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Physico-chemical Characteristics of Two Different Varieties of Sesame (*Sesamum indicum* L.) Seed Oil

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Sesame (*Sesamum indicum* L.) is an important oil seed crop. In the present research work, the physical and chemical characteristics of the oils extracted from indigenously available two varieties of sesame seeds are investigated. Proximate composition of sesame seeds (var. Pb-89 & TS-3) was as follow: oil content, 26.91, 30.48 %; moisture content, 17.30, 21.80 %; protein content, 31.00, 30.00 %; fiber content, 11.50, 10.00 % and ash content 5.50, 5.50 %, respectively. The extracted oils of the two varieties showed iodine value of 112,109; refractive index (40 °C), 1.4665, 1.4640; density (24 °C), 0.923,0.918; free fatty acid content, 0.45,0.51 %; saponification value, 183.0,178.8 mg of KOH /g of oil and unsaponifiable matter, 0.59,1.2 %, respectively. The oils analyzed exhibited good oxidative stability as evident from the determinations of peroxide value 2.20, 1.20; conjugated diene, 1.92, 0.91 and triene value, 0.95, 0.13, respectively. The level of α- and δ-tocopherol in the oils of sesame (var. PB-89 & Ts-3) were 54.0, 62.0 and 0.0, 159.0 mg/kg, respectively. The fatty acid composition of sesame oil was as follows: palmitic acid (C16:0) 10.05 and 9.72 %, stearic acid (C18:0) 5.37 and 4.33 %, oleic acid (C18:1ω-9) 41.29 and 36.22 %, linoleic acid (C18:2 ω-6) 41.77 and 48.07 %, respectively. The present results showed the oil from both the sesame seed varieties varied significantly with regard to physico-chemical attributes.

Key Words: Sesame Seeds, Physico-chemical parameters, Fatty acids, Oxidative stability, Tocopherols.

INTRODUCTION

Sesame (*Sesamum indicum* L.), a member of the family Pedaliaceae, is one of the most ancient oilseeds crop known to mankind. It is mostly grown around the world in the zones extending from 35°N to 25°S latitude. India, Sudan, China and Burma are considered as the major sesame producers of the world with contribution of 60 % in the total production[1,2].

Sesame (*Sesamum indicum* L.) has long been used as a traditional food in eastern countries. The sesame seeds are not only a source of edible oil, but also widely used in baked goods and confectionery products[3,4]. The seeds are also used in paste (tehineh) and food formulations such as Halaweh (sweetened tehineh), Java beans and salads[1,5]. Roasting sesame seeds and pressing these seeds without further refining are common process for production of sesame oil in Asia[6,7].

Studies have shown health-promoting effect of sesame seed and its oil[2]. Sesame seed consumption appears to increase plasma γ-tocopherol and enhanced vitamin E activity, which are believed to prevent cancer and heart disease[8]. It was noted that sesame oil is highly stable to oxidation compared with other plant oils[9]. The main sesame lignans, namely sesamin and sesamolin, which are found in sesame oil, do not possess as such any considerable antioxidative activity[10]. During sesame oil manufacturing, however, sesamilin can be converted to other lignans, such as sesamol, sesaminol and sesamol dimer[11]. These components are believed to play an important role in the oxidative stability of sesame oil[2]. In addition, sesame oil contains large amounts of linoleate in triglyceride form, which has beneficial role in maintaining cardiovascular health[12].

In Pakistan sesame is widely cultivated, especially, in the province Punjab[13]. Analytical characterization of oils or fats is important from standpoint of their nutritional or industrial uses. It is well recognized that the physico-chemical characteristics of an oil or fat determination its potential uses. The subject varieties of sesame oilseed gown under local environment have not yet been fully characterized for physico-chemical attributes. So the present research work is undertaken with the main objective to investigate the composition and characteristics of oils produced from two local varieties of sesame (*Semamum indicum* L.) seeds.

EXPERIMENTAL

Sesame (*Sesamum indicum* L.) seeds were procured from Ayub Agricultural Research Institute (AARI), Jhang Road, Faisalabad, Pakistan. The seeds were further identified and

authenticated from the Department of Botany, University of Agriculture, Faisalabad, Pakistan.

All reagents used were from Merck (Darmstadt, Germany) or Sigma Aldrich (Buchs, Switzerland). Pure standards of tocopherols [DL-α-tocopherol, (+)-γ-tocopherol, (+)-δ-tocopherol] and fatty acid methyl esters (FAMEs) standards were obtained from Sigma Chemical Co. (St. Louis, MO).

Extraction of oils: Samples of dried sesame seeds were crushed using a commercial blender (TSK-949, Westpoint, France). 100 g of well crushed seeds (80 meshes) sample of each variety of sesame were fed into a Soxhlet extractor fitted with a 1 L round bottomed flask and a condenser. The extraction was executed on a water bath for 6 h with 0.50 L of *n*-hexane. The solvent was distilled off under vacuum using a rotary evaporator (EYELA, N-N Series; Rikakikai Co Ltd., Tokyo, Japan).

Analysis of oilseed residues: The oilseed residues (meals), left after the extraction of oil from the seeds, were analyzed for protein, fiber and ash contents. Protein content (N × 6.25) was determined according to the Association of Official Analytical Chemists AOAC standard method 976.06[14] using a Kjeldahl apparatus.

The fiber content was determined according to the ISO method 5983[15]. Briefly, the meal sample (2.5 g) was freed from fat by extracting it with *n*-hexane. Then the test portion was boiled with sulfuric acid solution (0.255 mol/L), followed by separation and washing of the insoluble residue. The residue was then boiled with sodium hydroxide (0.313 mol/L), followed by separation, washing and drying. The dried residue was weighed and ashed in a muffle furnace (EYELA, TMF-2100, Tokyo, Japan) at 600 ºC. The loss in mass was calculated.

The contents of ash were determined according to ISO method 749[16]. Two grams of the test portion was weighed and carbonized by heating on a gas flame. The carbonized material was then ashed in an electric muffle furnace (EYELA, TMF-2100, Tokyo, Japan) at 600 ºC, until constant mass was achieved.

Physical and chemical parameters of oils: Determinations of density, refractive index, iodine value, peroxide value, acidity, saponification value and unsaponifiable matter of the extracted oil were made following 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[17]. The colour of the tested oil was determined by a Lovibond Tintometer (Tintometer Ltd., Salisbury, Wiltshire, United Kingdom), using a 1-in. cell. For the determination of specific extinctions, the oil samples were diluted with isooctane to and absorbance values at 232 and 270 nm were recorded using a spectrophotometer (U-2001; Hitachi, Instruments Inc., Tokyo, Japan). Specific extinctions ($^{1\%}\varepsilon_{1\ cm\ (\lambda)}$) were then calculated following an IUPAC method II D.23[18]. The *p*-anisidine value was determined following an IUPAC method II. D. 26[18]. The oil samples dissolved in isooctane were allowed to react with *p*-anisidine reagent for 10 min. On completion of the reaction a coloured complex was produced; the absorbance of which was recorded at 350 nm, using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan).

Fatty acid composition: The oil samples were derivatized into fatty acid methyl esters (FAMEs) following IUPAC standard method 2.301[18]. The analysis of FAMEs was carried out by Shimadzu gas chromatograph (model 17-A), fitted with SP-2330 (Supelco, Inc., Supelco Park, Bellefonte, PA, 16823-0048 USA) methyl-lignocerate-coated (film thickness 0.20 μm), polar capillary column (30 m × 0.32 mm) and an FID. Oxygen-free nitrogen gas at a flow rate of 3.5 mL min^{-1} was used as mobile phase. The column oven temperature was programmed from 180 to 220 °C at the ramp rate of 5 °C/min. The column initial and final temperatures were held for 2 and 10 min, respectively. Injector and detector temperatures were set at 230 and 250 °C, respectively. The identification of the FAMEs was based upon matching the relative and absolute retention times of unknowns with those of with those of authentic standards. All of the quantification was done by a built in chromatography station for Windows (CSW32) data-handling program (Data APEX Ltd., Pague 5, The Czech Republic).

Tocopherols content: Analysis of the tocopherols (α, γ and δ) was carried out by HPLC following the Current Protocols in Food Analytical Chemistry method[19]. Oil sample (0.1 g) and 0.05 g ascorbic acid were placed in a test tube. Five mL of 90 % ethanol and 0.5 mL of 80 % aqueous KOH solution were added to the test tube and vortexed for 30 s. The test tube was flushed with nitrogen, caped and incubated in a water bath (70 ºC) for 0.5 h with periodical vortexing. The tube was cooled down by placing in an ice bath for 5 min, then 3 mL deionized water and 5 mL *n*-hexane were added sequentially and vortexed for 30 s. The contents of the test tube were then centrifuged at 1,000 × g for 10 min at room temperature. The upper hexane layer was transferred to another test tube. The aqueous layer and the residue were re-extracted as described earlier. The upper hexane layers from both the extractions were pooled and evaporated to dryness under reduced pressure. Finally, 1 mL of the mobile phase was added to the tube and vortexed for 30 s to re-dissolve the extract and then transferred to an HPLC sample vial. A 20 μL sample was injected into a Supelcosil LCSi column (250 mm × 4.6 mm, Supelco Inc.). A mobile phase of ethyl acetate/acetic acid/hexane (1:1:98, v/v/v) was used at the flow rate of 1.5 mL min^{-1}. Detection was performed at 295 nm. The tocopherols were identified by comparing their retention times with those of pure standards of α and δ-tocopherols and were quantified on the basis of peak areas of the pure standards (Sigma Chemical Co.). Quantification was based on an external standard method. A D-2500 Hitachi Chromatointegrator model with a built-in computer program for data handling was used for quantification.

Statistical analysis: All the measurements were made in triplicate and the data statistically analyzed by analysis of variance (ANOVA)[20]. The values are reported as mean ± SD.

RESULTS AND DISCUSSION

Proximate composition of sesame seeds: Proximate composition of sesame seeds involved the determinations of oil, ash, fiber, protein and moisture contents of the samples. The results of proximate composition of two different varieties

of sesame seed (Pb-89 and TS-3) are depicted in Table-1. The hexane-extracted oil contents of sesame seeds were ranged from 26.91-30.48 %. The highest (30.48 %) yield of oil was exhibited by sesame var.TS-3, whereas lowest (26.91 %) by variety Pb-89. Significant variation ($p < 0.05$) was observed in oil and moisture contents between the tested varieties of sesame seeds while ash, protein and fiber contents showed non-significant ($p > 0.05$) differences. The variation in oil contents of sesame seeds may be the result of varied genetic makeup of the varieties as well as to the differences of agro climatic conditions employed for growing. The present oil contents in the tested sesame seeds was quite lower than that reported in the literature (40-60 %)[21]. The analysis of oilseed residues (Table-1) revealed that seeds of both of the tested sesame varieties are a good source of protein (30-31 %). The seeds also exhibited considerable amounts of fiber (10-11.5 %).

TABLE-1
PROXIMATE COMPOSITION OF SESAEM SEEDS

Constituents (%)	Variety	
	Pb-89	TS-3
Oil	$26.91^{b} \pm 0.53$	$30.48^{a} \pm 0.30$
Ash	5.50 ± 0.11	5.50 ± 0.11
Protein	31.00 ± 0.62	30.00 ± 0.90
Moisture	$17.3^{b} \pm 0.51$	$21.8^{a} \pm 0.21$
Fiber	11.50 ± 0.30	10.00 ± 0.23

Values are mean ± SD of triplicate determinations; Different alphabets in superscript within the same row indicate significant differences ($p < 0.05$) between tested varieties of sesame seeds.

Physico-chemical characteristics of oils: The results of various physico-chemical parameters of the extracted oils from two varieties (Pb-89 and TS-3) of sesame seeds are presented in Table-2. The sesame seed oil investigated exhibited non-significant ($p > 0.05$) variation between the varieties with regard to the value of refractive index (40 ºC) and density (24 ºC), which were 1.4665, 1.4670 and 0.902, 0.935 mg/mL, respectively. The present refractive index and density values were quite comparable to those investigated (1.4650-1.4690 and 0.874-0.954 mg/mL, respectively) for melon seeds[22]. Among the tested varieties, Pb-89 showed the lowest value of density 0.902 and highest of refractive index (1.4670), while TS-3 having the highest density (0.932) showed the lowest value of refractive index (1.4650). Pure oils have characteristic range of refractive index and density. Thus the degree of variation of typical oil from true value of refractive index and density may be considered as supportive for determination of its relative purity.

The colour of the investigated sesame seed oils (1.60-1.35 red and 23.0-22.0 yellow units) showed significant ($p < 0.05$) variation within the varieties analyzed. The colour measurements revealed that these oils can be employed for edible applications after slight bleaching. Colour development in vegetable oils is mostly due to the presence of different types of pigments such as chlorophyll and carotenoids which are extracted alongwith the oil during extraction. Such colourings pigments have to be effectively removed during refining and bleaching process of oils. The vegetable oils with minimum colour intensity are useful for edible and industrial uses.

TABLE-2
PHYSICOCHEMICAL CHARACTERISTICS OF SESAEM SEED OILS

Parameters	Variety	
	Pb-89	TS-3
Free fatty acid (% as oleic acid)	0.45^{a}±0.090	0.51^{b}±0.050
Iodine value (g of I/100 g of oil)	112.0±1.100	109.0±2.100
Density (24 ºC, mg/mL)	0.923±0.027	0.918±0.018
Refractive index (40 ºC)	1.4665±0.014	1.4640±0.029
Saponification value (mg of KOH/g of oil)	183.0^{a}±1.800	178.8^{b}±2.000
Unsaponifaiable mater (%)	0.59^{b}±0.010	1.20^{a}±0.010
Color (yellow unit)	23.0±2.200	22.0±2.000
Color (red unit)	1.60^{a}±0.140	1.35^{b}±0.950

Values are mean ± SD of triplicate determinations; Different alphabets in superscript within the same row indicate significant differences ($p < 0.05$) between tested varieties of sesame seed oils.

The free fatty acids value for the analyzed oils of sesame seed varieties (Pb-89 and TS-3) was found to be 0.45 and 0.50 % (% as oleic acid), respectively. Free fatty acids are the measure of extent to which hydrolysis has liberated fatty acids from ester linkage of their parent molecule. The free fatty acid values as determined in our present analysis were found to be in good agreement with that given in the literature[23].

The saponification number (mg of KOH/g of oil) determined in the present analysis of sesame oil Var. Pb-89 was 183.0 while 178.8 for Var. TS-3 indicating significant variation ($p < 0.05$) between the varieties tested. These values were found to be higher than those reported (0.51- 0.85 %) for pumpkin and melon seeds[24]. The contribution of unsaponifiable matter of the investigated varieties Pb-89 and TS-3 of sesame seed oil was 0.59 and 1.20 %, respectively showing significant variation ($p < 0.05$) (Table-2). These values were in good agreement to those of *Moringa oleifera* oil[25] and sesame oil reported in the literature[23].

The iodine value of the oils derived from both the investigated varieties of sesame seed was ranged from 109 g/100 g of oil to 112 g/100 g of oil (Table-2) showing no considerable variation ($p > 0.05$). The present iodine values were in agreement to those reported for sesame seed oil in the literature[23]. It is well recognized that iodine value is the measure of the degree of unsaturation of the fatty acids present in oil, principally oleic and linoleic acids. High iodine value means that oil is more unsaturated and could be used for edible purposes. This value is helpful in determining the quality of oil, whether as drying, semi-drying or non-drying oils. Iodine value is also related to the melting point or hardness of an oil or fat. Iodine value decreases with the aging of oil due to oxidative deterioration of unsaturated fatty acids.

Oxidative stability of sesame seed oils: Seed oils from the tested sesame varieties showed very good oxidative stability as is depicted by the determinations in Table-3. The specific extinction at 232 and 270 nm, which revealed the oxidative deterioration and purity of oils[26] of sesame seeds (var.Pb-89 and TS-3) oils, were 1.92, 0.19 and 0.95, 0.13, respectively. The present results revealed the values of conjugated diene and triene to be varied significantly ($p < 0.05$) between the tested varieties of sesame seeds oils. The peroxide value is a measure of those substances in an oil or fat, expressed in terms

TABLE-3
OXIDATIVE STABILITY OF SESAME SEED OILS

Determination	Variety	
	Pb-89	TS-3
Conjugated diene $\varepsilon^{1\%}_{1cm}$ (λ_{232})	$1.92^{a} \pm 0.10$	$0.91^{b} \pm 0.13$
Conjugated triene $\varepsilon^{1\%}_{1cm}$ (λ_{268})	$0.95^{a} \pm 0.10$	$0.13^{b} \pm 0.01$
Peroxide value (meq/kg of oil)	$2.20^{a} \pm 0.20$	$1.20^{b} \pm 0.10$
para-Anisidine value	$0.50^{a} \pm 0.02$	$0.41^{b} \pm 0.02$

Values are mean ± SD of triplicate determinations; Different alphabets in superscript within the same row indicate significant differences ($p < 0.05$) between tested varieties of sesame seed oils.

of milli equivalents of active oxygen per kilograms of sample, which oxidize potassium iodide under conditions of test. It measures the magnitude of primary oxidation products of an oil or fat. Peroxide values of sesame seed oils var. Pb-89 and TS-3 were 2.20 and 1.20 meq/kg, respectively, revealing the oil from latter variety to be very stable. The present peroxide values varied significantly ($p < 0.05$) between the varieties analyzed. The *p*-anisidine values of the tested sesame seed oils were quite low < 0.50 indicating high resistance to secondary oxidation of these oils. *p*-Anisidine value is a measure of the α and β-aldehydic secondary oxidation products (principally 2-alkenals) in oils[27]. Rancid and off-flavours in an oil or fat are generally originated due to the presence of such aldehydes of short or medium chain.

Tocopherol contents of sesame seed oils: The data for the quantification of tocopherols (α and δ) of sesame seed oils is presented in Table-4. The level of α-tocopherol in sesame oil of variety Pb-89 was found to be 54.0 mg/kg. The other sesame seed oil (variety TS-3) was devoid of this tocopherol isomer. The concentration of δ-tocopherol in the sesame seed oil of variety Pb-89 and TS-3 was determined to be 62.0 and 159.0 mg/kg of oil, respectively. Significant variations ($p < 0.05$) were observed for tocopherols contents between two varieties of sesame seed oils tested.

TABLE-4
TOCOPHEROLS CONTENTS (mg/kg OF OIL) OF SESAEM SEED OILS

Tocopherols	Variety	
	Pb-89	TS-3
α-Tocopherol	$54.00^{a} \pm 3.20$	Not detected^{b}
δ-Tocopherol	$62.00^{b} \pm 1.24$	$159.00^{a} \pm 2.59$

Values are mean ± SD of triplicate determinations; Different alphabets in superscript within the same row indicate significant differences ($p < 0.05$) between tested varieties of sesame seed oils.

The level of α-tocopherol, which exhibits greater vitamin E potency[23], in the present analysis of sesame (variety Pb-89) seed oil was noted to be lower than those reported for soybean (99 mg/kg), palm (89 mg/kg), groundnut (178 mg/kg), rapeseed (202 mg/kg), cotton seeds (338 mg/kg) and maize (282 mg/kg) seed oils[23]. The concentration of δ-tocopherol which has the strongest antioxidant potency than either of the α- or γ-tocopherols, in sesame seed oils was higher than those of sunflower (0.6), cotton seeds (3.3), groundnut (7.6), maize (54) and low erucic acid rapeseed (9.0) oils but was lower than that of soybean (421) oil[23].

Fatty acids composition: The fatty acid composition of two varieties of sesame seed oils as determined by GLC is depicted in Table-5. The sesame oils contained fatty acid with carbon chain ranging from C16-C22. Medium chain saturated fatty acids such as lauric acid (C12:0) and myristic acid (C14:0) were not present in these oils while the percentage of palmitic acid (C16:0) in sesame seed oil of variety Pb-89 and TS-3 was 10.05 and 9.72 %, respectively. The contents of the stearic acid (C18:0), a saturated acid of octadecanoic fatty acid family, was found to be 5.37 and 4.33 %, respectively. Significant variations ($p < 0.05$) were observed for most of the fatty acids contents between two varieties of sesame seed oils. It is widely accepted that both of these saturated fatty acids are negatively linked with cardiovascular diseases[22]. The concentration of oleic acid (a common monounsaturated fatty acids fatty acid present in most of the vegetable oils) was 41.29 and 36.22 %, respectively. Oleic acid (C18:1, ω-9) is currently gaining importance in view of its cholesterol lowering effects. The amounts of an essential fatty acid *i.e.* linoleic acid (C18:2 ω-6) with potential health benefits was found to be 41.70 and 48.07 %, respectively showing no considerable variation between the varieties tested. Small concentration of minor fatty acids such as C18:3 (linolenic acid), C20:0 (arachidic acid) and C22:0 (behenic acid) with contribution less than 1.5 % were also established.

TABLE-5
FATTY ACID COMPOSITION (g per 100 g OF FATTY ACIDS) OF SESAME SEED OILS

Fatty acids	Variety		
	Retention time	Pb-89	TS-3
C16:0	1.34	10.05 ± 0.50	9.72 ± 0.58
C18:0	1.79	5.37 ± 0.10	4.33 ± 0.17
C18:1	1.89	$41.29^{a} \pm 0.41$	$36.22^{b} \pm 1.44$
C18:2	2.07	$41.77^{b} \pm 1.50$	$48.07^{a} \pm 0.96$
C18:3	2.31	$0.38^{b} \pm 0.10$	$0.67^{a} \pm 0.10$
C20:0	2.70	$0.25^{b} \pm 0.10$	$0.39^{a} \pm 0.02$
C22:0	4.00	0.13 ± 0.02	0.13 ± 0.06

Values are mean ± SD of triplicate determinations; Different alphabets in superscript within the same row indicate significant differences ($p < 0.05$) between tested varieties of sesame seed oils.

Conclusion

It could be concluded from the present findings on sesame seeds that both the local varieties have appreciable potential for yield of good qiality oil. It is suggested that these varieties should be cultivated on large scale production under local agroclimatic regimes to benefit from their oil.

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