The Structure of the Glycerides of Pistachio Kernel Oil

ATAOLLAH DANESHRAD, Faculty of Engineering, University of Teheran, Teheran, Iran

ABSTRACT

Pistachio kernel oil has been analyzed by separating the triglycerides according to their degree of unsaturation by means of thin layer chromatography on silica gel plates impregnated with silver nitrate. The total fatty acids and the fatty acid on position 2 of each individual triglyceride were determined. The distribution of oleic acid on the different positions of triglycerides varies according to the unsaturation of these triglycerides. Oleic acid in the case of the less unsaturated triglycerides locates in the 2-position, and, as the degree of unsaturation increases due to linolenic or linoleic acids, it tends to be on external positions. Linoleic and linolenic acids usually are esterified in the middle position.

INTRODUCTION

Recent studies on intraglyceride distribution of fatty acids by lipase-catalyzed deacylation have revealed a selective distribution of fatty acids in lipids of oil seeds (1-4). Such oils most commonly contain palmitic, stearic, oleic, linoleic, and linolenic acids, and its seems that the secondary hydroxyl group is acylated by the unsaturated C_{18} acids in preference to the saturated acids (1). Different studies made on the glyceride structure of rape oil (1,5,6,7) show that monounsaturated fatty acids of over 18 carbon atoms behave like saturated fatty acids. However, the unsaturated acids such as eicosenoic (20:1) and docosenoic (22:1) generally accompany the saturated acids in the 1- and 3-positions (1). Oleic, linoleic, and linolenic acids do not, in fact, compete equally for the 2-position, and linoleic acid is usually found at this position slightly more than oleic or linolenic acids (8,9). These widely accepted generalizations are based on results obtained from many plant species and from investigations conducted in several laboratories.

Investigations carried out on pistachio oils, have revealed fatty acid composition (10-12) and unsaponifiable content (10). In this paper the distribution of fatty acids in the triglycerides of pistachio oil has been investigated.

MATERIALS AND METHODS

The oil was extracted from the pistachio kernel by petroleum ether (40-60 C b.p.) in a soxhlet apparatus for 2 hr. The extract was filtered, and the solvent was evaporated under reduced pressure. The triglycerides were separated from the more polar substances by column chromatography over aluminum oxide which had been activated at 26 C for 2 hr (13); the solvent used was petroleum ether.

The pancreatic lipase was prepared and purified from fresh pancreas of pork by the method of Marquis et al. (14).

Hydrolyses of the triglycerides were carried out following the method used by Luddy et al. (15) on small quantities of triglycerides. In this method pancreatic lipase brings about the preferential hydrolysis of fatty acids in the terminal positions of triglycerides.

Triglyceride (30 mg) was weighed in a 10 ml stoppered centrifuge glass tube. Pancreatic lipase (12 mg) and 1.2 ml 1 M trishydroxymethylaminomethane buffer, pH 8, were added while shaking the mixture gently. Sodium cholate (bile salts) (0.3 ml 0.1% solution) and 0.12 ml calcium chloride (22%) solution were then added to the contents of the centrifuge tube. The stoppered tube and its contents were warmed in a water bath at 40 ± 0.5 C for 1 min, while shaking slowly. The tube was then removed from the water bath and shaken vigorously for 2 min. The tube was cooled, and the contents, were acidified with 1 ml (6N) hydrochloric acid and extracted with ethyl ether. The ether extract was washed with distilled water and evaporated; the residue was preserved for separation and analysis.

Thin layer chromatography (TLC) was carried out on 20 x 20 cm plates coated with 0.5 mm acetone washed Silica Gel G (E. Merck). For the separation of the triglycerides of the original oil, 20% silver nitrate was added to the silica gel. The plates were made and then activated according to the method used by Stahl (16). The solvent system used for separation was composed of benzene and ethyl ether (90:10). Compounds were made visible by spraying the plates with an ethanolic solution (0.2%) of 2,7-dichlorofluorocein and viewing under UV light. After scraping the different bands from the plates, triglycerides were eluted by ethyl ether.

The monoglycerides produced by pancreatic lipase hydrolysis were separated from other glycerides and free fatty acids by developing TLC plates with a mixture of petroleum ether and ethyl ether (70:30) to which 1.5% formic acid was added (13,15). The components were separated into four well-defined zones. The monoglycerides were collected and extracted from the lowest zones (around the base line) by ethyl ether.

TABLE I

Fatty Acid Composition (mol %) of Unfractionated Triglycerides of Pistachio Kernel Oil and Triglyceride Fractions Separated by Thin Layer Chromatography

Fatty acid	Unfractionated triglyceride	Fraction I	Fraction II	Fraction III	Fraction IV	Fraction V	Fraction VI
$C_{14:0}$	0.1	2.9	0.1	—	—	—	0.6
$C_{16:0}$	10.4	48.6	16.4	8.3	6.6	5.7	2.6
$C_{16:1}$	1.1	1	6	2.2	1.1	1	0.6
$C_{18:0}$	1	7.3	2.1	0.8	0.4	0.4	0.1
$C_{18:1}$	58	38.2	59.1	66.5	61.6	49.4	22.4
$C_{18:2}$	29.1	1	16.2	21.2	30.3	38.1	60.6
$C_{18:3}$	0.2	1	0.1	1	—	5.2	13
$C_{20:0}$	0.1	—	trace	—	—	—	0.1
Proportion of different fractions (%)		5.1	19.8	24.2	25.5	18.8	6.6

TABLE II

Fatty Acid Composition (Mol %) of 2-Monoglycerides (MG) and the Percentage of Fatty Acid Esterified in the 2-Position

Fatty acid	Unfractionated oil		Fraction I		Fraction II		Fraction III		Fraction IV		Fraction V		Fraction VI	
	2-MG	% 2-Position	2-MG	% 2-Position	2-MG	% 2-Position	2-MG	% 2-Position	2-MG	% 2-Position	2-MG	% 2-Position	2-MG	% 2-Position
C14:0	—	—	4.2	48.3	—	—	—	—	—	—	—	—	—	—
C16:0	1.3	4.1	18.6	2.7	4.9	8.9	1.3	5.2	1.1	5.5	5.2	30.4	2.5	32
C16:1	0.6	18.2	—	—	1.9	10.5	1.3	19.6	0.7	21.2	0.5	16.6	1	55.5
C18:0	0.1	3.3	18.7	85.4	2.5	41.2	trace	—	0.3	25	1	83.3	0.1	33.3
C18:1	62.9	36.1	58.5	51	80.4	4.5	77.2	38.7	57.6	31.2	39.4	26.6	16.5	24.5
C18:2	34.9	39.9	—	—	9.9	20.3	18.8	29.6	40.3	44.3	53.4	46.6	74.3	40.8
C18:3	0.2	0.2	—	—	0.2	66	1.3	43.3	trace	—	0.5	3.2	5.8	14.3
C20:0	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Methyl esters of fatty acids of the original oil and the separated glycerides were prepared by transmethylation catalyzed by p-toluene sulfonic acid (17).

Gas liquid chromatography analysis of methyl esters was carried out on a Varian Aerograph 2800 double column, with flame ionization detector. The column was a 3m x 2.5 mm ID stainless steel coiled tube packed with 60-80 mesh Chromosorb W coated with 20% ethylene glycol succinate polyester. The temperatures were 185 C for the columns, 210 C for injectors, and 220 C for detectors. The flow rate of nitrogen carrier gas was 25 ml/min.

RESULTS AND DISCUSSION

Triglyceride Fractions of Oil

The pistachio kernel triglycerides were separated by TLC on silica gel plates impregnated with silver nitrate to six fractions called fraction I to VI. Fraction I, close to solvent front, was the least unsaturated. Fraction VI was the most unsaturated and remained on the base line.

Fatty acid compositions of the unfractionated triglycerides and of each the six fractions are shown in Table I. In fraction I, which is monounsaturated, fatty acids of the triglycerides consisted mainly of palmitic and oleic acids. This fraction contains the triglycerides of the types SSO and SOS (18). [Symbols used for fatty acid: S = saturated fatty acids ($C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{20:0}$); O = monounsaturated fatty acids ($C_{16:1}$, $C_{18:1}$); L = diunsaturated acids ($C_{18:2}$); Le = triunsaturated acids ($C_{18:3}$).

In fractions II, III, and IV, which are the major ones, the amount of saturated fatty acids decreases from fractions II to IV, respectively, while linoleic acid increases. The types of triglycerides in these fractions are di-, tri-, and tetra-unsaturated, respectively. The principal constituents of fraction II are SOO, OSO; fraction III, OOO, SLO, SOL; and fraction IV, OLO, OOL, SLL.

In fractions V and VI there is a decrease in the amount of saturated fatty acids and oleic acid while linoleic and linolenic acids increase. The main triglycerides of the last two fractions are five and six unsaturated compounds respectively. The types of triglycerides in fraction V are OLL, LOL, and the types in fraction VI are LLL and OLe L.

Monoglycerides Produced from Pancreatic Hydrolysis

Table II summarizes the fatty acid composition of the 2-monoglycerides. The percentage of these fatty acids were also calculated according to Mattson and Volpenhein (1) as follows:

$$\%\ \text{fatty acid in the middle position} = \frac{\%\ \text{fatty acid in the 2-monoglycerides}}{3x\ \%\ \text{this fatty acid in the triglycerides}} \times 100$$

In fraction I oleic acid and stearic acid are esterified in the middle position. In spite of the fact that almost half of the fatty acid composition of triglycerides in fraction I (48.6%) are composed of palmitic acid (Table I), only a small amount of this acid is esterified in position 2; that means, normally it is esterified in positions 1 and 3.

In fractions II, III, IV, V, and VI almost all the middle positions are occupied by unsaturated acids (oleic and linoleic acids). In these fractions, as the amount of oleic acid decreases, linoleic acid increases. For example, in fraction V fatty acids esterified in the 2-position consist of 39.4% oleic acid, 53.4% linoleic acid; and in fraction VI these quantities are 16.5% for oleic acid, 74.3% for linoleic acid, and 5.8% for linolenic acid.

Summing up, the distribution of oleic acid in the pistachio kernel oil depends on the total unsaturation of tri-

glycerides. When the degree of unsaturation is less than three double bonds, the $C_{18:1}$ is more likely to be esterified in the middle position. When unsaturation of triglyceride is more than three double bonds, it will be esterified in the external positions, and internal position will be occupied by linoleic and linolenic acids. It seems that with equal chain length, the degree of unsaturation determines the type of distribution. This agrees well with the results obtained by Catalano (19) on olive oil.

Linoleic and linolenic acids, regardless of the unsaturation of triglycerides, usually occupy the middle position. This conclusion agrees with the results obtained by Appelqvist and Dowdell (20) and Sergiel (7).

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