

Variation in Lipid Composition and Physico-chemical Constituent Among Six Cultivars of Grape Seed

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Summary: The oil extracted from the six cultivars of grape seeds (Perlette, Anib-e-Shahi, Madess Field, Black Hobbage, South Columbia and Autumn) was analyzed for its physico-chemical properties and fatty acid composition. The iodine value, saponification value, free fatty acid, peroxide value and ester value etc were different in each cultivar. Similarly, fatty acid compositions of all the cultivars was found to be different from each other. Oil yield was also different in each cultivar, which ranged from 4.3 to 13%. But the different physico-chemical parameters oil content and fatty acid composition of the oils of all the cultivars do not appear to affect the nutritional value of the grape seed oil.

Introduction

Grapes, commonly known as angur belong to the family vitaceae cultivated originally in Asia. Minor grows in south Europe, North Africa and Middle East [1]. Grape vine is sarmetose shrub. Its trunk can reach 1 m in circumference at its base and branches can be 30 m long. Its climbing stem has tendrils opposite to leaves. The seeds of grapes are two celled with two ovules in each cell which develops into succulent pedicellate berry of spherical or ovoid form in which the cells are obliterated [2].

Fruit contains grape sugar (glucose), gum, tannin, tartaric acid, citric acid, racemic and malic acid. Chloride of potassium and sodium, sulphate of the potash, tartaric of lime alum, iron. Some albumins, ozotised matters and acids and tartarate of potassium. Grapes are demulcent laxative, refrigerant, stomachic, diuretic and cooling. Raisins (dried grapes) are laxative demulcent and expectorant. They are also considered as attenuant, suppurative, nutritious and blood purifier and also recommended in certain form of anemia and wasting diseases and also useful in bilious dyspepsia, haemorrhages, dysuria ardour urinae and strangury. Grapes are beneficial in chronic bronchitis, heart diseases, brights diseases and gout. It is also a good diet given in morning and evening during jaundice. Grapes are also recommended in certain form of anemia and wasting diseases. It is also useful in bilious dyspepsia, haemorrhages, dysuria ardour urinae and strangury. Grapes are beneficial in chronic bronchitis, heart diseases, brights diseases and gout. It is also a good diet given in morning and evening during jaundice. Ripe fruits

partly dried in the sun are useful in thirst attendant on fever, in cough catarrh jaundice, constipation and subacute cases of enlarged liver and spleen [3].

Juice of unripe grapes and leaves are astringent while juice of sour grapes is useful for bruises and sprains.

Grape seed oil is a natural agent, which raises serum HDL level [4]. Oil of grape seeds having smoke point 320 °F (160 °C) can safely be used in cooking as low fat diet and also used in salad dressing, margaren, deep frying, baking and massage oil. It helps in skin retain (the normal structure of the epithelium and nerve cells) and is also used in sunburn lotions, hair products, body hygiene cream, lip balm and hand cream [5]. Grape seed oil has synergistic anticancer effect [6]. It is also helpful in protection of cardiac muscle [6,7]. Due to the medicinal importance of the oil, six varieties of the grapes namely Perlette, Anib-e-shahi, Madess Field, Black Hobbage, South Columbia and Autumn were selected to study the oil contents and the quality of oil with respect of essential fatty acids, EFAs. Omega-3, Omega-6 and Omega-9 have biomedical importance and they regulate the key functions of body and are also essential for the body growth and cannot be synthesized by the human body. The present study would not only help in exploring the non conventional sources of lipids but also play a vital role in promoting the health of population of Pakistan. The dietary requirement is only based on food cooked in oil/ghee which are the main sources for growth and

energy. Specific techniques have been used for extraction, purification and characterization of oils from all the cultivars of grapes. The extracted oils were then hydrogenated, methylated and their fatty acid composition was determined by GLC.

Result and Discussion

By using methanol and chloroform mixture, the lipids were extracted from the seeds of six varieties of grapes. The solvent was then removed at reduced pressure to avoid changes like polymerization and decomposition of oils. The % age yields of these six varieties are shown in Table-1. The maximum yield was in perlette (13.0 %) and minimum yield was in Black Hobbage (4.3 %).

Table-1: Percentage of oil of different cultivars of grape seeds.

Cultivars	% age of oils
Perlette	13%
Anib-e-shahi	6.6%
Madess Field	8.8%
Black Hobbage	4.3%
South Columbia	9.6%
Autumn	11.7%

The remarkable difference in the yields of oil among different cultivars of grape seeds may be due to the different climatic condition, nature of soil, ripening time and the colour of berry. These results seem to be according to an earlier investigation of

Holeapek [8] who observed a high yield of oil in light colour berry than dark colour berry.

The extracted oil was examined for its physico-chemical values because chemical constituent are valuable to evaluate oils with respect to their utilization for edible or industrial purposes. Some important physicochemical properties of grape seed oil determined are given in Table-2. The refractive indices of these six cultivars are in the range of 1.4653 - 1.5154 at 40 °C, which shows that molecular arrangement of all the six cultivars are more or less same. Iodine value is in the range of 125.3 to 132.27, which shows that it is a drying oil. Saponification value (188.24 - 201.5) shows that oils have fatty acids of similar molecular weights. Low free fatty acids present in the oils indicate that oils are fit for human consumption. Low peroxide value of the oils minimizes the chances of the formation of epoxide and slows down the process of oxidation. The oil of this range of peroxide value even in crude form is considered to be fit for edible purpose.

The fatty acid profile of grape seed oil of all the six varieties are given in Table-3. The oils contain higher % age of unsaturated fatty acids which is main characteristic of a good quality vegetable oil. This makes oil more important and suitable for edible purpose. Nash [4] reported that grape seed oil is a

Table-2: Physicochemical values of oil of different six varieties of grape seeds.

Physicochemical values	Cultivars					
	Perlette	Anib-e-Shahi	Madess Field	Black Hobbage	South Columbia	Autumn
Refractive index	1.4653	1.5126	1.5017	1.5059	1.5075	1.5154
Iodine value	127.4	131.96	132.27	129.06	125.3	126.4
Saponification value	194.68	210.0	188.24	196.4	197.29	201.5
Free fatty acid (% age as Oleic acid)	1.95	2.6	3.3	1.3	2.3	1.3
Peroxide value meq/kg	9.68	10.1	8.2	5.6	10.6	6.16
Acid value	3.88	5.17	6.56	2.58	4.57	2.58
Ester value	190.8	204.63	181.68	193.82	192.72	198.92
INS value	87.29	98.02	75.97	87.34	81.99	85.1

Table-3: Fatty acid composition of oil of different cultivars of the grape seeds.

Sample Cultivars	Fatty Acids								
	Capric Acid C _{10:0}	Lauric Acid C _{12:0}	Myristic Acid C _{14:0}	Palmitic Acid C _{16:0}	Palmitoleic Acid C _{16:1}	Stearic acid C _{18:0}	Oleic Acid C _{18:1}	Linoleic Acid C _{18:2}	Linolenic Acid C _{18:3}
Perlette	0.3	0.2	1.3	7.7	T	3.2	18.2	71.7	0.9
Anib-e-Shahi	0.1	0.1	0.3	7.3	T	4.8	16.7	73.3	0.8
Madess Field	0.5	0.3	0.2	8.9	T	3.5	17.1	76.6	1.1
Black Hobbage	0.9	0.7	1.5	10.3	T	4.3	19.8	73.2	1.5
South Columbia	T	T	0.6	11.1	T	15.0	17.2	76.6	0.8
Autumn	0.1	0.3	0.4	9.8	T	3.9	18.7	74.1	0.9

T* = Traces

natural agent, which raises the serum HDL level. Consequently the fatty acid composition of grape seed oil has been the focus of attention in determining its nutritional adequacy in relation to coronary heart disease (CHD) risk factors. Linoleic acid in all the cultivars is in huge amount along with appreciate amount of oleic and linolenic acid. Among saturated acids palmitic acid is present in good amount along with stearic acid. Lower fatty acids are also present in lesser amount in all the cultivars of grape seeds.

Due to its health promoting fatty acid composition it is a good alternative of vegetable ghee saturated fat. Grape seed oil satisfies almost all the properties of edible oil. The %age of lauric acid in the grape seed oils of all the cultivars is less, so chances of cholesterol raising are minimized. Stearic acid is found in such amount which generates no effect on blood lipids and lipoprotein. Palmitic acid also present in fewer amounts so lesser triglycerides are synthesized, because palmitate is preferentially synthesized into triglycerides [9]. Oleic acid is present in good amount, which lower low-density lipoproteins (LDL) while maintaining HDL (high density lipoprotein) [10,11] and also helpful in the synthesis of essential fatty acids. Linoleic acid reduces plasma lipoprotein by directing fatty acid metabolism away from synthesis and towards oxidation, it down regulate gene that control fatty acid and cholesterol synthesis [12,13]. The reason is that liver mitochondria oxidize linoleate faster than other common fatty acid so hypocholesterolemic action of linoleate was principally due to changes in its metabolism rather than changes in cholesterol metabolism [14,15]. It also improve diabetic neuropathy and aid in cancer.

Linolenic acid is also present in the grape seed oil in lesser amount but sufficient to suppress lipid synthesis.

Linolenic acid of grape seeds accomplished this by up regulation of lipid oxidation and a down regulation of lipid synthesis. So the out come is an improvement in the symptoms of the metabolic syndromes and a reduced risk of heart disease. Moreover linolenic acid alongwith linoleic acid (polyunsaturated fatty acid, (PUFA) reduce triglyceride accumulation in skeletal muscles and potentially in cardio myocytes and β -cells [16]. Linolenic acid is used in the formation of cell walls making them flexible along with improving circulation and oxygen uptake with proper red blood cell.

Table-4 shows the yield of neutral and polar lipids of six cultivars of grape seed.

Table-4: Yield of neutral and polar lipids of six cultivars of grape seeds.

Cultivars	Neutral lipids	Polar lipids
Perlette	96.8%	3.2%
Anib-e-Shahi	93.9%	6.1%
Madess Field	93.2%	6.8%
Black Hobbage	98.3%	1.7%
South Columber	91.6%	8.4%
Autumn	94.5%	5.5%

The present study indicates that Perlette cultivar of grape seed contained maximum yield of lipid along with ideal and health promoting fatty acid composition and positive values linked to grape qualities.

Experimental

Extraction of oil

The grape seeds of above varieties were procured from agriculture department. They were dried in oven at 105 °C and crushed into fine powder. The lipids were extracted with 500ml chloroform: methanol (2;1 v/v) [17] mixture at room temperature by shaking on magnetic stirrer for 2 hours. After filtration the residual material was treated three times with 100 ml of same mixture. All the extracts were combined and three consecutive washing with Folch solution [18] were given to remove the-lipid impurities. After removal of solvent under reduced pressure, the oil was stored in an inert atmosphere.

Physicochemical values of the oil

The physicochemical value was determined according to British standard specification and [19-21] procedure.

Refractive index was determined by Abbe's refractometer.

The oil was refluxed with 0.5 N alcoholic KOH (potassium hydroxide) solution for 3 hour. The soap solution reacted with 2N H₂SO₄ to liberate fatty acids after separation of unsaponifiable matter by diethyl ether [19]. The methyl ester of fatty acid was prepared with BF₃ Methanol reagent [21].

Methylation of fatty acid and purification of methyl ester.

The lipids grape seed were treated with boron trifluoride-methanol for recommended time in test

tube with Teflon lined screw cap for the formation of methyl ester. The ester were extracted with n-hexane and dried over anhydrous sodium sulphate. After the removal of solvent, the dried methyl ester of the acids of oil stored at low temperature for GLC analysis.

Identification of fatty acids by GLC

The methyl esters of grape seed oil of all the cultivars were analyzed on Shimadzu GC-4A gas chromatograph equipped with FID(flame ionization detector) and polar (PEG) capillary column (25 m x 0.2 mm i.d). The temperature programme of column oven was 180 °C- 3 °C/min-220 °C. Nitrogen was used as carrier gas with a flow rate of 2 ml/min. The temperature of injector and detector was 230 °C and 250 °C respectively. The peaks were recorded on Shimadzu CR-4A chromatopac and identified by comparing their retention time with those of standards run under the same parameters.

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