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Full Length Research Paper

Isolation and characterization of edible oil from wild olive

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We report for the first time new edible oil extracted and quantified from fruit samples of wild olive (*Olea cuspidata*) possibly use for human consumption. Fruits and oil of wild olive were assessed for physical and chemical properties, while saturated and unsaturated fatty acids were quantified using gas chromatography. Results indicate that fruits of wild olive contain moisture (42.1 ± 1.6 - 60.7 ± 2.6%), crude protein (0.5 ± 0 to 1.1 ± 0.01%), total oil (32.1 ± 1.1 to 38.6 ± 1.2%), fiber (2.6 ± 0.4 to 6.5 ± 0.3%), ash (1.7 ± 0.2 to 2.1 ± 0.4 %) and carbohydrate (0.5 ± 0.0 to 14.3 ± 0.7%). The refractive index of the oil (1.331 to 1.372), specific gravity (0.91 to 0.93), pH values (5.1 to 5.5), iodine value (75.2 ± 1.2 to 91.4 ± 1.5), peroxide value (14.2 ± 0.2 to 20.3 ± 0.8 mg/kg oil), saponification number (175.6 ± 1.2 to 187.3 ± 1.8 mg KOH /g), unsaponificable matter (12.6 ± 0.4 to 15.6 ± 0.8 g/kg), acid value (0.7 ± 0 to 1.3 ± 0 mEg/ kg and total phenol (23.6 ± 1.5 to 92.4 ± 2.1 mg/kg) were also determined. Concentration of fatty acids; oleic acid (69.3 to 74.5%), linoleic acid (1.3 to 3.2%), linolenic acid (11.2 to 15.2%) palmitoleic acid (1.10 to 2.10%), palmitic acid (11.2 to 14.0%) and stearic acid (0.1 to 0.2%) remained closed to those reported for the commercially available olive oil extracted from *Olea europea*. The new oil can be used as alternative to olive oil in human diet after toxicological studies.

Key words: Wild olive, edible oil, total fat, polyunsaturated fatty acids, total phenol.

INTRODUCTION

Olive belongs to family *oleaceae* which comprised of 30 genera with 600 species. The olive industry is chiefly dependent upon *Olea europea* L, where as in the Indian Sub-continent, wild olive (*Olea cuspidate* Wall), is widely distributed in north west Himalayas and adjoining hills. In Pakistan, *O. cuspidata* (locally known as khao) is found in the hilly areas of Rawalpindi, N.W.F.P and Azad Kashmir (Ginai, 1968). The tree is 10 - 50 feet tall with ever green leathery entire leaves and black fruit when ripe. Farmers in these areas believed that fruits of wild olive are rich in oil and use dried fruit to fatten their animals (Nisar et al., 2002).

Continued increase in demand of edible oils under increasing human population requires that alternative non conventional sources of oils including under exploited (Salunke et al., 1992; Omode et al., 1995) or alternative oil bearing seeds (Agbaji et al., 1993) may be searched for their potentials to produce edible oil which is more important for developing countries. There are hundreds of trees which may provide food for people but not explored fully as compared to some annual crops (Cannel, 1999).

The chemical composition of olive oil varies with growing conditions, locality and variety of olive. Virgin olive oil is unique among other vegetable oils having high level of unsaturated fatty acids and phenolic compounds (Sunga and Whitby, 1992; Visioli and Galli, 1998) including phenolic acid, phenolic alcohol and flavonoids (Tsimidou, 1999; Olivaria et al., 2005). It contains mostly unsaturated fatty acids such as oleic acid, linoleic acid and linolenic acid, etc. However, due to presence of 80% of oleic acid, olive oil is at the top of monounsaturated fat (Karleskind and Wolf, 1998; Murkovic et al., 2004).

Phenols also contribute to the characteristic taste and the high stability of olive oil against oxidation. The saponifiable material of olive oil made up of triglycerides, partial glycerides, esters of fatty acids with saturated fatty alcohols of linear chain, terpenic alcohols and free non-

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esterified fatty acids (Pitchard, 1991). The present study was conducted to analyze chemical composition of fruit and oil of wild olive (*Oliva cuspidata*).

MATERIALS AND METHODS

Plant materials

Samples (1000 g) of ripe fruits collected from three heavily fruit tree of *O.cuspidate* from five locations of Kotli Sattian (Rawalpindi, Pakistan) in 2006.

Sample preparation

Forty randomly selected fresh fruit from each sample were collected and average weight of fruits was calculated. The fruits were air dried and ground into a paste using cleaned and dried mortar and pestle and stored in an air tight container in a refrigerator (4°C) prior to use.

Proximate analysis

Proximate analysis of fruit samples consist of moisture, total oil, crude protein, crude fiber and ash contents (Duke and Atchley, 1984). Nitrogen content was estimated by the Kjeldhal method (AOAC, 1984) and level of crude protein was estimated by using factor (N x 6.25). Ash content and crude fiber contents were determined by using methods of AOAC (1990). Carbohydrates content was estimated by difference (Al-Khalifa, 1996). Total oil was extracted by using solvent extraction in which, 150 g of powdered sample was placed into a cellulose paper cone and extracted using light petroleum ether (b. p 40 - 60°C) in a 5 litre Soxhlet extractor for 8 h (AOAC, 1984). The oil was then recovered by evaporating off the solvent using rotary evaporator Model N-1 (Eyela, Tokyo Rikakikal Co., Ltd., Japan) and residual solvent was removed by drying in an oven at 60°C for 1 h and flushing with 99.9% nitrogen.

Physical properties

Parameters like pH, refractive index and colors of oil were assessed. The colors and state of oil at room temperature were observed by visual inspection, while density was determined by using method of AOAC (1980). pH was determined by using pH meter and refractive index of oil at room temperature was measured by using the Abbe refractometer (Pearson, 1982).

Chemical composition

Free fatty acids, iodine, peroxide and acid values, saponification number, unsaponifiable matter were analyzed according to methods of AOAC (1990). About 10 g of the oil was heated under reflush and saponified with 5 ml of ethanolic potassium hydroxide solution (20% w/v) for 3 h. The unsaponifiable matter was extracted three times with 15 ml of petroleum ether, and the extracts were combined and evaporated in a rotary evaporator at 40°C under reduced pressure and residue was weighed (Bastic et al., 1978). For peroxide value, about 5 g of wild olive oil was dissolved in a mixture of acetic acid/chloroform (3:2, v/v) and a saturated solution of KI (1 ml) was then added. The liberated iodine was titrated with sodium thiosulphate solution (0.01 M) in the presence of starch as indicator. Acid value was of oil sample was determined by dissolving 0.2 g of oil in 2.5 ml of 1:1 (v/v) ethanol : diethylether solvent and titrating with 0.1 N sodium hydroxide, with phenolphthalein used as indicator. For the free fatty acid, a known weight of olive oil was dissolved in a mixture of petroleum ether/ethanol (1:1, v/v). The mixture was titrated with potassium hydroxide in methanol (0.01 M) in the presence of phenolphthalein as indicator. For total phenol 50 g of oil was extracted three times with 200 ml of methanol (water: methanol; 60:40). The total phenol contents of oil extracts were measured by the Folin-Ciocalteu assay (Tsimidou et al., 2005). The measurement of absorbance was carried out at 765 nm using UV spectrophotometer (Shimadzu). Results were expressed as mg of gallic acid equivalent in one kg oil yields of wild olive (mg/kg).

Fatty acid analysis

The fatty acid composition was determined by conversion of oil to fatty acid methyl esters prepared by adding 950 µl of n-hexane 50 mg of oil followed by 50 µl of sodium methoxide using the method of Cocks and Van (1966). The mixtures were vortexed and allowed to settle for 5 min. The top layer (1 µl) was injected into a gas chromatograph (Shimadzu, Japan) equipped with a flame-ionization detector and a polar capillary column (BPX70 0.25), 0.32 mm internal diameter, 60 m length and 0.25 µm film thickness (SGE Incorporated, USA) to obtain individual peaks of fatty acid methyl esters. The detector temperature was 240°C and column temperature was 110°C held for one minute and increased at the rate of 8°C/min to 220°C and held for one minute. The run time was 32 min. The fatty acid methyl esters peaks were identified by comparing their retention time with those of standards. Percent relative fatty acid was calculated based on the peak area of a fatty acid to the total peak areas of all the fatty acids in the oil sample (AOCS, 1990).

Statistical analysis

Values represented are the means and standard deviations for three replicates. Statistical analysis was carried out by Student's *t*-test using SPSS Version 11.0 software and ANOVA (Duncan multiple range test) using SAS system Version 8e. Significance was defined at $P < 0.05$.

RESULTS AND DISCUSSION

Proximate analysis

Proximate analysis (Table 1) of olive samples shows the moisture contents, crude protein, total oil, fiber and ash contents ($p < 0.05$). The samples were found to have quite low crude protein (0.5 ± 0 - 1.1 ± 0.01%) but higher crude oil contents (32.7 ± 1.1 to 38.6 ± 1.2%). Crude fiber content of olive fruits contributed 2.6 ± 0.4 to 6.5 ± 0.3%, ash 1.7 ± 0.2 to 2.1 ± 0.4%, and carbohydrates content 0.5 ± 0 to 14.3 ± 0.7% of the total content. The lower values of protein and carbohydrates and higher values of total oil validated suitability of this oil for edible purposes like olive oil. Our findings of proteins and other parameters of wild olive oil are almost similar to those reported in literature for olive oil (Salunk et al., 1992). The variation in protein, oil and moisture contents are probably due to variety of fruit, growth conditions, harvest time and processing of oil (Pitchard, 1991).

Table 1. Proximate composition of wild olive fruit.

Sample*	Weight (g)	Moisture (%)	Crude protein (%)	Crude oil (%)	Crude fiber (%)	Ash (%)	Carbohydrate (%)
Kotli 1	2.3	58.4 ± 2.2	1.1±0.01	32.1 ± 1.1	5.1± 0.6	1.8± 0.1	1.5± 0.2
Kotli 2	2.1	42.1± 1.6	1.0± 0.02	34.4*± 0.8	6.5± 0.3	1.7± 0.2	14.3± 0.7
Kotli 3	2.2	60.7± 2.6	0.5±0.0	32.7± 0.6	2.6± 0.4	1.8± 0.3	1.7± 0.3
Kotli 4	2.3	52.6± 1.8	0.7±0.0	37.5± 0.5	3.5± 0.7	2.1± 0.4	3.6± 0.1
Kotli 5	1.8	54.3± 2.1	1.0±0.0	38.6*± 1.2	3.8± 0.4	1.8± 0.1	0.5± 0.0

*Kotli 1-5 fruit samples of wild olive (*Olea cuspidata*) collected from 3 heavy fruit bearing trees of 5 different locations.
Mean values of triplicate analysis.
*Significant ($p<0.05$).

Table 2. Physical properties of Fruit and wild olive oil.

Sample No.	Refractive index at RT	Specific gravity	pH	State at 25°C	Color
Kotli 1	1.331	0.92	5.5	Liquid	Yellow
Kotli 2	1.342	0.91	5.2	Liquid	Yellow
Kotli 3	1.372	0.92	5.1	Liquid	Dark yellow
Kotli 4	1.332	0.93	5.1	Liquid	Light yellow
Kotli 5	1.332	0.91	5.2	Liquid	Yellow

Mean values of triplicate analysis; *significant ($p<0.05$); n = 3.

Table 3. Chemical properties of wild olive oils.

Sample	Free fatty acids (% oleic acid)	Iodine value (mg/100 g)	Peroxide value (mg/kg oil)	Saponification number (mg KOH/g)	Unsaponificable Matter (g/kg)	Acid value (mEg/kg)	Total phenol (mg/kg)
Kotli 1	1.5* ± 0.1	75.2*± 1.2	16.2*± 0.5	186.5*±1.6	11.3*±0.6	1.2 ±0.2	64.2*±1.2
Kotli 2	1.0 ± 0.01	82.3*± 1.8	20.3*± 0.8	180.2 ±1.5	14.5 ± 0.5	0.8± 0.1	23.6 ± 1.5
Kotli 3	1.5 ± 0.1	91.4 ± 1.5	14.2 ± 0.2	187.3 ±1.8	12.6 ± 0.4	0.7± 0.0	46.3 ±1.4
Kotli 4	0.8 ± 0.0	78.3 ± 0.6	14.3 ± 0.1	175.6 ±1.2	15.6 ± 0.8	1.3± 0.0	47.2 ±1.3
Kotli 5	0.6 ± 0.0	80.2 ± 0.5	14.8 ± 0.5	182.3*±1.6	14.3 ± 0.6	0.9± 0.0	92.4*±2.1

Mean values of triplicate analysis, *significance $p<0.05$, n = 3.

Physical properties

Results of physical properties of oil are shown in Table 2. Color of oil obtained from *O. cuspidat*a was light to dark yellow and was liquid at 25°C. Fruit weight of all samples ranged 1.8 to 2.3 g. Aparicio and Launa (2002) observed that fruit weight of *O. euorpea* L. ranged from 3.5 g - 3.9 g; however, no single study in literature is available about fruit weight of *O. cuspidata*. It was observed that fruit weight of this olive species is lower than that was reported for *O. euorpea.* Differences in weight of various fruit of olive may be due to soil characteristics, fertilization and cultivar differences. Refractive index of 1.331 to 1.372, specific gravity 0.91 to 0.93 and pH values 5.1 to 5.5 was observed for oil of wild olive. Variance analyses indicates presence of significant ($p<0.05$) difference in different sample for these parameters in wild olive oil (Kritsakis and Markakis, 1984).

Chemical properties

The characteristics of the chemical properties of oils extracted from all wild olive samples are shown in Table 3. Regard to of free fatty acid (0.6 ± 0 to 1.5 ± 0.1%), iodine value (75.2 ± 1.2 to 91.4 ± 1.5), peroxide value (14.2 0.2 to 20.3 ± 0.8 mg/kg oil), saponification number (175.6 ± 1.2 to 187.3 ± 1.8 mg KOH /g), unsaponificable matter (12.6 ± 0.4 to 15.6 ± 0.8 g/kg), acid value (0.7 ± 0 to 1.3 ± 0 mEg/ kg and total phenol (23.6 ± 1.5 to 92.4 ± 2.1 mg/kg). Total phenol content as determined by the Folin Ciocalteu assay (Bastic et al., 1978; Tsimidou,

Table 4. Fatty acid (%) contents of oil obtained from wild olive.

Sample	Oleic acid (24.13 min)	Linoleic acid (25.37 min)	Linolenic acid (20.16 min)	Palmitoleic acid (20.16 min)	Palmitic acid (19.77 min)	Stearic acid (23.56 min)
Kotli 1	71.3*	12.0*	1.5	1.31	13.9*	0.2
Kotli 2	72.4	11.2	1.3	1.92*	13.4	0.1
Kotli 3	69.3*	15.2*	3.2*	1.10	11.2	0.1
Kotli 4	74.5*	11.3	1.5	1.89	11.5	0.1
Kotli 5	71.2	11.4	1.4	2.10	14.0	0.1

Mean values of triplicate analysis; *significant ($p<0.05$); n = 3.

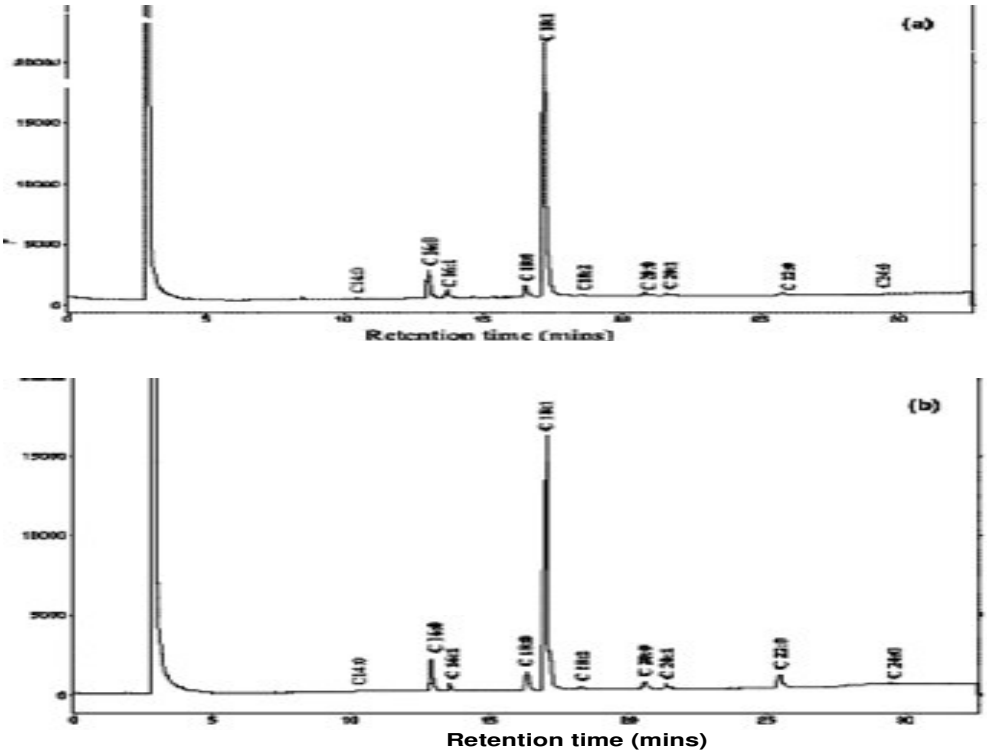

Figure 1. (a) GC Peaks of fatty acids from wild olive oil (b) commercial olive oil.

1999) and expressed as gallic acid equi-valents were significant for different samples ($p<0.05$) (Table 3).

The phenolic values of oils samples extracted from Kotli 2, 3 and 4 samples were lower as compared to those recorded for oil from Kotli 1 and Kotli 5. Montedoro et al. (1992) reported that total phenol contents of Italian olive oils (50 to 1000 ppm) were lower than oil produced in other countries (400 mg/kg) as caffeic acid equivalent (Garcia et al., 2003). Although phenol contents of wild olive were lower than those reported for *O. europea* oil, but quantity of phenols found in wild olive fruits are reliable for a good quality of edible oil because phenols are very important due to its antioxidant properties.

The level of free fatty acids in the oil of wild olive indicates its good quality and can be stored for a long time without spoilage via oxidative rancidity. The peroxide values of wild olive oil (Table 3) is comparable to commercially available oil and indicates its suitability for edible purposes (Person, 1982). The saponification number and iodine value of this oil suggests that the oil is not suitable for soap formation and it is most probably non drying group of oils. Furthermore lower acid value of wild olive oil (Table 3) indicates the oxidization tendency of the oil and is highly desirable from industrial point of view.

Fatty acid compositions of olive oils

The fatty acid compositions of olive oils determined using gas chromatography (Table 4 and Figure 1 a and b) suggested that oleic acid (69.3 - 74.5%) was present as the highest concentration followed by linoleic acid (11.2 -

15.2%), palmitic acid (11.2 - 14.0%), linolenic acid (1.3 - 3.20%), palmitoleic acid (1.31 - 2.10%) and stearic acid (0.1 - 0.2%), and also differences in fatty acids contents were significant ($p<0.05$) (Table 4). Comparison of oil and fatty acids contents of wild olive are comparable with those *O. europea* (Aparicio and Launa, 2002; Kiritsakis and Markakis, 1984). The fatty acid composition of oils is affected by species, genetics, variety, growing conditions, locality, climatic conditions and post harvest treatment (Warner and Knowlton, 1997). On the basis of present study it is concluded that oil obtained from wild olive (*O. cuspidata*) in the present study is almost comparable to edible oil of *O. europea* and have all physical and chemical properties required for any seed oil used for edible purposes, especially due to the presence of unsaturated fatty acids and phenolic contents. However, toxicological studies of this new oil are required before it can be recommended for public consumption.

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