL with acetonitrile in a volumetric flask, wrapped in aluminum foil to inhibit photooxidation. 20- μL of filtered sample were injected into an analytical Hypersil (Thermo Hypersil, GmbH, Germany) ODS reverse phase (C18) column (250 \times 4.6 mm; 5 μm particle size) fitted with a C18 guard column. The mobile phase comprising a mixture of HPLC grade methanol and

acetonitrile (65:35 v/v) was applied at a flow rate of 1.3 mL min^{-1} at 30°C under isocratic elution. Detection was made at 292 nm. Tocopherols (α -, γ -, and δ) were identified by comparing the retention times and quantified on the basis of the peak area percent of the unknowns compared with those of pure standards (Sigma Chemical Co., St. Louis, MO). The quantification was done on a computer using SRI peak simple chromatography data acquisition and integration software (SRI Instrument, Torrance, California, USA).

2.5. Statistical Analysis

Three canola oilseed samples were assayed for each cultivar and analyzed individually in triplicate. Data is reported as mean ($n = 1 \times 3 \times 3$) \pm SD ($n = 1 \times 3 \times 3$). One way ANOVA was used to determine significant differences among the cultivars considering a level of significance less than 5% ($p < 0.05$), using the statistical software STATISTICA (StatSoft, OK, USA).

3. RESULTS AND DISCUSSION

The data for the analysis of seeds and seed oils of five indigenously grown canola cultivars: Zafar-2002, Bulbul (Frontier), Dunkeld, Con-II and Oscar are summarized in Tables 1-5. The hexane-extracted oil content of canola seeds from the five investigated cultivars ranged from 34.3% (Con-II) to 39.3% (Dunkeld) (Table 1). The variation in the oil content within different canola oilseed cultivars may be attributed to their varied genetic make-up, and to different growing and agro-ecological conditions. The range of oil content (34.3-39.3%) in the present analysis of canola oilseeds was comparable to that reported in the literature (Pritchard, 1991). Our findings showed the highest concentration of oil in Dunkeld and the lowest in Con-11, however, Hassan *et al.*, (2005) reported somewhat higher oil contents in Bulbul (Frontier) and lower in Zafar-2002. The variations in the oil contents of different canola cultivars in the present study could be supported from the investigations of Pritchard *et al.* (2000).

The analysis of canola oilseed residues (Table 1) revealed that the protein content (22.0-41.0%) of the seeds varied significantly ($p < 0.05$) among cultivars analyzed. Whereas the fiber, ash and moisture contents of the seeds, 12.0-14.0%, 3.0-3.5% and 4.0-7.5%, respectively did not vary considerably. The protein content in the present analysis (22.0-41.0%), when compared with conventional oilseed crops was found to be higher than those of safflower (20-22%), sunflower (16-19%), cottonseed (19%), linseed (24%) and sesame (20-25%) (Pritchard, 1991). The rapeseed/canola meal is already reported to be a rich source of essential amino acids such as lysine, methionine, cystine, threonine and tryptophan; the concentrations of which are quite similar to other cereals (Sarwar *et al.*, 1984; Larsen and Sorensen, 1985).

Table 1
Proximate composition of canola oilseeds from different cultivars

Constituents	Zafar-2002	Bulbul (Frontier)	Dunkeld	Oscar	Con-11
Oil content (%)	35.3 ± 0.3 ^b	38.3 ± 0.2 ^a	39.3 ± 0.7 ^a	38.2 ± 0.8 ^a	34.3 ± 0.3 ^b
Moisture content (%)	4.0 ± 0.1 ^d	4.0 ± 0.1 ^d	4.5 ± 0.2 ^a	6.0 ± 0.1 ^c	7.5 ± 0.2 ^b
Fiber content (%)	12.5 ± 0.2 ^{bc}	13.5 ± 0.3 ^{ab}	12.0 ± 0.2 ^c	13.0 ± 0.4 ^b	14.0 ± 0.3 ^a
Ash content (%)	3.1 ± 0.1 ^{ab}	3.4 ± 0.2 ^{ab}	3.0 ± 0.2 ^b	3.0 ± 0.1 ^b	3.5 ± 0.2 ^a
Proteins content (%) (N × 6.25)	30.2 ± 1.3 ^b	22.1 ± 1.4 ^d	41.0 ± 2.1 ^a	29.0 ± 1.5 ^b	26.0 ± 1.3 ^c
Glucosinolates content (μ moles/g)	49.7 ± 3.4 ^c	51.5 ± 4.3 ^c	58.7 ± 3.0 ^b	62.4 ± 3.2 ^b	78.1 ± 4.0 ^a

Values (mean ± SD) are from three canola seed samples of each cultivar, analyzed individually in triplicate. Mean values followed by different superscript letters in the same row indicate significant difference ($p < 0.05$) among cultivars

Table 2
Physicochemical characteristics of canola seed oils from different cultivars

Constituents	Zafar-2002	Bulbul (Frontier)	Dunkeld	Oscar	Con-11
Refractive index (40°C)	1.464 ± 0.03 ^a	1.460 ± 0.02 ^a	1.464 ± 0.04 ^a	1.462 ± 0.03 ^a	1.463 ± 0.03 ^a
Density (mg /mL) (24°C)	0.919 ± 0.02 ^a	0.917 ± 0.04 ^a	0.914 ± 0.03 ^a	0.919 ± 0.02 ^a	0.916 ± 0.02 ^a
Iodine value (g of I/100g of oil)	121.6 ± 1.6 ^{ab}	118.2 ± 2.6 ^b	120.5 ± 1.9 ^b	122.6 ± 2.0 ^{ab}	124.6 ± 1.9 ^a
Saponification value (mg of KOH/g of oil)	193.0 ± 3.3 ^{ab}	189.0 ± 2.9 ^{ab}	187.0 ± 3.2 ^b	188.0 ± 4.0 ^{ab}	195.0 ± 4.0 ^a
Unsaponifiable matter (%)	0.51 ± 0.04 ^c	0.70 ± 0.04 ^b	1.10 ± 0.10 ^b	0.90 ± 0.10 ^b	0.70 ± 0.05 ^a
Color (1-in. cell)					
Red unit	1.71 ± 0.16 ^a	1.62 ± 0.06 ^{ab}	1.60 ± 0.14 ^{ab}	1.35 ± 0.12 ^b	1.73 ± 0.12 ^a
Yellow unit	35.0 ± 0.5 ^b	21.0 ± 1.0 ^c	23.0 ± 0.7 ^c	22.0 ± 0.3 ^c	38.0 ± 0.5 ^a
Acidity (% as oleic acid)	0.40 ± 0.02 ^d	0.68 ± 0.03 ^c	1.21 ± 0.04 ^b	0.66 ± 0.03 ^c	1.40 ± 0.04 ^a

Values (mean ± SD) are of three canola oil samples from each cultivar, analyzed individually in triplicate. Mean values followed by different superscript letters in the same row indicate significant difference ($p < 0.05$) among cultivars.

The glucosinolate (GSL) contents of the canola seeds of the different cultivars ranged from 49.7 to 78.1 mmol g⁻¹ of dry seed weight (Table 1). The lowest content of GSL (49.7) was detected in cultivar Zafar-2002, whereas, that of Con-II offered the highest level (78.1). The present levels of GSL for Oscar, Dunkeld, and Con-II were in agreement with the findings of Hassan *et al.*, (2005), while for Zafar-2002 and Bulbul (Frontier) the present values were quite different. True canola cultivars should have GSL contents less than 30 mmol/g of air-dried meal (Pritchard, 1991). However, the present analysis showed that none of the canola cultivars examined here were a true canola because of the presence of quite high contents of GSL. The significant differences ($p < 0.05$) of GSL contents among the cultivars examined could be due to the diverse genetic makeup of the cultivars, and to specific agroecological and environmental conditions of the regions. Glucosinolates are anti-nutritional components present in high amounts in rapeseed/canola meal, but not in considerable amounts in canola oil. Glucosinolate accumulation is known to be affected by water/moisture

availability, particularly at the seed development and maturation stages. The glucosinolate levels in seeds can be controlled during growth stages through applications of jasmonic acid (Pritchard *et al.*, 2000; Harbans and Hamama 2003).

Various physicochemical attributes of the extracted canola seed oils are given in Table 2. The values determined for iodine, refractive index (40°C), density (24°C), saponification value and unsaponifiable matter for the canola seed oils of the investigated cultivars were found to be 118.2 to 124.6 (g of I/ 100 g of oil), 1.462 to 1.464, 0.914-0.919, 187 to 195 (mg of KOH/g of oil), and 0.51-1.10%, respectively. The iodine values (118.2-124.6) for canola oils in the present analysis were somewhat comparable with those reported in the literature (Rossell, 1991). The refractive indexes (1.462-1.464) for the investigated canola seed oils were almost comparable with those of cottonseed (1.458-1.466), corn (1.465-1.468), mustard seed (1.461-1.469), high and low erucic acid rapeseed (1.465-1.467) oils (Rossell, 1991). The saponification values were noted to be comparable with those of cottonseed (189-198), olive (184-189), pumpkin seed (185-198)

Table 3
Oxidation state of canola seed oils from different cultivars

Constituents	Zafar-2002	Bulbul (Frontier)	Dunkeld	Oscar	Con-11
Conjugated dienes $\epsilon_{1\text{cm}}^{1\%} (\lambda 232)$	2.40 \pm 0.20 ^b	2.17 \pm 0.20 ^b	2.26 \pm 0.15 ^b	2.29 \pm 0.18 ^b	3.16 \pm 0.28 ^a
Conjugated trienes $\epsilon_{1\text{cm}}^{1\%} (\lambda 270)$	0.51 \pm 0.04 ^d	0.91 \pm 0.10 ^a	0.44 \pm 0.04 ^c	0.54 \pm 0.05 ^d	0.64 \pm 0.10 ^b
Peroxide value (meq/kg of oil)	7.08 \pm 0.19 ^a	4.70 \pm 0.20 ^c	6.00 \pm 0.11 ^b	4.10 \pm 0.17 ^d	2.00 \pm 0.21 ^e

Values (mean \pm SD) are of three canola oil samples from each cultivar, analyzed individually in triplicate. Mean values followed by different superscript letters in the same row indicate significant difference ($p < 0.05$) among cultivars.

Table 4
Fatty acid (FA) composition (g/100g of FA) of canola seed oils from different cultivars

Fatty Acids	Zafar-2002	Bulbul (Frontier)	Dunkeld	Oscar	Con-11
C16:0	4.80 \pm 0.39 ^c	3.47 \pm 0.40 ^d	5.24 \pm 0.24 ^b	4.84 \pm 0.22 ^c	6.00 \pm 0.30 ^a
C18:0	1.80 \pm 0.13 ^b	1.62 \pm 0.05 ^c	2.10 \pm 0.11 ^a	1.51 \pm 0.10 ^d	1.67 \pm 0.10 ^c
C18:1	59.71 \pm 0.81 ^b	49.16 \pm 1.10 ^c	59.00 \pm 1.00 ^b	58.62 \pm 0.74 ^b	62.14 \pm 0.85 ^a
C18:2	19.38 \pm 0.45 ^c	14.61 \pm 0.81 ^d	21.00 \pm 0.50 ^b	23.45 \pm 0.70 ^a	21.25 \pm 0.65 ^b
C18:3	9.10 \pm 0.51 ^a	6.97 \pm 0.32 ^c	8.00 \pm 0.36 ^b	9.00 \pm 0.29 ^a	6.98 \pm 0.49 ^c
C20:0	0.55 \pm 0.10 ^a	0.51 \pm 0.10 ^a	0.65 \pm 0.09 ^a	0.60 \pm 0.14 ^a	0.56 \pm 0.10 ^a
C20:1	1.61 \pm 0.28 ^c	10.63 \pm 0.40 ^a	1.50 \pm 0.12 ^c	1.85 \pm 0.12 ^b	1.30 \pm 0.23 ^d
C22:1	3.05 \pm 0.18 ^b	13.03 \pm 0.21 ^a	2.51 \pm 0.10 ^c	tr	tr

Values (mean \pm SD) are of three canola oil samples of each cultivar, analyzed individually in triplicate, (tr traces)? Mean values followed by different superscript letters in the same row indicate significant difference ($p < 0.05$) among cultivars.

and safflower seed (186-198) oils (Rossell, 1991). The free fatty acid contents (as oleic acid) of the tested canola oils ranged from 0.40 to 1.40%, which could not be compared with literature reports. Of the canola seed oils, cultivar Zafar-2000 exhibited the lowest acid value, indicating better resistance to hydrolysis for this oil. Generally, higher acid values indicate a high magnitude of hydrolytic deterioration and thus poor quality oils (Rossell, 1991).

The color values of the canola seed oils were found to be 1.35-1.71 red units and 21.0-38.0 yellow units. Color growth in vegetable oils is mainly due to the occurrence of a variety of pigments such as chlorophyll and carotenoids. Such pigments are efficiently removed using different treatments like refining and bleaching of oils. From the viewpoint of commercial applications, the oils with the least coloration are more appealing.

The peroxide value, which is generally used to express the magnitude of primary oxidation products in oils (McGinley, 1991), ranged from 2.00 to 7.08 meq kg⁻¹ in the canola seed oils investigated (Table 3). The specific extinctions at 232 and 272 nm, which are important indicators of oxidative deterioration of oils (McGinley, 1991; Manzoor *et al.*, 2007), ranged from 2.17-3.16 and 0.44-0.91, respectively (Table 3). There are no previously reported data on these canola cultivars to compare the results of peroxide value and conjugated diene and triene contents with our findings reported here.

Table 4 depicts the fatty acid composition of the seed oils from different canola cultivars. The tested

canola seed oils mainly consisted of oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids at levels of 49.16-62.14, 14.61-23.45 and 6.97-9.10%, respectively. The concentrations of palmitic (C16:0), stearic (C18:0), erucic (C22:1) and gadoleic (C20:1) acids ranged from 3.47 to 6.00, 1.51 to 2.10, traces to 13.03 and 1.30 to 10.63%, respectively. A small amount of arachidic acid (20:0) with a contribution below 1% was also detected.

The levels of C16:0 and C18:0 in the present analysis of canola seed oils was quite comparable with those for canola oil reported in the literature (Rossell, 1991). The contents of oleic acid (C18:1 *n*-9), ranging from 49.16-62.14%, varied significantly ($p < 0.05$) among the canola cultivars analyzed. The highest amount of oleic acid was detected in Con-11 (62.14%), whereas, the lowest in Bulbul (Frontier) (49.16%). The content of oleic acid in our present analysis was slightly different from that reported by Hassan *et al.*, (2005). However, the present range of oleic acid was in agreement with those reported in the literature (Rossell, 1991; Pritchard *et al.*, 2000). Oleic acid is the most abundant fatty acid in nature and is present in almost all vegetable oils and fats. Oleic acid is gaining importance in human nutrition owing to its potential health benefits against cardiovascular heart diseases and its cholesterol lowering effects (Kris-Etherton 1999; Lovejoy 2002).

The contents of C18:2 *n*-6 in the tested canola seed oils (14.61-23.45%) varied significantly ($p < 0.05$) among cultivars analyzed with the highest

levels (23.45%) in Oscar and the lowest (14.61%) in Bulbul (Frontier). The highest level (9.10%) of C18:3 *n*-3 was observed in Zafar-2002, whereas the lowest (6.97%) was observed in Bulbul (Frontier), showing significant ($p < 0.05$) variations among cultivars. Our present analysis showed that the contents of C18:3 *n*-3 determined in the canola cultivars were comparable with those reported by Hassan *et al.*, (2005). Both, C18:2 *n*-6 and C18:3 *n*-3 are the essential fatty acids that the human body is unable to synthesize. The potential health benefits of essential fatty acids are well documented elsewhere (Markwell *et al.*, 1998; Schaefer 2002).

Erucic acid (C22:1) was detected in the range of traces to 13.03% with the highest level (13.03%) in Bulbul (Frontier), with the lowest (traces) in Oscar and Con-11. True canola contains less than 2% erucic acid (C22:1). It was observed from the present fatty acid data that only two of the five tested canola cultivars exhibited erucic acid levels below 2% and could therefore be defined as true canola.

The data also revealed significant ($p < 0.05$) differences among cultivar means for gadoleic acid (C20:1), ranging from 1.30% in Con-11 to 10.63% in Bulbul (Frontier). The exceptionally high amount of gadoleic acid (10.63%) determined in the present analysis of Bulbul (Frontier) as compared to the other canola cultivars tested might be attributed to the presence of higher levels of erucic acid. This fact is supported by the findings of Rossell (1991), who reported higher levels of gadoleic acid (2.6-9.4%) in those canola cultivars, which possess elevated amounts of erucic acid (6.5-51.6%) as compared to those containing low erucic acid (tr-4.7%).

Table 5 presents the levels of tocopherols in the seed oils of different canola cultivars. The contents of α -tocopherol, γ -tocopherol, and δ -tocopherol ranged from 77.1-270.3, 190.6-500.0, 3.5-15.6 mg kg⁻¹ of oil, respectively. The concentration of α -tocopherol, which has greater vitamin E effectiveness (Rossell, 1991) was found to be the lowest in Zafar-2000, and the highest in Dunkeld. The amount of γ -tocopherol was noted to be higher in Oscar (500.0 mg kg⁻¹) and Con-11 (420.0 mg kg⁻¹), whereas Dunkeld offered the lowest level of this isomer of tocopherol (190.0 mg kg⁻¹). The concentration of δ -tocopherol having superior antioxidant activity over γ - or α -tocopherol (Anwar *et al.*, 2006), was quite low (3.5-15.6 mg kg⁻¹). No previously reported data on the tocopherol contents of these cultivars of canola seed oils with which to compare the values of the present analysis

have been reported. Overall, the tocopherol profiles of Oscar and Con-11 seed oils were quite comparable to those reported for canola oils in the literature (Rossell, 1991).

From the values of erucic acid and GSL contents of the investigated canola cultivars, grown in Pakistan, it is possible to declare that none of the tested cultivars is true canola because the contents of both of these antinutritional components are above the permissible limits as established by current specifications of the Canola Council of Canada. Of course these cultivars are in the developing stage in Pakistan and such variation in the contents of components may be attributed to the varied geographical and cultivation regimes. This necessitates the need to further reduce and control the contents of GSL and erucic acid in these locally grown canola cultivars, where applicable. The present information might be useful in controlling and maintaining the required attributes of growing canola cultivars under indigenous conditions and environment for the on-going breeding programs and for researchers.

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Table 5
Tocopherol contents (mg/kg) of canola seed oils from different cultivars

Tocopherol	Zafar-2002	Bulbul (Frontier)	Dunkeld	Oscar	Con-11
α -tocopherol	77.1 \pm 3.9 ^e	101.0 \pm 5.0 ^d	270.3 \pm 4.0 ^a	170.0 \pm 4.3 ^b	150.5 \pm 6.0 ^c
γ -tocopherol	276.5 \pm 8.3 ^d	300.3 \pm 7.3 ^c	190.6 \pm 10.0 ^e	500.0 \pm 12.5 ^a	420.1 \pm 15.0 ^b
δ -tocopherol	6.0 \pm 0.3 ^c	11.7 \pm 0.3 ^b	6.3 \pm 0.3 ^c	15.6 \pm 0.6 ^a	3.5 \pm 0.4 ^d

Values (mean \pm SD) are of three canola oil samples of each cultivar, analyzed individually in triplicate.

Mean values followed by different superscript letters in the same row indicate significant difference ($p < 0.05$) among cultivars.

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