NEUTRAL LIPID COMPOSITION OF OIL FROM TWO VARIETIES OF OIL PALM IN INDIA

Keywords : Oil palm varieties; Mesocarp; Neutral lipids; Glycerides; TLC; GLC; Fatty acids; Sterols; *Elaeis guineensis* Jacq.

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The neutral lipids were isolated from palm oil from dura and pisifera varieties of Elaeis guineensis *grown in India. On examination by TLC, they were found to be composed of triacylglycerols (87.7% and 89.8% respectively), diacylglycerols (4.5%, 3.8%) monoacyglycerols (1.2%, 1.0%), free fatty acids (4.0%, 3.0%) and sterol esters (1.6%, 1.4%), along with hydrocarbons and free sterols (1.0%, 1.0%).*

GLC analysis of the sterols and sterol esters revealed the presence of β-sitosterol, stigmasterol, campesterol and brassicasterol, identified by reference to the relative retention time values of the standards. GLC analysis of the triacylglycerols, diacylglycerols, monoacyglycerols, free fatty acids, and sterol esters showed the presence of mainly palmitic and oleic acids, along with some stearic, linoleic and linolenic acids.

INTRODUCTION

In our earlier communications, a quantitative study of the phospholipids and glycolipids in oil from the *dura, pisifera and tenera* varieties of oil palm in India was reported (Kulkarni *et al.*, 1991a, 1991 b and 1991c; Khotpal *et al.*, 1991; Bhakare *et al.*) Some other communications on minor components of palm oil have also appeared (Goh *et al.*, 1985; Goh *et al.*, 1991).

In the present investigation, neutral lipids from the *dura* and *pisifiera* varieties of *Elaeis guineensis* grown in India have been analyzed for their components, the fatty acid composition of the lipid materials and the composition of the sterol fraction for the first time.

EXPERIMENTAL

Materials

Palm fruits from the two varieties were obtained from Oil Palm (I) Ltd., Kottayam, India. All the solvents used were of analytical grade (Merk-Darmstadt, Germany) and were used without further purification. TLC plates (20 x 20 cm) were coated with silica gel G (0.25 mm thick) in the laboratory. The reference standards were obtained from the Sigma Chemical Corporation and Analabs of the USA.

PROCEDURE

Isolation of total lipids

The total lipids (TL) were extracted from the mesocarp of the palm fruits with chloroform-methanol (2:1, v/v) by the procedure of Folch *et al.* (1957).

Separation of neutral lipids

Six grams of the TL fraction were eluted successively with chloroform, methanol and acetone from a silica gel column (4x70 cm) using 2:1, 4:1 and 2:2:1 solvent to column ratio. The neutral lipids (NL) were eluted in chloroform, and then characterized by TLC and photodensitometry (Blank *et al.*, 1964).

TLC analysis of neutral lipid fraction

The NL fraction was analyzed by TLC using the solvent system: petroleum ether (60°C-80°C) - diethyl ether-acetic acid (90:10:1, v/v/v) (Mangold and Malilns, 1960) and the behaviour of the components in the fraction with different detection reagents (Helverson and Quist, 1974). Seven classes of lipids were identified, namely triacylglycerols (TG), diacylglycerols (DG), monoaclyglycerils (MG), free fatty acids (FFA), free sterols (S), sterol esters (SE) and hydrocarbons (H).

The TG, DG, MG, FFA, S and SE fractions were further fractionated by TLC against reference standards.

The sterols (5 mg) were also converted into their trimethyl silyl ether (TMS) derivatives (Pierce, 1967). These were analyzed by GLC using a glass column (0.3 x 200 cm) packed with 1.5% OV-17 on Chromosorb -(60–100 mesh) at 250°C. Nitrogen was used as the carrier gas at 20 ml/min. The detector temperature was 280°C. Under these conditions, the retention time of β–sitosterol was 30 minutes (*Figure 1*). The sterol esters were saponified (Miyazawa *et al.*, 1974) with 0.4 N KOH in methanol (4.5 ml) for 4 hours at 37°C; the mixture was then cooled and chloroform (4 ml) and water (2 ml) were added. The sterols in the chloroform layer were converted to their TMS derivatives and analyzed by GLC. The aqueous methanol layer was acidified to pH 1.2 with concentrated HCl. Fatty acids were extracted using hexane. These were converted to methyl esters and subsequently analyzed by GLC.

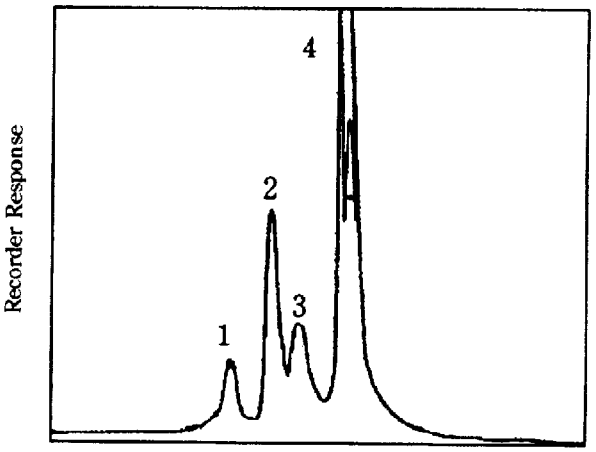

Figure 1. GLC profile of free sterols from an oil palm variety (dura).

1 = Brassicasterol 2 = Campesterol
3 = Stigmasterol 4 = ß-diyodyrtol

The authentic reference standards for the sterols were also converted to their TMS derivatives by the procedure of Pierce (1967) and analyzed by GLC under similar conditions. Comparison of the relative retention time values with those of the sterol fractions separated confirmed the identity of the latter.

GLC analysis of fatty acid methyl esters (FAME)

The fatty acids from TG, DG, MG, FFA and SE were converted into their respective FAME by the method of Kulkarni *et al.*, (1991a). The FAME were analyzed by GLC using a Perkin-Elmer Gas Chromatography equipped with a glass column packed with 15% EGSS-X on Chromosorb-W (40–60 mesh) and a flame ionization detector at 280°C.

The conditions were: chart speed 60 cm/hr, injection port temperature 300° C and column temperature 200° C, with a nitrogen flow rate of 60 ml/min. The peak area and the percentage of FAME were obtained by the integration method with the help of a computer. The component of each peak was identified on the basis of retention data compared with those of the authentic standards.

RESULTS AND DISCUSSION

Samples of mesocarp from the fruit of *dura* and *pisifera* varieties of oil palm grown in India were found to contain 45.7% and 50.2% respectively of total lipid (TL). Silica gel fractionation of TL showed the major portion to be NL (87.4% and 89.7%). When the NL fraction from the (*Table 1*) two varieties were analyzed by TLC and photodensitometry, the major components were found to be triacylglycerols (TG) (87.7% and 89.8%) followed by diacylglycerols (DG) (4.5% and 3.8%), free fatty acids (FFA) (4.0% and 3.0%), sterol esters (SE) (1.6% and 1.4%), monoacylglycerols (MG) (1.2% and 1.0%) along with free sterols and hydrocarbons (1.0% and 1.0%). Palmitic and oleic acids were the major fatty acids in the NL fraction, along with linoleic acid (*Table* 1). The fatty acid composition of the monoacylglycerols and diacylglycerols of palm oil is being reported for the first time, although Goh and Timms (1985) determined the content of mono- and diglycerides. Sterol esters have also been analyzed for their fatty acids content for the first time. The sterol composition of the oil from the two varieties (*Table 2*) showed that β-sitosterol (70.1% and 69.5%) were the major sterols present, followed by campesterol, stigmasterol and brassicasterol. Brassicasterol was detected for the first time in palm oil, but cholesterol, which was reported to be present in palm oil in very small amounts (Goh *et al.*, 1985), could not be detected in the oil from our samples. Nevertheless this study does provide increased knowledge concerning the neutral lipids of oil from the two varieties we studied.

TABLE 1. NEUTRAL LIPIDS OF OILS FROM OIL PALM VARIETIES AND THEIR FATTY ACID COMPOSITION

Variety	Neutral[a] Lipid Component	Weight[b] %	Fatty acids[b] (Weight%) 16:0	18:0	18:1	18.2	18:3	Others[c]
Dura	NL	87.4	50.4	3.2	28.8	12.3	0.7	4.6
	TG	87.7	53.0	3.2	30.1	11.0	0.6	2.1
	DG	4.5	60.4	2.6	24.1	10.2	0.4	2.3
	MG	1.2	43.6	6.2	22.4	26.7	0.8	0.3
	FFA	4.0	38.0	5.8	24.6	25.9	1.0	4.7
	SE	1.6	43.7	4.7	25.4	21.8	1.2	3.2
	S,H	1.0	—	—	—	—	—	—
Pisifera	NL	89.7	50.7	4.2	30.7	13.5	0.7	0.2
	TG	89.8	52.5	4.0	31.0	12.1	0.4	—
	DG	3.8	60.2	3.2	26.2	10.4	—	—
	MG	1.0	45.2	6.4	22.5	24.7	1.2	—
	FFA	3.0	40.2	8.4	22.1	22.9	0.8	5.6
	SE	1.4	41.2	4.8	24.8	25.4	1.0	2.8
	S,H	1.0	—	—	—	—	—	—

[a]NL – Neutral lipids, TG – Triacylglycerols, DG– Diacylglycerols, MG–Monoacyglycerols, FFA – Free fatty acids, SE– Sterol esters, S, H- Free Sterol and hydrocarbons.

[b]Means of triplicate analysis. 'Others' include 12:0, 14:0, 20:0, 22:0 and 24:0 fatty acids.

TABLE 2. COMPOSITION (weight %) OF THE STEROL FRACTION FROM OIL PALM VARIETIES

Variety	Sterols[a]			
	I (Peak I)	II (Peak II)	III (Peak III)	IV (Peak IV)
Dura	3.2	18.4	8.3	70.1
Pisifera	4.3	19.8	6.4	69.5

[a]I – Brassicasterol (RRT = 0.70)
II – Campesterol (RRT = 0.81)
III – Stigmasterol (RRT = 0.88)
IV – β-sitosterol (RRT = 1.00)
RRT– Relative retention times, with retention time of β-sitosterol (30 min) taken as 1.00.

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