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Gas chromatographic coupled mass spectroscopic study of fatty acids composition of *Nigella sativa* L. (KALONJI) oil commercially available in Pakistan

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Abstract

The present study was conducted on physicochemical properties of oil extracted from Kalonji seeds (*Nigella Sativa* L.) and commercial oil samples available in Pakistan. The analyzed commercial oil samples were compared with the oil extracted from seeds samples for their fatty acid composition and some important physicochemical parameters. Gas chromatograph coupled with mass spectrometer detector (GC-MSD) was employed for the determination of fatty acid composition, while for the some physical and chemical analysis such as density, refractive index, iodine value, saponification value and unsaponifiable matter were carried out by AOCS official methods. Among the saturated fatty acids (SFA), palmitic and stearic acid were main fatty acids; the mean value of total SFA in commercial kalonji oil samples ranged at 16.22-31.65, where as among the unsaturated fatty acids (UFA) oleic and linoleic acid were major fatty acids. Mean values of UFA were ranged at 68.34-83.75. Fatty acid profiles and physicochemical parameters demonstrated that two commercial oil samples were adulterated with other source of oils. However results revealed that kalonji oil is a good source of essential fatty acid.

Introduction

Herbal products are increasingly used as alternatives to traditional chemical drugs. *Nigella sativa* L. plant belonging to the *Ranunculaceae* family native to south and southwest Asia, north Africa, and southern Europe has been used traditionally as an important medicinal plant and spice since ancient times (Padhye *et al*., 2008). In South Asia particularly Pakistan and India, it is cultivated as an annual herb and is commonly known as Kalonji (Randhawa and Al-Ghamdi, 2002), while in English it is called black cumin, black seed, Roman coriander, nutmeg flower or fennel flower (Weiss, 2002). The seeds are used as seasoning for vegetables, legumes and different types of baked products (Atta and Imaizumi, 1998). Several authors have investigated the essential oil of *Nigella* seeds and isolated and identified active constituents that have beneficial clinical effects (Karawya *et al.*, 1994). *N. sativa* oil or extract has protective and curative actions. The holy book Sahih al-Bukhari mentions kalonji as "a cure for every disease except death" (Butt and Sultan, 2010). The extracts of *N. sativa* have been used as a natural treatment for hypertension, asthma, inflammation, diabetes, eczema, bronchitis, fever, headache, dizziness, cough and influenza (Ali and Blunden, 2003; Ramadan, 2007).

The quality of fats plays a very important role in food processing technology (Kandhro *et al*., 2008). The *N. sativa* seeds contain valuable nutrients, such as fixed and volatile oils besides protein, ash, minerals, essential amino acids and some vitamins (Takruri *et al.*, 1998). *N sativa* oil is a rich source of linoleic fatty acid (ω6) which has the ability to boost human immune system significantly. The present work makes special data and comparison on the characteristics of kalonji seed oil and commercially available oils in Pakistan.

Method and Material

Samples and reagents

Kalonji seed and oil samples were purchased from local supermarkets of Hyderabad, Pakistan. The choice of the commercial oil brands were based on the

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highest availability/vending among those available in the market. All reagents, chemicals and solvents used were from E. Merck (Darmstadt, Germany).

Extraction of oil from Kalonji seeds

The oil content of the *N. sativa* seed was extracted by soxhlet extraction method by taking 100 g of ground seed with 500 ml of n-hexane for 6 h at 70°C. The fixed oil was pooled and concentrated in a rotary evaporator (Buchi Rotavapor-RE 111), and then placed in a vacuum oven at 105°C for 15 min; cooled in a desiccator and refrigerated until further analysis.

Physical and chemical parameters

Physical and chemical parameters such as density, refractive index, iodine value (Wij's), saponification value and unsaponifiable matter of the extracted and commercial oil samples were determined by standard AOCS methods (AOCS, 1997).

Determination of fatty acid composition

For the determination of fatty acids composition of the oil, fatty acid methyl esters (FAMEs) were prepared using standard method 2.301 (IUPAC, 1979). Agilent GC-MS was used with ChemStation Scale Mode software. GC-MS chromatogram obtained were compared with two libraries (NIST & Wily) which provide best information about the identification of fatty acid present in Kalonji oil samples to avoid the use of costly standards.

GC-MS conditions

The GC-MS analysis for FAMEs was performed on Agilent 6890 N gas chromatography instrument coupled with an Agilent MS-5975 inert XL mass selective detector and an Agilent autosampler 7683-B injector (Agilent Technologies, Little Fall, NY, USA). A capillary column HP-5MS (5% phenyl methylsiloxane) with dimension of 30 m × 0.25 mm i.d × 0.25 µm film thickness (Agilent Technologies, Palo Alto, CA, USA) was used for the separation of fatty acid methyl esters. The initial temperature of 150°C was maintained for 2 min raised to 230°C at the rate of 4°C/min, and kept at 230°C for 5 min. The split ratio was 1:50, and helium was used as a carrier gas with the flow rate of 0.8 ml/min. The injector and detector temperatures were 240 and 260°C, respectively. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV in the scan range of 50–550 m/z.

Calculations and statistical analyses

Peak identification of the fatty acids in the analyzed Kalonji samples was carried out by the comparison with retention times and mass spectra used for the confirmation of GC-MS libraries result. All analytical determinations were performed at least in triplicate and reported as mean (n = 2×3).

Results and Discussion

The results of fatty acid composition of analyzed commercial Kalonji oil samples are given in Table 1. The Kalonji oil brands were coded as CKO-1, CKO-2, CKO-3, CKO-4 and CKO-5, where as the oil extracted from Kalonji seeds were coded as EKO. All analyzed Kalonji oil samples (extracted and commercial) contained seven saturated fatty acids and five unsaturated fatty acids including (C14:0, C15:0, C16:0, C17:0, C18:0, C20:0 and C24:0) and (C16:1, C18:1, C18:2, C20:1 and C20:2), respectively. Saturated fatty acids with the chain length of (C12:0–C16:0) carbon atoms have been reported to be atherogenic, stearic acid has found neutral effects, while oleic and polyunsaturated fatty acids produced a blood lipid lowering effect (Aro *et al.*, 1997; Hu *et al*., 1999). Among the saturated fatty acids, palmitic acid (C16:0) was the main fatty acid ranging from 12.17 - 20.54%, the highest amount of palmitic acid was found in CKO-3, while smaller in CKO-1. Stearic acid (C18:0) was present at 3.68–3.99%, the highest amount of stearic acid was found in EKO, while lowest in CKO-4. Meanwhile, myristic acid (C14:0) at 0.12–3.40%. Some odd number fatty acids like pentadecanoic acid (C15:0) at 0.50% and margaric acid (C17:0), 0.23% were also determined in considerable amounts in CKO-3.

The other members of saturated fatty acids such as arachidic acid (C20:0) was also determined in considerable amount ranging from 0.18-0.25%, while lignoceric acid (C24:0) was found only in one sample CKO-4 (0.29%). The dominant fatty acid among the unsaturated group including monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) of Kalonji oil was linoleic acid (C18:2) and its range varied from 37.11 to 52.34%, the highest amount of linoleic acid was found in CKO-1, while lowest in CKO-3. Among the MUFA, oleic acid (C18:1) was ranged from 27.8-29.1%, the highest and lowest amount were found in CKO-2 and CKO-4, respectively. Oleic acid is considered to be responsible for lowering the LDL (bad) cholesterol levels. Whereas polyunsaturated fatty acids have beneficial effects on both normal health and chronic diseases, such as regulation of lipid levels (Mori *et al*., 2000) cardiovascular (Kris-Etherton and Appel, 2002) and immuno functions (Hwang, 2000). The other

Table 1. Saturated and unsaturated fatty acids composition (mean percentage FAMEs) of Kalongi oil samples

Samples	EKO	CKO-1	CKO-2	CKO-3	CKO-4	CKO-5
C14:0	0.12±0.004	0.12±0.003		3.40±0.12	0.33±0.005	0.18±0.004
C15:0				0.50±0.02		
C16:0	12.68±0.5	12.17±0.25	13.40±0.5	20.54±0.58	16.97±0.45	12.64±0.45
C17:0				0.23±0.01		
C18:0	3.99±0.15	3.74±0.15	3.78±0.15	6.73±0.25	3.68±0.15	3.82±0.08
C16:1n9cis	0.12±0.005	0.32±0.01		0.42±0.02		
C18:1n9cis	28.55±0.75	28.12±0.75	29.1±0.75	28.44±1.05	27.8±1.05	28.7±1.35
C18:2n9, 12cis-cis	51.80±1.45	52.34±1.54	51.14±1.25	37.11±1.25	47.72±1.25	51.95±2.15
C:20:0	0.21±0.003	0.19±0.005	0.18±0.005	0.25±0.01	0.19±0.005	0.18±0.005
C20:1 n11	0.48±0.01	0.51±0.02	0.50±0.02	0.73±0.02	0.59±0.02	0.54±0.02
C20:2n11, 13	2.05±0.05	2.46±0.12	1.90±0.05	1.64±0.05	2.44±0.06	1.97±0.05
C24:0					0.29±0.01	

Table 2. Groups and ratio between the types of fatty acids from the composition of kalongi oil samples

Samples (%)	EKO	CKO-1	CKO-2	CKO-3	CKO-4	CKO-5
Total SFA	17.00	16.22	17.36	31.65	21.46	16.82
Total	83.00	83.75	82.64	68.34	78.55	83.16
Total MUFA	31.20	31.41	31.50	31.23	30.83	31.21
Total PUFA	53.85	54.80	53.04	38.75	50.16	53.92
SFA/UFA	0.20	0.19	0.21	0.46	0.27	0.20
Cis-MUFA+cis-PUFA	85.05	86.21	84.54	69.98	80.96	85.13
Cis-PUFA/SFA	3.17	3.38	3.06	1.22	2.34	3.21

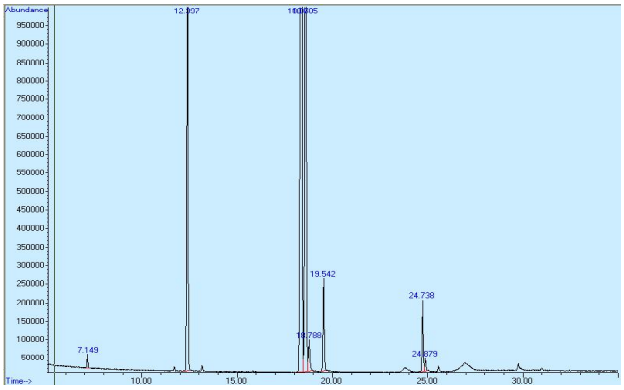

Figure 1. Representative chromatograph of fatty acids of CKO-1 analysed by GC-MS

members of MUFA such as palmitoleic acid C16:1 and eicosenoic acid (C20:1) were also determined in the range of 0.12–0.42% and 0.48–0.73% in EKO and CKO-3, respectively. Where as among the PUFA, eicosadienoic acid (C20:2) was also determined in considerable amount ranging from 1.64-2.46% in CKO-3 and CKO-1.

The fatty acid composition of three commercial Kalonji oil samples CKO-1, CKO-2 and CKO-5 significantly similar with extracted sample EKO. Whereas two samples CKO-3 and CKO-4 showed different fatty acids composition. In CKO-3 some unusual fatty acids (C15:0 and C17:0) were detected, naturally these both fatty acids are not very common in the vegetable oils, whereas the contents of these fatty acids present in the animal fats (Shoji and Masatoshi, 2005). Figure 1 shows the representative chromatogram of fatty acids. The analysed oil sample chromatogram was in a good peak shape under the optimized chromatographic conditions.

Table 2 represents the total fatty acid groups and ratios. The saturated fatty acids (SFA) were ranged from 16.22-31.65%, highest amount was found in CKO-3, while lowest in CKO-1. Total unsaturated

Table 3. Some important physical and chemical parameters of kaloni oils samples

Samples (%)	Oil Content (%)	Density at 20°C (g/ml)	Refractive Index at 40°C	Iodine Value (Wij's)	Free Fatty Acids (%)	Saponification Value (mg of KOH/g oil)	Unsaponifiable Matter (%)
EKO	36.2	0.9158±0.02	1.465±0.05	123.4±1.52	4.69±0.15	205.21±2.46	0.33±0.01
CKO-1	-	0.9157±0.02	1.462±0.03	121.9±1.56	16.05±0.65	204.15±2.54	0.45±0.01
CKO-2	-	0.9159±0.02	1.471±0.02	122.5±2.51	14.60±0.27	205.12±2.64	0.34±0.01
CKO-3	-	0.9152±0.02	1.462±0.05	110.6±2.41	16.10±0.23	195.92±2.55	0.51±0.01
CKO-4	-	0.9152±0.02	1.468±0.04	115.3±1.78	20.46±0.79	201.27±2.94	0.31±0.01
CKO-5	-	0.9153±0.02	1.468±0.03	122.1±2.31	9.79±0.21	206.12±2.87	0.65±0.01

fatty acids (UFA) was ranged from 68.34-83.75%, highest and lowest values were found in CKO-1 and CKO-3, respectively. From the total saturated and unsaturated fatty acids composition the two commercial kalonji samples (CKO-3 and CKO-4) showed dissimilar results with the composition of EKO sample which indicates that the these commercial kalonji oils may be adulterated with other oils. The saturated/unsaturated FA (SFA/UFA) shows the relation between two major fatty acid groups of the Kalonji oil. Its value varies from 0.19 to 0.46. These ratios indicate the dominancy of saturated fatty acid over unsaturated fatty acids. The prevalence of unsaturated over saturated fatty acids (smaller ratio) is considered to be positive from the nutritional point of view. All the samples contain smaller ratios, only one sample had a higher ratio of 0.46 (CKO-3), which clearly indicated a high proportion of saturated fatty acids. Furthermore, these results also indicate a great variation between extracted sample (EKO) and commercial samples (CKO-3 and CKO-4). The mean ratio of cis-PUFA/SFA recommended by the British Department of Health is 0.45 (Da Silva *et al.*, 2002). The ratio of cis-PUFA/SFA ranged from 1.22 to 3.38, which is less than the recommended value.

Table 3 shows some important physical and chemical characteristics of the extracted oil and commercial kalonji oils samples. The EKO (hexane extracted kalonji oil) sample contained 36.2% oil content. The density at 20°C for EKO was 0.9158 g/ml, where as the commercial samples ranged at 0.9152 to 0.9159, there is no any noticeable differences between the densities of extracted and commercial samples. The high relative densities could be an indication of high molecular weight and unsaturation as the density of an oil increases with increase in molecular weight and unsaturation (Onyeka *et al.*, 2005). The relative densities of kalonji oils are significant different with the reported vegetable oils (corn oil; 0.916 g/cm^3), (cottonseed oil; 0.914 g/cm^3), (olive oil; 0.909 g/cm^3), (rapeseed oil; 0.903–0.907 g/cm^3), (sunflower oil; 0.9178 g/cm^3), (soybean oil; 0.9148 g/cm^3) (Noureddini, 1992; Hui, 1996). The average values of refractive index for EKO (1.465 at 40°C) were comparable with commercial Kalonji oil samples ranging from 1.462-1.471. Oxidation or

polymerization reactions tend to lower the iodine value of vegetable oils (Eckey, 1954). These values indicate a high degree of unsaturation and therefore a high susceptibility to oxidative rancidity. The iodine value determined in EKO was 123.4, while for the commercial kalonji oils it ranged at 110.6-122.5.

Free fatty acid content is one of the main criteria for checking the quality of edible oil. Its value varied from 4.69 to 20.46%. The highest amount of FFA, 20.46%, was observed in CKO-4 and was lowest in CKO-5. The level of FFA indicates a higher level of oil hydrolysis and usually freshly processed edible oils contain less than 0.1% FFA. Mehran (1974) reported that prolonged storage of vegetable oils does not show significant change in saponification values. Saponification value of EKO oil was 205.21, whereas CKO-1, CKO-2, CKO-3, CKO-4 and CKO-5 were at 204.15, 205.12, 195.92, 201.27 and 206.12 mg of KOH/g oil, respectively. Unsaponifiable matter includes those substances frequently dissolved in fats and oils, which cannot be saponified by the caustic alkalies but are soluble in the ordinary fat solvents; these include higher aliphatic alcohols, sterols, pigments and hydrocarbon. Mostly refined, bleached and deodorized (RBD) fats and oils contains very low amount of unsaponifiable matter. The unsaponifiable matter for EKO was at 0.33%, while for CKO-1, CKO-2, CKO-3, CKO-4 and CKO-5 were at 0.45, 0.34, 0.51, 0.31 and 0.65%, respectively.

Conclusions

In conclusion, the data on the fatty acid composition and physicochemical properties of commercial Kalonji oil are lacking in the scientific literature. GC-MS was used for the fatty acid composition to achieve more accurate peak identification. The results of this study showed that the kalonji oil is rich in PUFA. No significant differences observed in the fatty acid composition of commercial oil samples with extracted seed oil, except two samples. This variation may be due to the adulteration with other cheaper oils. All the oils indicated desirable quality apart from FFA which was higher in all commercial samples.

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