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Effect of Cold-Pressing and Soxhlet Extraction on the Physico-Chemical Attributes of Sunflower (*Helianthus annuus* L.) Seed Oil

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Abstract

This study investigates variation of physicochemical characteristics between sunflower seed oil extracted by cold pressing (CP) and Soxhlet extraction (SE) methods. SE exhibited higher sunflower seed oil yield (42.5%) as compared with the CP (21.4%). There were significant variation observed in saponification value, iodine value and color, whereas, density, refractive index and unsaponifiable matter (%w/w) did not differ between sunflower oils produced by both the methods. The cold pressed (CP) sunflower oil had relatively good oxidation state than the Soxhelt extracted (SE) oil. The fatty acids profile of SE and CP oils showed considerable variation for the contents of palmitic, oleic and arachidic acids while the amount of the principal fatty acid (linoleic acid 61.26 and 60.12 g/100g FA) was almost similar for both the oils. Significantly ($P< 0.05$) higher tocopherol contents (α, δ and γ) were observed in CP oil (570, 210 and 3.8 mg kg^{-1}) as compared with SE oil (525, 170 and 2.2 mg kg^{-1}), respectively. Overall, it could be concluded that CP sunflower oil has superior nutritional characteristics compared with SE sunflower oil which might be attributed to the mild extraction conditions employed during cold pressing.

Key words: Sunflower oil; Comparative extraction methods; Quality characteristics, Linoleic acid; Tocopherols; HPLC; GLC

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1. Introduction

Sunflower (*Helianthus annuus* L.) is an oil seed crop belonging to *Compositae* family and grows in several regions around the world. Sunflower is considered as a potential source of nutrients such as protein, minerals and lipids. The whole sunflower seed contains roughly 25 % protein, 2.9% crude fiber, 4.8% ash and minerals such as phosphorous, potassium, calcium, magnesium, iron sodium and zinc as well as vitamin C, B_1, B_2, A, E and K [1]. Sunflower seed oil is good quality edible oil which is light in taste and appearance. Due to high content of linoleic acid and high ratio of polyunsaturated/saturated fatty acids, the oil is nutritionally regarded as a valuable food commodity [2, 3]. At present, among all world vegetable oil production, sunflower seed oil is ranked at fourth position after soybean, palm and rapeseed. Sunflower seed oil is also one of the best sources of lecithin, tocopherols, carotenoids and polyunsaturated fatty acid (linoleic (66.2%) [4].

The impressive features such as high content of antioxidant vitamin E, light color, mild in taste, and low in saturated fats makes sunflower oil (SFO) to be a good choice for cooking especially for frying foods. SFO, due to its wide ranging medicinal and nutritional benefits, is regarded as a healthy vegetable oil. SFO has cleansing properties; it can be used both as diuretic and an expectorant so is useful in the treatment of bronchial, laryngeal and pulmonary infections, whooping coughs and colds. Moreover, SFO is easily absorbed and moisturizes the skin and thus has potential for cosmo-nutraceutical applications [5].

Two main processes mostly employed for oil extraction from seeds on industrial scale are cold pressing and solvent extraction (Soxhlet method). Soxhlet method, involving the use of *n*-hexane or petroleum ether as extraction solvent, although gives higher oil yield but a higher

temperature employed in this method may cause undesirable effects on the quality of oil [6]. Cold pressing method of oil extraction, although with lower oil yield, is advantageous with regard to mild operational temperature conditions, process safety and product quality [7].

Keeping in view of the growing demand and consciousness about the functional and nutritional properties of oils, the present work was therefore designed with the main objective to evaluate and compare the physicochemical properties and nutritional quality of sunflower seed oil extracted by two different methods.

2. Materials and Methods

2.1. Reagents and chemicals

All the reagents and chemicals used were HPLC and analytical grade and were got from Merck (Darmstadt, Germany) or Sigma Aldrich (Buchs, Switzerland). The pure standards of tocopherols [DL-α-tocopherol, (+)-δ-tocopherol, (+) -γ-tocopherol] and fatty acid methyl esters (AMEs) were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Samples

Sample of sunflower seeds were obtained from Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. The seeds were further identified and authenticated by the Department of Botany, University of Agriculture, Faisalabad.

2.3. Apparatus

Apparatus used for the present experiments experiment were: blender (TSK-949, Westpoint, France), Soxhlet extractor, seed oil presser apparatus, rotary evaporator (model SB-651,EYELARikakikai Co. Ltd. Tokyo, Japan), Kjeldahl apparatus, HPLC (Sykam GmbH, Kleinostheim, Germany) equipped with a S-1121 dual piston solvent delivery system and S-3210 UV/Vis diode array detector, a SHIMADZU gas chromatograph (model 17-A, SHIMADZU, Japan) fitted with a SP-2330 (SUPLECO, INC., Bellefonte, PA, USA) methyl lignocserate coated (film thickness 0.20µm), polar capillary column (30 m x 0.25 mm) and equipped with flame ionization detector, Lovibond Tintometer (Tintometer Ltd, Salisbury, U. K) using 1 in. cell, automated Metrohm Rancimat apparatus, model 679 and a spectrophotometer (model U-2001, Hitachi, Inst. Inc., Tokyo, Japan).

2.4. Extraction of oil

After removing seed impurities, dried sample of sunflower seeds, were crushed using a commercial blender and then subjected to cold pressing and Soxhlet extraction. For Soxhelt method, the well-crushed seed samples were placed in a paper thimble and fed to a Soxhlet extractor which was fitted with a 500 mL round bottom flask and a condenser. The oil was extracted by using n-hexane (as an extracting solvent) on water bath for 8 h. After extraction, the extra hexane was distilled off under vacuum in a rotary evaporator at 45°C. The extracted oil was weighed and the yield was calculated. The extracted oil was kept in a fridge until further analyses [8-9]. In cold pressing, oil was extracted by continuous screw-pressers (Carver Press, USA) without using any chemicals for 20 min at a pressure range of 29.4–49.0 MPa.

2.5. Proximate analysis of oilseed residues

The sunflower seed residue was analyzed for fibre, protein, and ash contents. Protein content (percent N × 6.25) was estimated using a Kjeldahl apparatus [10, 12]. The fiber and ash contents were determined according to the ISO method 5983 and ISO method 749, respectively [13-15]. All the determinations were made in triplicate.

2.6. Physical and chemical parameters of oils

The physicochemical properties such as the color, density (Cc 10a–25), refractive index (Cc 7–25), saponification value (SV) (Cd 3-25), iodine value (IV) (Cd 1– 25), free acid content (FA) (Ca 5a-40), unsaponifiable matter (Ca 61–40) and peroxide value (Cd 8–53) of the extracted oils were analyzed according to AOCS standard methods [2]. The color of oils was checked by a Lovibond Tintometer. The oxidative stability of the oil was tested by using automated Metrohm Rancimat apparatus. Specific extinctions at 232 and 270 nm, representing the magnitude of primary and secondary oxidation products of oils, were determined using a spectrophotometer. The oil samples were diluted with *iso*-octane to get the absorbance readings within limits (0.2-0.8) and $\varepsilon^{1\%}_{1cm}(\lambda)^{11}$. The oil samples were then dissolved in *iso*-octane and allowed to react for 10 min to react with *para*-anisidine. The absorbance of the colored complex formed was recorded at 350 nm, using a spectrophotometer [13].

2.7. Analysis of tocopherols

For tocopherols (α, γ and δ tocopherols) determination, an appropriate amount of the SE and CP sunflower oil, dissolved in 1 mL of 2-propanol, was vortexed to form a homogenous phase. Vortexed oil samples were injected into a Hypersil ODS (C18) reverse phase column (250 × 4.6 mm) (Supelcosil LC-Si column (250 mm × 4.6 mm, Supelco Inc., Supelco Park, Bellefonte, USA). Standard solutions of tocopherols were also prepared in 2-propanol and used for calibration curve construction. A 20-uL sample volume was injected, whereas, elution was made using a mobile phase consisting of a mixture of acetonitrile and methanol (35:65 V/V) at a flow rate of 1.3 mL/min. A UV detector was used for detection of tocopherols at wavelength of 292 nm. For identification of tocopherols, the retention times (RT) of the unknown samples were compared against absolute/ pure tocopherol compounds (α-, γ- and δ -tocopherols). A Hitachi Chromatointegrator model D-2500 (Hitachi Instruments, Inc.) was used for quantification purposes using standard calibration curves.

2.8. Analysis of fatty acid composition

The oil obtained by soxhlet extraction and cold pressing was converted into their fatty acid methyl esters (FAMEs). The FAMEs were then analyzed on a gas

chromatograph (Agilent technologies 7890A, USA). A polar capillary column (SP-2330; 30 m × 0.25mm; Supelco Inc., Supelco Park Bellefonte, PA) was used for the separation of fatty acids. Mobile phase gas was nitrogen and was used at a flow rate of 1.5 mL/min. Column oven initial temperature was set at 180 °C and programmed by the linear increment of 5°C/ min to final temperature of 220 °C whereas hold up 2 min before and 10 min after the run were employed. Other conditions were set as under: injector temperature, 230 °C; detector (FID) temperature, 250 °C. Identification of targeted fatty acid compounds was based upon matching their relative and absolute RT (retention times) against those of absolute/pure standards of FAMEs [16, 17]. The fatty acid composition was reported as relative percentage of the total peak area.

2.9. Statistical Analysis

The statistical analysis of the data was performed by analysis of variance (ANOVA) using the statistical software Minitab 13 for Windows. A probability value at $P < 0.05$ was considered statistically significant. Data presented in tables is mean values ± standard deviation calculated from triplicate determinations.

3. Results and Discussion

3.1. Proximate composition

The results for proximate composition of sunflower oil extracted by two different methods are depicted in Table 1. As expected, the oil contents from sunflower seed was higher (42.5%) with Soxhlet method compared with cold pressing (21.4%). The present sunflower oil yield was lower than that reported by Latif and Anwar [18]. The amount of seed fiber, protein and ash did not varied significantly (P>0.05) between soxhlet and cold pressing method. The present results for crude fiber were quite comparable (2.99 g/100g) whereas for protein and ash content were lower (24.9 and 4.8g/100g, respectively) than the results reported earlier for sunflower seeds by Ingale and Shrivastava [19].

Table 1. Proximate composition of sunflower seeds extracted by two methods

Parameter (%)	Soxhelt extraction	Cold pressing
Oil content	42.5 ± 1.5^{d}	21.4 ± 1.2^{a}
Protein content	16.7 ± 0.6	16.5 ± 1.0
Fiber content	2.9 ± 0.2	2.9 ± 0.1
Ash content	2.6 ± 0.1	2.5± 0.10

Values are mean ± SD of triplicate measurements. Means with different superscript letters (if any) show significant ($p < 0.05$) difference.

Table 2. Comparison of physic-chemical properties of Soxhelt extracted and cold pressed sunflower seed oils

Parameter	Soxhlet extracted	Cold pressed
Refractive Index (40 °C)	1.4652 ± 0.002	1.4679 ± 0.003
Density (mg/mL)	0.90 ± 0.03	0.89 ± 0.04
Saponification value (mgKOH/g of oil)	190 ± 3	186 ± 4
Free fatty acid contents (% as oleic acid)	0.92 ± 0.08	0.75 ± 0.02
Iodine value (g of I/ 100g of oil)	126.2 ± 3.5	133.7 ± 2.9
saponifiable matter (%w/w)	0.53 ± 0.04	0.45 ± 0.02
Color (1-in. cell)		
Red units	1.9 R ± 0.4	2.7 R ± 0.2
Yellow units	19.5 Y ± 0.03	25.6 Y ± 0.7

Values are means ± SD of triplicate determinations

Table 3: Comparison of oxidative state of Soxhelt extracted and cold pressed sunflower seed oils

Parameter	Soxhlet extracted	Cold pressed
Conjugated diene $\varepsilon^{1\%}_{1cm}$ (λ232)	3.22 ± 0.09	3.06 ± 0.09
Conjugated triene $\varepsilon^{1\%}_{1cm}$ (λ270)	0.77 ± 0.04	0.63 ± 0.01
Peroxide value (millieq/kg)	1.85 ± 0.06	1.42 ± 0.11
para-anisidine	1.90 ± 0.05	1.67 ± 0.07

Values are means ± SD of triplicate determinations

Table 4: Comparison of tocopherols of Soxhelt extracted and cold pressed sunflower seed oils

Tocopherols (mg kg^{-1})	Soxhelt extracted	Cold pressed
α	525± 17	570 ± 22.0
δ	170 ± 9	210 ± 11.0
γ	2.2 ± 0.2	3.8 ± 0.4
Total	697.2	783.8

Values are means ± SD of triplicate determinations

Table 5. Comparison of fatty acid (FA) composition (g/100g FA) of Soxhelt- extracted and cold pressed sunflower seed oil

FA	Soxhelt extracted	Cold pressed
$C_{16:0}$	7.93± 0.13	9.86 ± 0.11
$C_{18:0}$	4.22 ± 0.07	4.07 ± 0.06
$C_{18:1}$	25.05 ± 0.43	22.68± 0.45
$C_{18:2}$	61.26 ± 1.19	60.12 ± 1.87
$C_{20:0}$	1.28 ± 0.07	ND

Values are mean ± SD of triplicate determinations

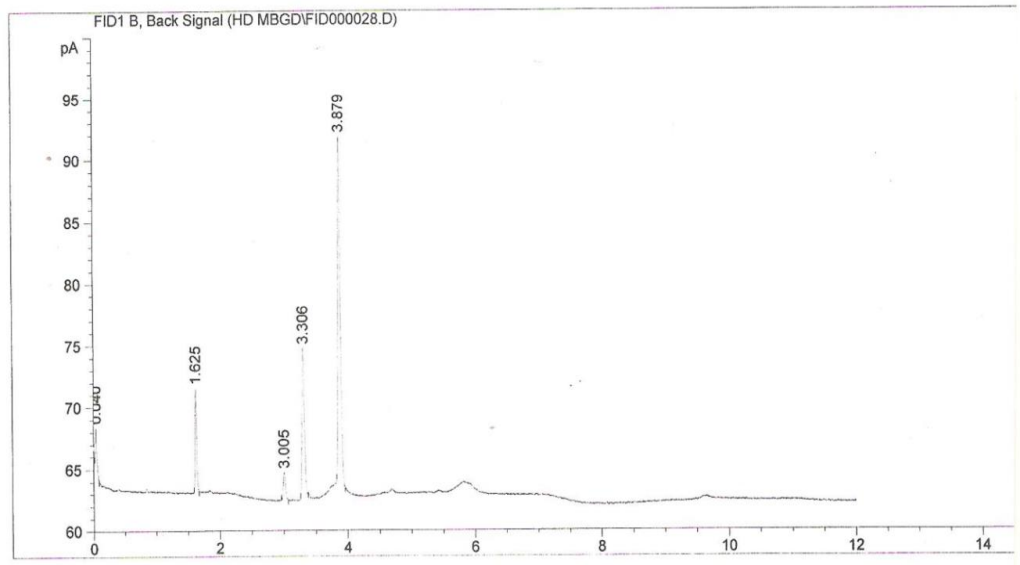

Fig 1. Typical GLC chromatogram showing the separation of FA of cold pressed sunflower seed oil.

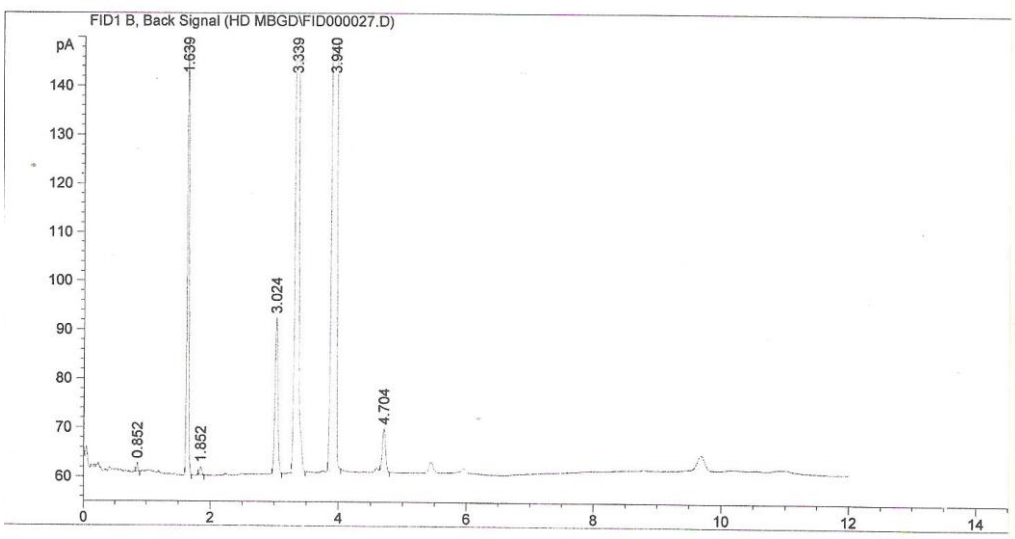

Fig. 2. Typical GLC chromatogram showing the separation of FA of soxhlet extracted sunflower seed oil.

3.2. Physico-chemical characterization of sunflower oil

The results for physico-chemical characteristics of cold pressed and Soxhlet extracted sunflower oil are given in Table 2. There was no significant (p>0.05) difference observed for refractive index, density, free fatty acid contents and unsaponifiable matter of the oil extracted by two methods. The saponification value (190 mg KOH/g of oil) of Soxhelt method oil was significantly higher (p<0.05) than those of cold pressed oil (186mg KOH/g of oil) whereas iodine value (126g of I_2/100g of oil), red unit (1.9 r) and yellow unit (19.5y) of Soxhelt method oil were significantly lower than those of iodine value (133.7 g of I_2/100g of oil), red unit (2.7r) and yellow unit (25.6y) of cold pressed oil. The present results for physic-chemical attributes of sunflower oil tested are in line to those reported in the literature for this oil (Latif and Anwar [18], Madhavi *et al*. [20], and Galucio *et al*. [16].

3.3. Oxidative state

The data for oxidative state of sunflower oil produced by cold pressing and Soxhlet method is presented in Table 3. The oxidative state of cold pressed sunflower oil was relatively good. The specific exterminations at 232 nm and 270 nm, which reveal the oxidative deterioration of sunflower seed oil was 3.22 and 0.77 in case of Soxhelt produced oil while 3.06 and 0.63, respectively for cold pressed oil. The *p*-anisidine value, which indicates the occurrence of secondary oxidation namely aldehydic products in Soxhelt extracted sunflower oil (1.90) was higher than that oil extracted by cold pressing (1.67) indicating improved oxidation state of the later. The values of *p*-anisidine, conjugated diene $\varepsilon^{1\%}_{1cm}$ (λ232) and conjugated triene $\varepsilon^{1\%}_{1cm}$ (λ270) as determined in the present analysis of sunflower oil were comparable to those reported values (1.903) and (3.28 and 0.82), respectively for this oil by Latif and Anwar [18].

Peroxide value is one of the widely used indicators for measurement of magnitude of primary oxidation products of oil. Actually, peroxide value, which is expressed in terms of millieq per kg of oil, is a measure of concentration of peroxides and hydroperoxides. As shown in Table 3, the Soxhelt extracted oil has higher peroxide value (1.85 millieq/kg) than the cold pressed oil (1.42 millieq/kg) revealing that the later method is better for yielding good quality oil due to mild operational conditions. The peroxide value of sunflower seed oil in the present analysis is relatively higher than the reported value (0.66 millieq/kg) by Galucio *et al*. [16].

3.4. Tocopherol contents

The tocopherols (α, γ and δ-tocopherols) content of sunflower oil produced by two methods as analysed by HPLC is depicted in Table 4. The result showed that cold pressed sunflower oil has significantly higher level of α, γ and δ tocopherols (570, 210 and 3.8 mg kg^{-1}) as compared to Soxhelt extracted sunflower oil (525, 170 and 2.2 $mgkg^{-1}$), respectively. A high amount of α tocopherol (average 547.5 $mgkg^{-1}$) detected in the present analysis of sunflower oils supports high vitamin E potency of this oil.

3.5. Fatty acid composition

The data pertaining to fatty acid composition ($C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{20:0}$) of sunflower seed oil determined by GLC is given in Table 5. Separation of fatty acids can be seen by GLC chromatograms (Figs. 1-2). Overall, there was no significant difference observed for the fatty acid composition of the sunflower oil obtained by two extraction methods. Little difference in the fatty acids composition was noted for palmitic, oleic and arachidic acids of oils tested in relation to cold pressing and Soxhelt extraction. The average content (60.69g/100g) of the major FA, i.e., 18:2, was considerably higher than the values in sunflower seed oils as reported by Galucio *et al*.[15] and Acko[1]. The concentration of $C_{16:0}$ $C_{18:2}$ and $C_{20:0}$ was notably higher but that of $C_{18:1}$ was lower than those reported by Galucio *et al*. [16] and Acko[1]. The contents of $C_{18:0}$ in the sunflower seed oils were lower (5.55 g/100g) than the values reported by Acko[1] but slightly higher (3.25g/100g) than that reported by Galucio *et al*. [16].

4. Conclusions

The findings of this study reveal that cold pressing of sunflower oil, although with lower oil yield, offered better quality oils in terms of oxidation state and tocopherols content and thus could be used for yielding such oils for functional food and nutraceutical applications.

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