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Fatty Acids and Volatile Compounds in Avocado Cultivated in North of Iran

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Abstract: Fatty acids and volatile constituents of avocado mesocarps of two varieties (Fuerte and Hess) of avocados cultivated in Babol, Iran, were extracted and isolated through solvent extraction in the soxhlet exelo 50/42 apparatus using n-hexane. The extracts were concentrated in a rotary. A greenish oil, with faint odor, bland taste with D = 0.92 Saponification value 179-195, Iodine value 70-92, refractive index 1.45-1.46 (40°C) was obtained. The separated Avocado oil was analyzed using gas chromatograph. A bonded phase, open tubular fused silica capillary column was employed (30 m×0.25 mm i.d.). Ions were collected or scanned over the range m/z 400-17 mass units at 1 s decade^{-1}. The temperature program was 2 min at 50°C increasing at 5 °C min^{-1} to 250 °C for the above column. Other operating conditions were: oven and injector temperatures, i.e., 50°C and 250°C, respectively. The results of fatty acids analysis showed that the main fatty acid was oleic acid with percentages of 54.5 and 58.13 in the avocado mesocarp of Hess and Fuerte, respectively. Other acids found in these fruits were palmetic acid (19.7 and 22.9%) Linoleic acid (15.7 and 10.6%) and Palmitoleic acid (7.6 and 8.30%). Linolenic and Stearic acid were present only in Hess variety though in low concentrations. As to volatile components, isolation of these components in Hess and Fuerte through solvent extraction in a soxhlet showed the presence of 22 aroma compound in Fuerte variety and only 8 in Hess variety. The influence of variety and region on the content and composition of lipids and volatiles in Iranian avocados has been tested.

Key words: Avocado · Fatty acids · GC · Soxhlet extraction · Volatiles · Fuerte · Hess

INTRODUCTION

The avocado (*Persia americana Mill*) belongs to the Lauraceae, a family of mainly (sub) tropical trees and shrubs; other well-known members are laurel, cinnamon, saffras and green-heart (a timber of the Guianas). The English name derives from the Spanish word abogada, which became avocet in French [1]. The avocado has been for thousands of years and still is, a popular food in Central America. It is a nutritious fruit but the sugar content is low; therefore, it can be recommended as a high energy food for the diabetic [1, 2]. Ancient civilizations all over the world have consumed this fruit, because of the beneficial unsaturated lipids and due to their high number cause the improvement of heart health and circulation of blood system. The chemical composition on the volatiles of avocado has been the subject of several studies [3, 4].

The chemical composition of the edible portion of the flesh fruit is water 65-80%; protein 1-4%; sugar about 1% and oil 3-30%. It is rich in vitamin B and moderately so in vitamins A and D. The oil, which is similar in composition to olive oil, is highly digestible. Because of the high oil content, avocados have the highest energy value of any fruit. The fatty acids found in the avocado mesocarp are long chain, with 16 carbon atoms or more [5].

Sinyinda and Gramshaw [6] considered volatiles of avocados. Sesquiterpenes were the predominant constituents present. The main component was β-caryophyllene and other volatiles consist of: Humulene, caryophyllene oxide, copaene, cadinene and farnesene. Gas chromatography is now the standard technique for detailed analysis of fatty acid (as fatty acid methyl esters) composition in avocados [5]. Avocado is an unusual fruit because its characteristic composition varies dramatically with the cultivar, time in the season, environment and variety. Thus, better knowledge of the variations in total lipid level and composition in the fruit is important for Iran.

The main objective of the present work is to determine the influence of variety and regional changes on the content and composition of lipids and volatiles in Iranian avocados. In this respect, the fatty acid composition of the extracted lipids was determined by gas chromatographic analysis of methyl esters of the

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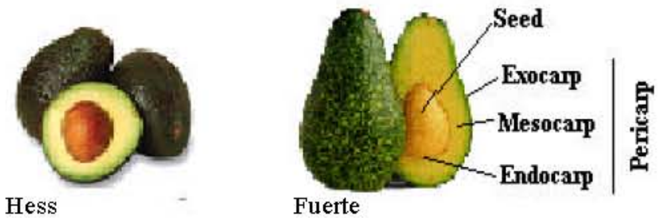

Fig. 1: Shapes of Hess and Fuerte avocados cultivated in North of Iran

derivatives of fatty acids. There is no research examining avocado lipids in Iran. In this research fatty acids and volatile aroma compounds in two varieties (Hess & Fuerte) of avocado fruit cultivated in Babol, Iran (Fig. 1), were determined and compared with each other.

MATERIALS AND METHODS

Fruits and Chemicals: Samples of avocados consist of Hess and Fuerte varieties collected from farm in North of Iran (Mazandaran, Babol) for the purpose of protection of similar region and climate conditions. All stages of sample preparation and analysis were carried out in a clean air environment. Prior to digestion or sample analysis, all glassware and plasticware were scrupulously cleaned, rinsed several times with double-distilled water and placed in a clean air environment until dry. All avocados were first cleaned and washed with water and then washed several times with double-distilled water. Both prepared varieties were sliced into small pieces and dried at 60°C for 48 h. Each solvent was distilled through a fractionating column before use. Methanolic potassium hydroxide and n-hexane, was supplied by Fluka chemicals and sodium sulfate was purchased from Merck chemicals.

Apparatus: Fatty acids and volatiles mesocarps of avocado fruits were extracted in soxhlet exelo 50/42. Sample size, 100 g of each variety of avocado mesocarp was sliced into small pieces and placed into an extraction thimble of soxhlet apparatus. Organic solvent, 250 ml n-hexane was used as an extracting agent. Extraction was carried out for 24 h and then the extracts were concentrated in a rotary evaporator and the avocado oil was separated. A greenish oil with faint odor and bland taste with D=0.92, Saponification value 179-195, Iodine value 70-92, refractive index 1.45-1.46 (40°C) was obtained. Oils were kept in samplers in an icehouse till analyzed. The extracts were analyzed using Shimadzu 9A gas chromatographs equipped with a flame ionization detector and linked gas chromatography-mass spectroscopy (GC-MS), employing a QP 2010 mass spectrometer. A bonded phase open tubular fused silica capillary column was employed (30 m × 0.25 mm i.d.). Ions were collected or scanned over the range m/z 400-17 mass units at 1 s decade^{-1}. Temperature program was used stating temperature at 50°C (2 min) and then increased by a rate of 5°C per minute till reach to final temperature to 250°C (40 min). Other operating conditions were: oven and injector temperatures, 50 and 250°C, respectively. Other devices were BP211D balance, electrothermal southend mantel and electrothermal furnace.

Identification by GC and GC-MS: Gas chromatography is the most common technique for detailed analysis of fatty acid (as fatty acid methyl esters) content of avocados [5]. It has been supposed that there were some compounds in the extractants which might be; thermally stable or thermally unstable but decomposed and have high molecular weight or containing polar functional groups. Therefore, we used chemical derivatization prior to analysis to increase the volatility and decrease the polarity of the compounds; reduce thermal degradation of samples by increasing their thermal stability; increase the volatility and decrease the polarity of compounds. Common derivatization methods can be classified into 4 groups depending on the type of reaction applied: Silylation, Acylation, Alkylation and Estrification.

Methylation of oils has been done by using a methanolic potassium hydroxide agent. For each variety we transferred about half ml (10 drops of oil) in a test tube, added 2 ml methanolic KOH and 8 ml of n-hexane as extracting solvent and then heated the sample in test tubes until two separated phases were obtained. The upper phase, the more transparent was methyl ester of fatty acids which has been separated from aqueous phase. The extracted oil was dehydrated by sodium sulfate and then, injected to GC and GC-MS for identification of volatile compounds.

RESULTS AND DISCUSSION

Fatty Acids Analysis in Avocado Mesocarps of Hess and Fuerte: In this research, the extracted fatty acids, methyl esters and the derivatives of fatty acids compositions were determined using gas chromatograph (Table 1 and Fig. 2). The analyzed samples were consisted of six fatty acids; 4 unsaturated fatty acids, i.e., oleic acid, palmitic acid, linoleic acid and palmitoleic acid and 2 saturated fatty acids, i.e., linolenic and stearic acids. The saturated fatty acids were in low quantities in Hess variety. The Fuerte sample contained only 4 unsaturated fatty acids as stated above. The percentage of oleic acid in both varieties were higher than other fatty acids. The percentages of oleic, palmitic and palmitoleic acids in Fuerte variety were higher than in Hess variety.

Table 1: The percentages of fatty acids for two varieties of avocados Hess and Fuerte

Type of fatty acid	Hass variety, Percentage	Fuerte variety, Percentage
Oleic acid	54.50	58.13
Palmitic acid	19.70	22.90
Linoleic acid	15.70	10.60
Palmitoleic acid	7.60	8.30
Linolenic acid	1.18	-
Stearic acid	1.05	-

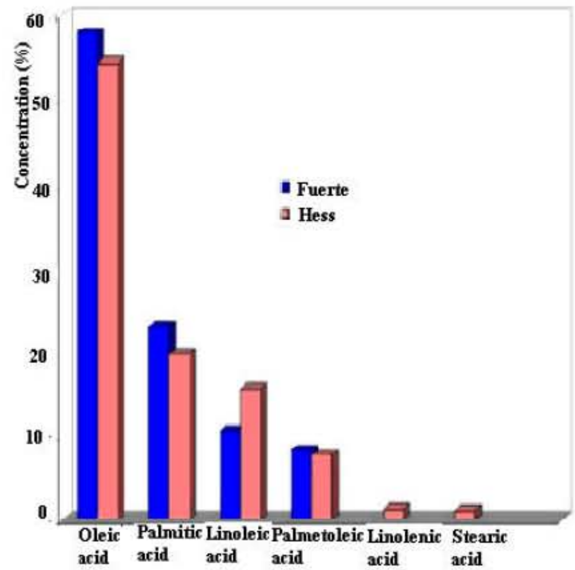

Fig. 2: Percentages of fatty acids analysis for two varieties of Hess and Fuerte avocados

Table 2: Volatile compounds identified by GC-MS from Hess avocado mesocarp

Number	Component name	Retention time
1	Alpha-Pinene	7.717
2	Beta-Pinene	9.283
3	Limonene	11.217
4	Gamma-Terpinene	12.367
5	Caryophyllene	23.567
6	Beta-Bisabolene	25.917
7	Gamma-Elemene	27.233
8	Caryophyllene Oxide	27.900

Table 3: Volatile compounds identified by GC-MS for Fuerte avocado mesocarp

Number	Component name	Retention time
1	Alpha-Pinene	N
2	Camphene	7.842
3	Beta-Pinene	8.825
4	Limonene	10.817
5	D-Limonene	10.842
6	Gamma-Terpinene	11.883
7	Terpinolene	12.842
8	Terpinene-4-ol	16.050
9	Alpha-Terpineol	16.533
10	Beta-Elemene	22.308
11	Trans-Alpha-Bergamotene	22.958
12	Caryophyllene	23.108
13	Alpha-Bergamotene	23.483
14	Alpha-Humulene	24.092
15	Alpha-Santalene	24.208
16	Gamma-Cadinene	24.875
17	Delta-Selinene	24.942
18	Beta-Selinene	25.175
19	Cis-Alpha-Bisabolene	25.317
20	Alpha-Farnesene	25.400
21	Beta-Bisabolene	25.483
22	Germacrene B	26.758

Volatile Compounds in Avocado Mesocarps of Hess and Fuerte: One il of fatty acid methyl esters from extracted oils of two varieties of avocado mesocarps cultivated in North of Iran were injected into GC-MS and retention time was reported for each peak. Tables 2 and 3 show lists of components obtained in Hess and Fuerte avocado mesocarps, respectively. The compounds are arranged with an increasing trend of the volatiles retention times. The constituents were identified by comparing their mass spectra with those data listed in the Wiley database and also that confirmed with many volatile compounds by their relative retention indices. In the Fuerte variety 22 aroma compounds, while only 8 aroma compounds in the Hess variety were obtained. It could be deduced that the structural composition of these fruits varies with variety and with climate as compared with those fruits cultivated in the other regions as reported in the literature [5-7]. In other words, there may be a regional or climatic effect influencing the synthesis and composition of lipids in fruits. Further research is necessary to determine the extent of the effects incorporated with compositions of fruits fatty acids.

CONCLUSION

The present investigation revealed that the compositions of fatty acids as well as the number of aroma in two major varieties of avocado, Hess and Fuerte were different. The differences may be attributed to the variety of the fruit and climatic condition in which it grows. The fruit shows great variability in weight and chemical composition, depending on the variety of the avocados and the climatic conditions.

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