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Fatty Acids Composition by (GC-MS) and Most Important Physical Chemicals Parameters of Seed Oil Pomegranate and Grape Seeds

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Abstract
Physical and chemical characteristics of Pomegranate and Grape seeds oil and fatty acids their oils were determined. It was found that the Pomegranate seed oil 13.% and Grape seed oil 12. % .Physicochemical parameters of the extracted oils were respectively as follow: refractive index (1.51 and 1.46),acid value(0.68 and 0.62 mg/KOH/g), peroxide value(1.27 and 3.11 meq O2 /kg oil), iodine value (130.2 and 124. 33 (g I2/100 g oil), saponification value (198 and 182 **mg KOH/ g oil),** chlorophylls(4.35 and 5.61 mg/kg), carotene (3.23 and 3.87 mg/kg). Specific extinctions at two wavelengths of 232 nm (K232) and 270 nm (K270) and R-value (K232/K270) were found in Pomegranate and Grape seeds oil (2.15, 2.10, 1.45, 0.79, 0.67 and 0.37respectively. For the fatty acid content in these oils, Gas Chromatography-Mass Spectrometry (GC-MS) was used. The constituents of fatty acids in Pomegranate seed oil were :Punicic acid (77.37%) , Oleic acid (7.36%) , Elaidic acid (6.87 %), Margaric acid (3.86 %) , Stearic acid, methyl e (0.60 %) , Linoleic acid (0.08 %) , Lignoceric acid (0.07%) , Palmitic acid (0.03%) . for Grape seed oil, the found fatty acids were :, Oleic acid (16.98%) , Palmitic acid (12.09%) , Stearic acid (8.85%) , Palmitoleic acid (0.28%) , Myristic acid, (0.21%) , Azelaaldehydic acid (0.12%) , Margaric acid (0.11%) , Lauric acid (0.05%) , Pentadecanoic acid (0.03%) Saturated lauric acid o.11%, Palmitic acid: o.42%, Stearic acid 46.93%.
Keywords: GC MS , Physicochemical properties, Pomegranate, Grape, oil

Introduction
Seed oil consists of fatty acids which has high commercial value in food industries, pharmaceuticals, lubricants, cosmetics and others. Pomegranate (*Punica granatum* L.) belongs to the Punicaceae family (Facciola et al., 1990).It is a fruit tree with deciduous leaves, which in recent years has seen a great expansion in several countries, especially those with a Mediterranean-like climate, where fruit of excellent quality can be obtained. There is growing interest in this fruit not only because it is pleasant to eat, but also because it is considered to be a functional product of great benefit in the human diet, as it contains several groups of substances that are useful in disease prevention (Melgarejo and Martınez, 1992; Melgarejo and Salazar, 2002). Pomegranate extracts have been used as anticancer agents and they contain a large number of potentially bioactive substances. Punicic acid is an omega-5 long chain polyunsaturated fatty acid found in Punica granatum (pomegranate) seed oil

Grape(*Vitis vinifera L.*) belongs to Ampelidasea family and is also called sarmantace or vistacea. Grape seed oil (GSO) is a rich recourse of vitamin E that has effective role in prevention of heart disease and blood clot in veins, tacking on spoon of grape seed's oil and also provides the daily need of this vitamin in the body. This oil is containing necessary fatty acid in the body (Breck, 2008). Extracted oils from plant seeds are mainly composed of triacylglycerols (95 to 98%) which are esters of glycerol and complex mixtures (2 to 5%) minor compounds (Aluyor *et al*., 2009). Those minor compounds include fat soluble vitamins, pigments such as chlorophylls and carotenoids, phenolic compounds, phospholipids, mono and diacylglycerols and free fatty acids (Kamal-Eldin, 2006). Diets which tend to be rich in plant antioxidants, consumed from fruits and vegetables have been associated with lower risks of coronary heart disease and cancer (Xu *et al.,* 2010).

Materials and Methods
Materials
Fresh pomegranate (*Punica granatum*) and grape (*Vitis viniferal L.*) were obtained from Basarh market . The seeds after juice extracting with no any other parts of fruits products such as peels. The seeds were dried at 40˚C for 24h in an oven and ground to a powder .The were stored at (-10C˚) in glass bottles until analysis .

Oil Extraction
Oils were extraction from 30g seeds with 300 ml of n- hexane (50-60 C˚) in a Soxhlet extraction. Then the solvent was removed (oven at 40 C˚),cured oils ware stored at(–10C˚) in glass bottles until analysis(Rashid *et al.,* 2008).

Physical and Chemical parameter of oil
Refractive index of oil seeds were determined at 25˚C with an Abbe refractometer(Alamu et al., 2008). The

solubility measure of 1g of oil dissolved is 5ml of solvent organic. The state, odor and color of the oil noted, using visual inspection at room temperature (Oderinde et al., 2009). Acid, peroxide, iodine and saponification values were carried out using AOAC(1997).Specific extinctions (E1%1cm) at 232 nm (*K232*) and 270 nm (*K270*) were determined according to the European Official Method of Analysis (Commision Regulation EEC N-2568/91). Chlorophyll and carotenoids content were measured according to the described by Minguez et al.(1991). The absorbency measure of 7.5g of oils dissolved in 25 ml of cyclohexane was realized at 470 nm carotenoid content and 670nm chlorophyll content.

Preparation of Fatty Acid Methyl Esters (FAME)

Totalfatty acid content and fatty acid composition were determined simultaneously in the fruits seed oil samples.Fatty acid analysis was performed in triplicate consisted two consecutive steps, preparation of fatty acidmethyl ester (FAME) and chromatographic analysis .The AOAC(1997).method was followed to esterify the lipidextract. FAME was prepared from the lipids extracted from samples by heating with the methanolic NaOH first and then with BF3 Methanol for esterification. 5 ml n-heptane was added to recover the methyl esters in organic phaseSaturated NaCl solution was added to the mixture and the aqueous and organic layers were separated using a profil separating funnel. The upper n-heptane phase was pipetted out and stored in 10 ml glass vials and store analy under sub-zero temperature till GC-MS analysis is done.

Fatty Acid Methyl Ester (FAME) Analysis by Gas Chromatography Mass Spectrometer (GC MS):

The gas chromatograph analysis of methylated fatty acids was performed on a Shimadzu QP2010 quadrupole Gas Chromatography Mass Spectrometer (GC-MS) instrument equipped with a carbowax (30 m × 0.25 mm ID; 0.25ìm film thickness) capillary column (intercut DB5MS . japan). One microliter of sample was injected into the capillary column. Helium was used as the carrier gas. Injector and detector temperatures were set at 280°C. Injection was performed in split mode (1:30). The column temperature was programmed initially at 50°C for 1 minutes and then to increase at a rate of 5°C per min a final temperature of 280°C. Fatty acid methyl esters were separated at constant pressure (100 kPa) and peaks were identified by comparing the mass spectra with the mass spectral database. The identification of compounds was based on the comparisons of their mass spectra with NIST Library 2008

Results and Discussion

Oil yield

The oil content of *Vitis viniferal L*. seeds (22.18 %) was more than twice of that of *Punica granatum* seeds which was (12.68 %). These variations between oil yields in seeds may be due to their different in kind, cultivation climate,ripening table cultivar and condition (Stevenson *et al.,* 2007)

Physical characterization

Physicochemical characterization of Punica granatum and Vitis viniferal L. seeds oil are showed in Table 1. Physical properties of lipids derived directly from their chemical structures and functional groups and greatly influence the functions of lipids in foods and the methods required for their manipulation and processing. They can also be used to assess the purity or quality of lipid material in reference to known standards or preferred characteristics (Nichols and Sanderson, 2003). The pomegranate seed oil was clear yellow and grapes seeds oil yellow in color. Color is a sensory property with a strong influence on food acceptance as it contributes decisively to the initial perception that one can acquire of the condition, ripeness, degree of processing, and other characteristics of foods (Alos, *et al*., 2006). They were liquid at room temperature and even in a refrigerator. Solubility of both of them have the ability to soluble in water, oil and some organic solvent.Refractive index of oils depends on their molecular weight, fatty acid chain length, degree unsaturation and degree of conjugation (Nichols and Sanderson, 2003). The pomegranate and grapes seeds oil showed at refractive index of 1.51 and 1.46 respectively, those results were in a good agreement with 1.46 in grape seed oil of Karimian (2012).

Table 1:Pysacal characterization of pomegranate and grape seeds oil

Parameter	pomegranate seedoil	grape seeds oil
Clear	Yellow	Yellow
Odor	light odor	light odor
Water solubility	Soluble	Soluble
Other solubility	hexane, ethanol, chloroform, ether and with oils	hexane, ethanol , chloroform, ether and with oils
Refractive index	1.51	1.46
State at room temperature	Liquid	Liquid

Chemical characterization

The chemical properties of oil are amongst the most important properties that determines the present condition of oil .

Acid value

The acid value of pomegranate and grape seeds oil were determined to be 0.68 and 0.72mg KOH/g oil respectively .Acid value represent free fatty acid content due to enzymatic activity, and is usually indicative of spoilage. Its maximum acceptable level 4mg KOH/g oil

(CODEX Alimantarius Commission, 1982) . Acid value of grape seed oil was higher than that reported by Karimian (2012).

Peroxide value

Peroxide value (PV) are in pomegranate 1.27 and grape 3.11 meq O2/kg oil. The low values of PV indicative of low levels of oxidative rancidity of the oils and also suggest strong presence or levels of antioxidant certain antioxidant may however be used to reduce rancidity (Eze, 2012). (Chander, 2010) reported similar PV in pomegranate oil 1.2 meq O2/kg oil. A low peroxide values are comparable to palm oil(4.0) and sunflower seed oil (4.2) (Gunstone, 2004) shows that oil is stable to relative oxidation. Instead, the oil which has peroxide value of more than 10.0 will go rancid (Abitogun *et al*., 2009).

Iodine values

Table 2. Shows the results for iodine value of the tow oils. The iodine values o f pomegranate and grape oils were 130.4 and 124.36 g of I2/100 g oil, respectively. These values close to 122 and 130 reported by respectively Karimian (2012) and Abou Rayan (1998). Iodine value is a measure of the degree of unsaturation in oils, and an identity characteristic of native oil. This value could be used to quantify the amount of double bonds present in the oil, which reflects the of oil to oxidation (Oderinde *et al.,* 2009).

Table 2:Chemical characterization of pomegranate and grape seeds oil

Parameter	pomegranate seedoil	grape seeds oil
Peroxide value meq O2/kg	0.68	0.72
Iodine values g of I2/100 g oil	130.4	124.36
Specification value mg KOH/g	198.1	191.1
Specific extinction at 232 nm(*K232)*	2.154	2.104
Specific extinction at 270 nm(*K270)*	1.456	0.795
R- Value (K232/ K270)	0.679	0.377
Chlorophyll (mg/kg)	4.355	5.611
Carotenoid (mg/kg)	3.35	3.875

Saponification values

The result obtained for the saponification value for tow oils were shown in Table 2. The saponification values of the pomegranate and grape oils about 198.1 and 191.5 mg KOH/g respectively. These values were comparable to grape sharodi seed oil (192.05 mg KOH/g),raspberry seed oil (191 mg KOH/g),grape seed oil (192.9 mg KOH/g) and pomegranate seed oil (200 mg KOH/g) (Karimian et al., 2012 and Oomah et al., 2000).Saponification values is an indicator of the average molecular weight of fatty acid in the oil fractions, and hence , chain length. It is inversely proportion to the molecular weight if the lipid (Abayeh et al., 1998).

Specific extinction

The Specific extinction at wavelengths of 232 and 270 nm as well as their ratio(*R-Value*)are shown in Table 2. As can be seen, the k232 , k270 and R-Value (*k232/k270*) of pomegranate and grape seed oil were 2.154, 2.104, 1.456, 0.95, 0.679 and 0.373 respectively. The *k232* is usually considered as an indicator of the oil autoxidation and has been well correlated with peroxide value, but the *k232* is amore useful quantity that measures the presence of conjugated dienes and trienes(Ogutcu *et al*., 2008).

Chlorophyll and carotenoids content

Chlorophyll and carotenoids are important quality parameters because they antioxidants naturally in vegetable oils and provide some protection against oxidation by terminating free radicals and correlate with color (Yoshida *et al.*, 2006 and Salvador *et al.*, 2003). Grape seed oil contained slightly more chlorophyll and carotene than pomegranate seed oil (5.611, 4.355 mg/kg and 3.875, 3.35 mg/kg).

Determination of fatty acids composition of using GC-MS spectrometry

The Gas-chromatography coupled with mass spectrometry analysis of fatty acids in the oil from seeds of pomegranate has been recorded in Fig2 . and Table 4 . It is found The eight identified fatty acids included saturated, monounsaturated and polyunsaturated fatty acids which accounted Punicic acid, methyl ester (77.37%) , Oleic acid, methyl ester(7.36%) , Elaidic acid, methyl ester (6.87 %) , Margaric acid methyl ester (3.86 %) , Stearic acid, methyl e (0.60 %) , Linoleic acid, methyl ester (0.08 %) , Lignoceric acid methyl ester (0.07%) , Palmitic acid, methyl ester (0.03%) . The saturated fatty acids identified include Palmitic acid ,Margaric acid ,Stearic acid and Lignoceric acid . There were only two Monosaturated fatty acids Oleic acid and Elaidic acid .The polyunsaturated were dominantly Punicic acid together with rare amounts of Linoleic acid . Punicic acid which is an 18- carbon fatty acid with 3 unsaturated double bond in the form of conjugated makes up over 78 percent of the existing fatty acid in the oils examined. punicic acid has been claimed to have nutritional and health improving effect(Deman,1999).Our results are similar to Habibnia *et al.,(*2012) who studied five different varieties of pomegranate. The results of their research indicated that the predominant fatty acid higher than 70% was punicic acid. .As reported previously(Melo *et al.,* 2014) pomegranate seed oil a source of punicic acid about 72%.

The GC-MS analysis of fatty acids in the oil from seeds of grapes has been recorded in Fig1 . and Table 3 It is found 10 FAMEs and the main Linoleic acid, methyl ester (59.97%) , Oleic acid, methyl ester (16.98%) , Palmitic acid, methyl ester (12.09%) , Stearic acid, methyl ester (8.85%) , Palmitoleic acid, methyl ester(0.28%) , Myristic acid, methyl ester (0.21%) , Azelaaldehydic acid, methyl ester (0.12%) , Margaric acid methyl este (0.11%) , Lauric acid, methyl ester (0.05%) , Pentadecanoic acid, methyl ester (0.03%) . As reported previously (Barron et al., 1988; Schuster, 1992), grape seed oil contained mainly palmitic, stearic,oleic, and linoleic acids. As reported previously (Baydar and Akkurt ,2001),grape seed were rich in linoleic and oleic acid ranging from 60.1 to 70.1 and 17.8 to 26.5 respectively. In our study, among the identified fatty acids, linoleic acid (C18:2) was predominant. The ratio of linoleic acid was 59.97%. Second compound was oleic acid (C18:1) and showed an amount 16.98%. These values are in general, in agreement to those determined in grape seed oil by Canbay and Bardkçi (2011).

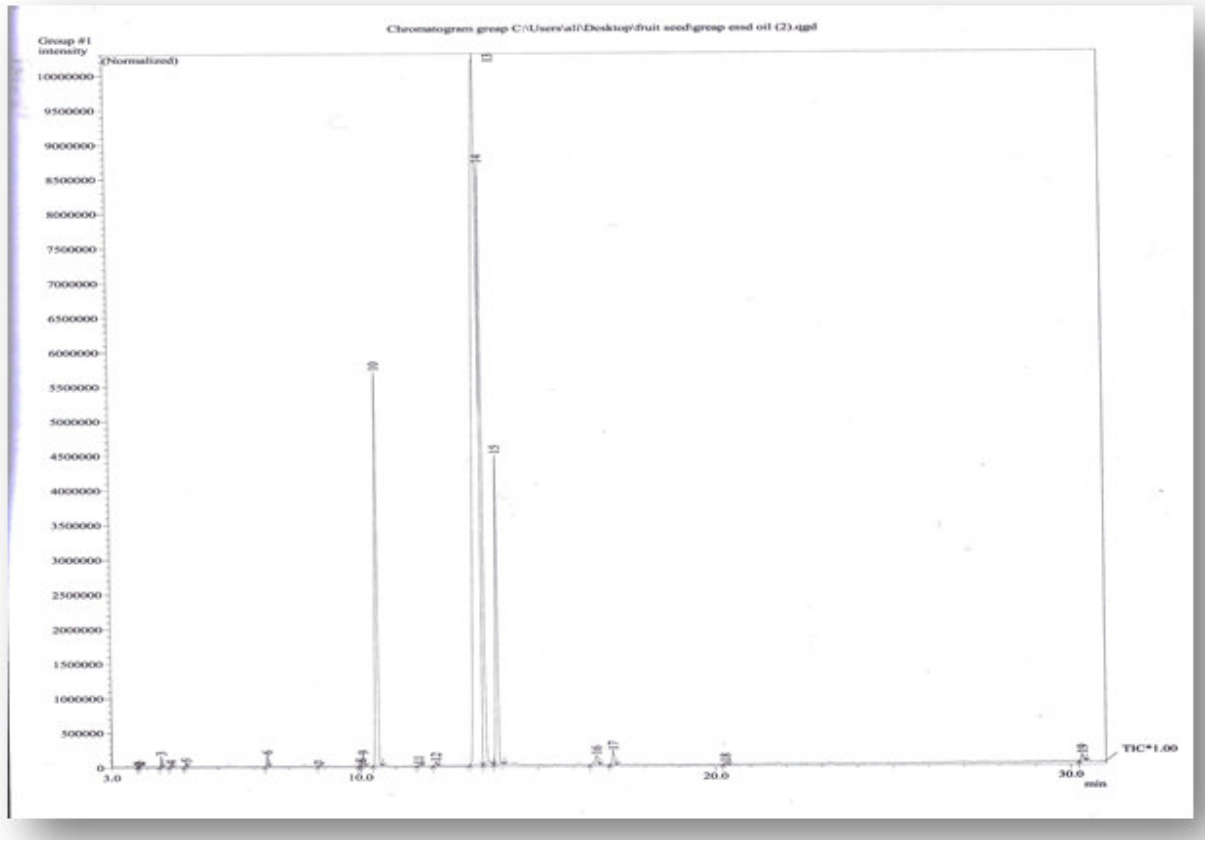

Fig.1 GC–MS total ion chromatogram of Fatty acid composition of the grapes seed oil

Table 3:fatty acid methyl estercomposition of the grapes seed oil

3	4.392	Azelaaldehydic acid, methyl ester	0.12	186
5	5.100	Lauric acid, methyl ester	0.05	214
6	7.383	Myristic acid, methyl ester	0.21	242
7	8.850	Pentadecanoic acid, methyl ester	0.03	256
9	10.083	Palmitoleic acid, methyl ester	0.28	268
10	10.500	Palmitic acid, methyl ester	12.09	270
11	11.642	cis-10-Heptadecenoic acid, methyl ester	0.05	282
12	12.125	Margaric acid methyl ester	0.11	284
13	13.392	Linoleic acid, methyl ester	59.97	294
14	13.500	Oleic acid, methyl ester	16.98	296
15	13.875	Stearic acid, methyl ester	8.85	298

RT: Retention time * Relative percentages obtained from the peak area in chromatogram

GC/MS analyses of the fatty acid methyl esters were replicated three times.(

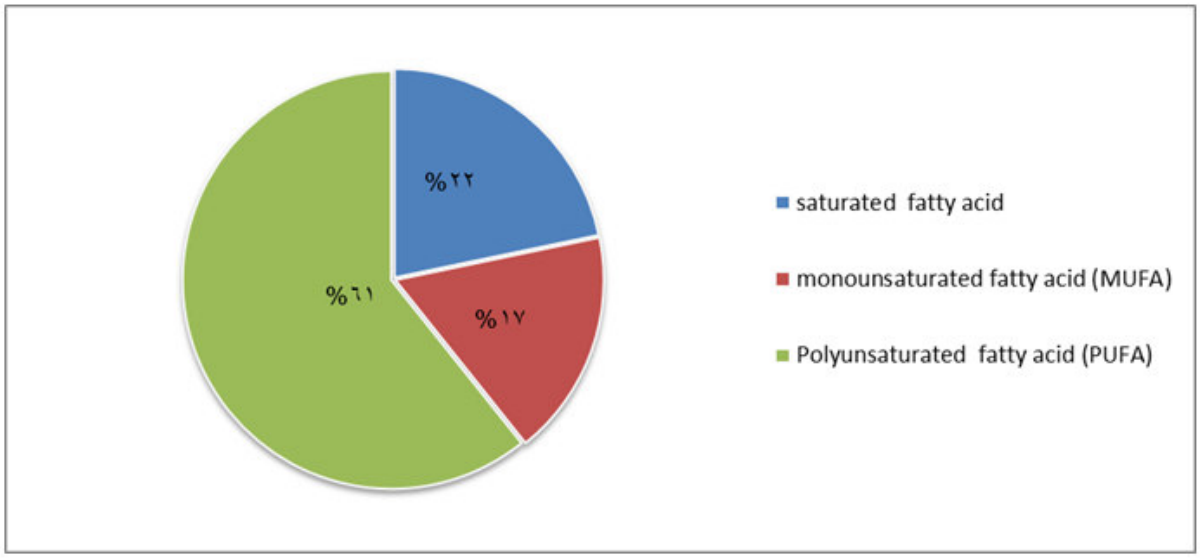

Fig.2:Types of fatty acid in grapes seed oil

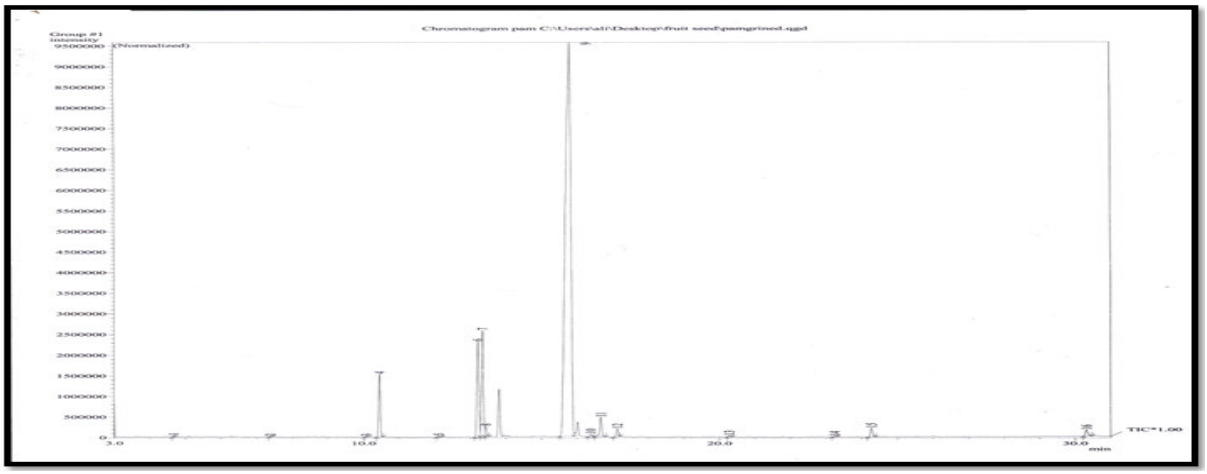

Fig.3 GC–MS total ion chromatogram of Fatty acid composition of the pomegranate seed oil

Table 4:. Fatty acid composition of the pomegranate seed oil

Peak #	R.T*	Fatty acid methyl esters	Area%	Mol Weight
2	10.075	11-Hexadecenoic acid, methyl ester	0.03	268
3	10.458	Palmitic acid, methyl ester	0.03	270
4	12.117	Margaric acid methyl ester	3.86	284
5	13.217	Linoleic acid, methyl ester	0.08	294
6	13.350	Elaidic acid, methyl ester	6.87	296
7	13.433	Oleic acid, methyl ester	7.36	296
8	13.817	Stearic acid, methyl ester	0.60	298
9	16.025	Punicic acid, methyl ester	77.37	292
14	23.233	Lignoceric acid methyl ester	0.07	382

RT: Retention time * Relative percentages obtained from the peak area in chromatogram

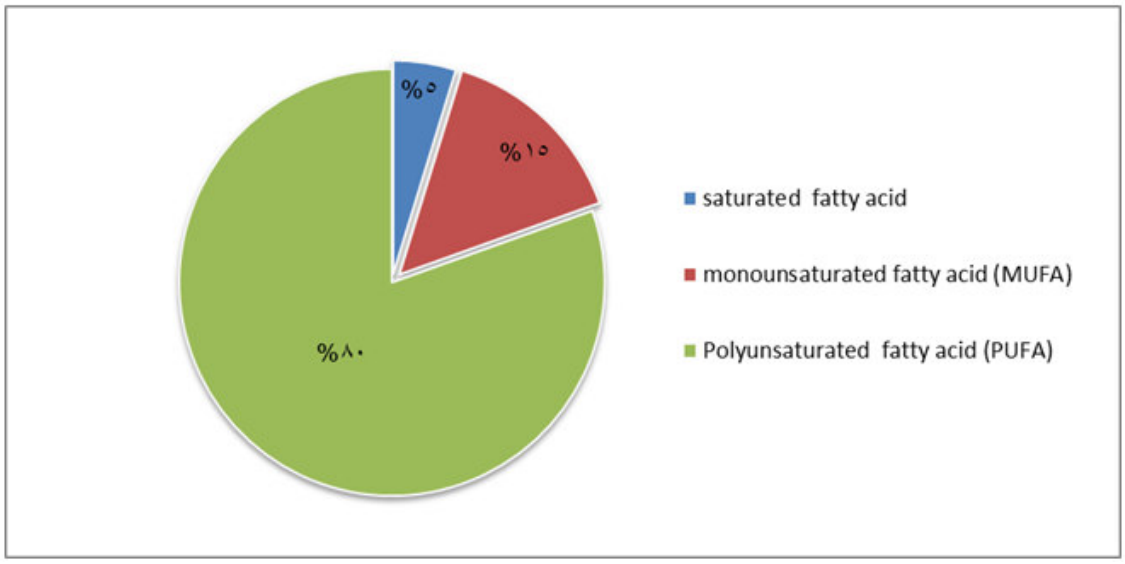

Fig. 4: Types of fatty acidin pomegranate seed oil

Conclusion

The seed oils pomegranate and grapes showed fatty acid compositionsuch as palmitic acid, stearic acid, oleic acid ,linoleic acid, punicic acid and eicosanoic acid

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