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Changes in the n-alkane composition of avocado pulp oil (*Persea americana*, Miller) during fruit ripening

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RESUMEN

Evolución de los hidrocarburos lineales saturados en el aceite de la pulpa de aguacate (*Persea americana*, Mill.) a diferentes estados de maduración.

Se ha analizado la composición en hidrocarburos lineales saturados del aceite de la pulpa de aguacate (variedad Hass). Tres muestras fueron recolectadas: el 3 de marzo 2003, el 18 de marzo 2003 y el 2 de abril 2003. La separación de los hidrocarburos lineales saturados se realizó mediante fraccionamiento del insaponificable por cromatografía gravimétrica de adsorción en columna y la determinación de los mismos hidrocarburos por cromatografía gaseosa. 14 compuestos fueron detectados del *n*-C_{21} al *n*-C_{34}. El *n*-C_{24} fue el mayoritario, seguido del *n*-C_{25} y el *n*-C_{23}. El porcentaje de *n*-C_{21}, *n*-C_{22}, *n*-C_{23}, *n*-C_{27} y *n*-C_{28}, aumentó durante la maduración, mientras que el porcentaje de *n*-C_{24}, *n*-C_{25}, *n*-C_{26}, *n*-C_{29}, *n*-C_{30} y C_{34} disminuyó desde el 3 de marzo 2003 hasta el 2 de abril 2003. Los hidrocarburos lineales saturados con número impar de átomos de carbono aumentaron (52.38%, 52.85% y 53.06% por las tres muestras respectivamente) con la maduración. Por el contrario, hidrocarburos lineales saturados con número par de carbono disminuyeron (47.62%, 47.15% y 46.94%). El contenido total en hidrocarburos lineales saturados disminuyó durante la maduración desde 25.20 mg/kg (3 de marzo 2003) hasta 16.77 mg/kg (2 de abril 2003). Para reducir el contenido total en hidrocarburos lineales saturados en el aceite de aguacate (variedad Hass) es necesario retardar la cosecha del fruto.

PALABRAS-CLAVE: Aceite vegetal – Aguacate – Alcanos – Hidrocarburos lineales saturados – Persea americana.

SUMMARY

Changes in the n-alkane composition of avocado pulp oil (*Persea americana*, Mill.) during fruit ripening.

The n-alkane composition of Avocado pulp oil (cv. Hass) was investigated during fruit ripening. Three samples of fruit were harvested on March 3, 2003, March 18, 2003 and April 2, 2003. Glass gravity column chromatography was employed to separate n-alkanes from other minor components contained in the unsaponifiable fraction. Gas chromatography was used to analyze the eluate. Fourteen compounds were detected ranging from *n*-C_{21} to *n*-C_{34}; mainly *n*-C_{24}, followed by *n*-C_{25} and then by *n*-C_{23}. Quantities of *n*-C_{21}, *n*-C_{22}, *n*-C_{23}, *n*-C_{27} and *n*-C_{28} progressively increased during ripening, whereas *n*-C_{24}, *n*-C_{25}, *n*-C_{26}, *n*-C_{29}, *n*-C_{30} and *n*-C_{34} decreased from the first harvest date to the third harvest date. While odd-numbered carbon n-alkanes increased (52.38%, 52.85% and 53.06% for the three samples respectively), even-numbered carbon n-alkanes decreased as the fruit ripened (47.62%, 47.15% and 46.94%). The total n-alkane content decreased during ripening, from 25.20 mg/Kg (first harvest date) to 16.77 mg/Kg (third harvest date). In order to minimize the n-alkane content in avocado pulp oil it is necessary to delay the fruit harvest date.

KEY-WORDS: n-Alkane – Avocado – Linear hydrocarbon – Persea americana –Vegetable oil.

1. INTRODUCTION

Climatic conditions characterizing the province of Reggio Calabria (Southern Italy) permit the cultivation of a large number of subtropical species. The avocado tree grows and fructifies easily if protected against strong wind. The oil extracted from the edible fruit is utilized for industrial purposes, especially in cosmetics. However, the principal utilization of the fruit is for fresh consumption. Avocado fruit contains a large quantity of oil. In the past, a great deal of attention has been paid to oil extraction (Martinez et al., 1988; Martinez et al., 1992; Bizimana et al. 1993;) and to avocado oil quality (Fedeli et al., 1967; Lozano et al., 1993; Martinez et al., 1994; Poiana et al., 1996; Poiana et al., 1999). In order to improve the studies on avocado pulp oil, new attention has been focused on the minor components of this vegetable oil. Minor components contained in the unsaponifiable fraction determine the quality of the oil. A high content of hydrocarbons with chain length > C_{29}, reduces the quality of vegetable oil. This happens because the short chain hydrocarbons are well absorbed by the mammalian small intestine. The shorter the hydrocarbon chain length, the easier the absortion. n-Alkanes of carbon number > C_{29} are not significantly absorbed. In this perspective, as avocado fruit is largely employed for human consumption, it becomes very important to know the hydrocarbon content in the avocado pulp oil as well as the hydrocarbon chain length. Little attention has been focused on n-alkanes of edible vegetable oils. There seems to be no previous studies on n-alkanes of avocado oil. In previous works, McGill (McGill A.S. et al., 1993) have found n-alkanes ranging quantitatively from 7 mg/Kg to 166 mg/Kg, in edible oils of the market. Neukom, (Neukom et al. 2002), have found that in edible vegetable oils more than 50 mg/Kg of mineral paraffins could be found, originating from extraction plants or from air pollution.

The aim of this study was to determine the n-alkane compounds present in the unsaponifiable fraction of avocado pulp oil (cv Hass) cultivated in the province of Reggio Calabria. Changes occurring in n-alkanes during fruit ripening were also determined.

2. EXPERIMENTAL

2.1. Plant Material

Avocado harvesting was conducted at three different dates, at biweekly intervals from March 3, when fruit was unripe, until April 2, 2003. The last sampling took place 3 weeks before full ripeness. Two fruits per plant were randomly collected from four 10-year-old trees, growing at Gioia Tauro, in the Reggio Calabria province (Italy).

2.2. Reagents

The n-alkanes used as standards were obtained from Sigma-Aldrich (Milan, Italy) and TCI (Tokyo, Japan), and all other reagents were of analytical grade and purchased from VWR International Merck (Darmstadt, Germany).

2.3. Oil extraction

To obtain the oil, 100 g of pulp per fruit was gently pestled in a mortar. Then the homogenate was treated with anhydrous sodium sulphate, and the oil was extracted for seven hours with petroleum ether by means of a Soxhlet apparatus. For the first five hours, the procedure was carried out without heating and the subsequent two hours with the temperature of the Soxhlet apparatus set at the boiling point of petroleum ether (60°C). The solvent was evaporated from the oil under vacuum in a rotary evaporator, and finally a stream of nitrogen was bubbled to eliminate the residual ether. The oil was dried over anhydrous sodium sulphate and stored at room temperature in a dark glass bottle. A stream of nitrogen was also used to eliminate the oxygen present in the space of the bottle, in order to prevent oxidative phenomena.

2.4. Analytical Procedures

Fatty matter, acidity and peroxide value were determined following the Official European Community Methods for olive oil analysis (E.E.C., 1991).

Isolation and determination of n-alkanes. The extraction and analysis of n-alkanes were conducted following the Official European Community Method for stigmastadienes in olive oil (E.E.C., 1995), modified as follows: the preparative chromatography was carried out using a glass gravity column packed with a 2 mm layer of anhydrous sodium sulphate and silica gel. After packing, n-hexane was used to elute any possible contaminating n-alkanes. One millilitre of a 1mg/mL solution of undecane ($C_{11}H_{24}$) was added as internal standard to a 250 mL round-bottom flask and dried under nitrogen. Subsequently, a 20 g aliquot of avocado oil was placed in the same flask and submitted to the saponification procedure, as prescribed in E.E.C., (1995).

Two millilitres of the unsaponificable fraction were loaded on the silica chromatographic column and eluted with 120 mL of n-hexane. The eluate was concentrated to ca. 1 mL on a vacuum rotary evaporator at 20°C. An aliquot (0.5 μL) was analyzed on a 3000 Series GC provided by Fisons Instruments, Milan, Italy, equipped with an on column injector, a SE 54 fused silica capillary column (25 m x 0.32 mm i.d. x 0.25 μm film thickness; Mega, Milan, Italy) and a FID. The oven temperature was programmed at 60°C for 1 min and then at 5°C/min up to 290°C (40 min). The detector temperature was maintained at 310°C. The eluting compounds were identified by comparison with the retention times of the authentic standards under identical experimental conditions. Response factors for all components were determined by injecting a complete pool of standards in a preliminary essay. n-Alkanes contents were reported as the mean of three replicates and expressed as mg of n-alkane per Kg of avocado pulp oil.

3. RESULTS

The Table I shows that the oil content of avocado pulp, its acidity and peroxide value increased during fruit ripening, whereas the unsaponifiable fraction decreased. The glass gravity column chromatography used in this study has been proven able to separate n-alkanes from the other components of the unsaponifiable fraction. The n-alkane composition of the avocado pulp oil is presented in Table II. Fourteen compounds were detected ranging from 21 to 34 carbon atoms. The total n-alkane content in the avocado pulp oil decreased a 33.45% from the first (March 3) to the last harvest (April 2), from 25.20 mg/Kg to 16.77 mg/Kg. Although in a higher magnitude, this reduction is consistent with the mentioned decrease of the unsaponifiable fraction. During ripening, odd-carbon n-alkanes increased, while even-carbon n-alkanes decreased. At all picking dates, *n*-C_{24} was the major component, followed by *n*-C_{25} and *n*-$C_{23,}$ being *n*-C_{34} and *n*-C_{32} the least representative compounds.

Results showed that the later the fruit was harvested, the lower the total n-alkane content found in the avocado pulp oil.

As the avocado oil was extracted by means of a Soxhlet apparatus, there was no possible contamination with paraffins originating from extracting machinery.

Table I
Physical and chemical characteristics of avocado pulp oil at different stages of ripening

	First Harvest Date 3 March 2003	Second Harvest Date 18 March 2003	Third Harvest Date 2 April 2003
Oil % of the whole fruit	13.77 ± 0.18	14.96 ± 0.11	15.01 ± 0.12
Oil % of the pulp	20.22 ± 0.14	21.12 ± 0.15	21.43 ± 0.12
Unsaponifiable matter (%)	1.16 ± 0.03	1.12 ± 0.04	1.02 ± 0.03
Acidity (as oleic acid %)	0.47 ± 0.00	0.64 ± 0.01	0.67 ± 0.01
Peroxide value (meq O_2/Kg)	2.99 ± 0.09	3.03 ± 0.03	3.62 ± 0.04

Data represent the mean ± standard deviation of three measurements.

Table II
n-Alkane composition of Avocado pulp oil at different stages of ripening

	First Harvest Date 3 March 2003		Second Harvest Date 18 March 2003		Third Harvest Date 2 April 2003	
	mg/Kg	%	mg/Kg	%	mg/Kg	%
n-C_{21}	0.64 ± 0.05	2.54	0.49 ± 0.05	2.66	0.45 ± 0.05	2.68
n-C_{22}	1.85 ± 0.05	7.34	1.37 ± 0.06	7.44	1.28 ± 0.07	7.63
n-C_{23}	3.64 ± 0.09	14.44	2.72 ± 0.07	14.77	2.50 ± 0.05	14.91
n-C_{24}	4.54 ± 0.13	18.02	3.30 ± 0.08	17.93	2.96 ± 0.09	17.66
n-C_{25}	4.20 ± 0.05	16.67	2.99 ± 0.08	16.24	2.69 ± 0.05	16.04
n-C_{26}	2.90 ± 0.07	11.51	2.03 ± 0.00	11.03	1.80 ± 0.09	10.73
n-C_{27}	2.27 ± 0.10	9.01	1.73 ± 0.05	9.40	1.63 ± 0.02	9.72
n-C_{28}	1.05 ± 0.05	4.17	0.82 ± 0.07	4.45	0.76 ± 0.04	4.53
n-C_{29}	1.30 ± 0.10	5.16	0.94 ± 0.02	5.11	0.86 ± 0.02	5.13
n-C_{30}	0.88 ± 0.12	3.49	0.62 ± 0.06	3.37	0.57 ±0.05	3.40
n-C_{31}	0.84 ± 0.04	3.33	0.62 ± 0.08	3.37	0.55 ± 0.04	3.28
n-C_{32}	0.49 ± 0.07	1.94	0.35 ± 0.06	1.90	0.33 ± 0.04	1.97
n-C_{33}	0.31 ± 0.06	1.23	0.24 ± 0.05	1.30	0.22 ± 0.06	1.31
n-C_{34}	0.29 ± 0.08	1.15	0.19 ± 0.04	1.03	0.17 ± 0.04	1.01
Total	**25.20 ± 0.45**	**100.00**	**18.41 ± 0.08**	**100.00**	**16.77 ± 0.11**	**100.00**
Σodd carbon numbers	13.20 ± 0.21	52.38	9.73 ± 0.11	52.85	8.90 ± 0.07	53.06
Σeven carbon numbers	12.00 ± 0.25	47.62	8.68 ± 0.14	47.15	7.87 ± 0.07	46.94
odd carbon/even carbon	1.10 ± 0.84		1.12 ± 0.82		1.13 ± 0.98	

Data represent the mean ± standard deviation of three measurements.

The average n-alkane content calculated on the fresh pulp, before oil extraction, per 100 g of avocado pulp, decreased as follows: 0.51 mg, 0.39 mg, and 0.36 mg from the first to the third sampling, respectively. No high chain length n-alkanes (C_{40}-C_{50}) characterizing a mineral origin (Neukom et al., 2002), were found.

4. CONCLUSIONS

Glass gravity column chromatography worked out for stigmastadienes separation in olive oil, well provided for n-alkanes separation of avocado pulp oil.

The study of n-alkanes together with other minor components of unsaponifiable fraction may be useful to better characterize the oil extracted from this fruit.

The n-alkane content of avocado pulp oil is comparable to the n-alkane content of other edible vegetable oils.

Further data are necessary in order to know the n-alkane composition of avocado pulp oil extracted from fruits of trees growing in different climatic

conditions. More varieties of avocado ought to be investigated.

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REFERENCES

Bizimana V., Breene W.M., Csallany A.S. 1993. Avocado oil extraction with appropriate technology for developing countries. *J. Am. Oil Chem. Soc.* **70**, 821-822.

EEC Commission Regulation n. 2568/91 Official Journal n° L 248 september 5, 1991.

EEC Commission Regulation n. 656/95 Official Journal n° L 69 march 28, 1995.

Fedeli E., Lanzani A., Jacini G. 1967. Composizione dell'insaponificabile dell'olio di avocado (*Persea americana*). *Riv. Ital. Sost. Grasse* **44**, 519-523.

Lozano Y.F., Dhuique Mayer C., Bannon C., Gaydou E.M. 1993. Unsaponifiable matter, total sterol and tocopherol contents of avocado oil varieties. *J. Am. Oil Chem. Soc.* **70**, 561-565.

Martinez Nieto L., Camacho Rubio F., Rodriguez Vives S., Moreno Romero M.V. 1988. Extracción y caracterización del aceite de aguacate. *Grasas Aceites* **39**, 272-277.

Martinez Nieto L., Barranco Barranco R., Moreno Romero M.V. 1992. Extracción de aceite de aguacate: un experimento industrial. *Grasas Aceites* **43**, 11-15.

Martinez Nieto L., Moreno Romero M.V. 1994. Compositión esterólica de la fracción insaponificable del aceite de aguacate de distintas variedades. *Grasas Aceites* **45**, 402-403.

Mc Gills A. S., Moffat C.F., Mackie P.R, Cruickshank P. 1993. The composition and concentration of n-alkanes in retail samples of edible oils. *J. Sci. Food Agric.* **61**, 357-362.

Neukom H.P., Grob K., Biedermann M., Noti A. 2002. Food contamination by C_{20}-C_{50} mineral paraffins from the atmosphere. *Atmospheric Environment* **36**, 4839-4847.

Poiana M., Giuffrè A.M., Mincione B., Gattuso A.M., Monastra F. 1996. Ricerche sulla frutta esotica – Avocado (Persea Americana, Miller). Caratterizzazione della frazione lipidica. *Riv. Ital. Sost. Grasse* **73**, 107-114.

Poiana M., Giuffrè A.M., Mincione B., Giuffrè F. 1999. L'olio di avocado. Evoluzione dei costituenti della frazione lipidica nel corso della maturazione dei frutti di alcune cultivar coltivate nel Mezzogiorno d'Italia. *Riv. Ital. Sost. Grasse* **76**, 257-275.

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