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Studies on the Lipid and Protein Composition of Guava Seeds (*Psidium guajava*)

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ABSTRACT

Chloroform–methanol extracted lipids of guava seeds amounted to 9·1% on a dry weight basis. Physical and chemical properties of the extracted oil are presented. Gas–liquid chromatographic analysis of the methyl esters of the fatty acids of the oil revealed the presence of twelve fatty acids. The fatty acid pattern obtained was generally similar to that of cottonseed oil. The unsaponifiable matter was also analysed by GLC which showed the presence of eight fractions.

The protein content of guava seeds was 9·73% on a dry weight basis. Qualitative and quantitative analyses revealed the presence of fifteen amino acids.

INTRODUCTION

The composition of the major biochemical constituents in the seeds of guava is of fundamental importance to an understanding of how such seeds could be utilised. Opute (1978) reported that guava seeds contained 9·4% lipids. He indicated that such lipids consisted almost entirely of neutral compounds, mainly triglycerides. Aly (1981) found that guava seeds contained 8·9% oil which was characterised by: $n^{26}C$, 1·4756; $d^{26}C$, 0·915; acid value, 0·52; iodine value, 124; saponification value, 190·2 and 1·09% unsaponifiable matter.

Unsaponifiable matter was isolated with diethyl ether and GLC

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fractionated which gave 2·77% cholesterol, 3·01% campesterol, 56·7% β-sitosterol, 6·55% Δ^7-avenasterol and 2·85% hydrocarbons ($C^{30} + C^{32}$). Five unidentified fractions accounted for 19·42%.

It is clear from the literature that data on the biochemical composition of guava seeds are scarce and limited to physical and chemical characteristics of the oil and some other constituents. The investigation described in this paper was performed to study the fatty acid, sterol and amino acid composition and other major biochemical constituents of guava seeds in order to evaluate their possible utilisation.

MATERIAL AND METHODS

Guava seeds were obtained from the fruit after blending. The seeds were then washed, air dried and finely ground.

Oil extraction and determination

The oil of guava seeds was extracted with $CHCl_3$: MeOH (2:1), as mentioned by Bligh & Dyer (1959). The oil content was determined according to the method of Dhopershwarker & Head (1961).

Specific gravity and refractive index were estimated at 26°C according to the AOAC (1970). Acid value, iodine value and saponification value were determined according to methods described by Dee Snell & Biffen (1972).

Gas–liquid chromatographic analysis of the oil

The total fatty acid fraction, unsaponifiables and standard materials were methylated using an ethereal solution of diazomethane as mentioned by Vogel (1975). The methyl esters of fatty acids and unsaponifiables obtained from guava seed oil samples and standard materials were analysed with a GVC Unicam gas chromatograph equipped with a dual flame ionisation detector.

The separation of fatty acid methyl esters was conducted using a coiled glass column (1·5 m × 4 mm) packed with 10% polyethylene glycol adipate (PEGA). The column was operated isothermally at 190°C with nitrogen at 30 ml/min. Detector and injection temperatures were 220°C and 200°C, respectively. The unsaponifiable matter was also separated

on a coiled glass column (2.8 m × 4 mm) packed with Diatomite C (100–120 mesh) and coated with 1% OV–17. The column was maintained at 270°C and the flow rate of nitrogen was 30 ml/min. Detector and injection temperatures were 300°C and 280°C, respectively. Hydrogen and air flow rates were 33 ml/min and 330 ml/min, respectively.

Peak identification was performed by comparing the relative retention time of each peak with those of standard materials. The relative retention times of methyl palmitate and β-sitosterol are given a value of 1·00. The peak area was measured by triangulation and the relative proportions of the individual compounds were, therefore, obtained by determining the partial areas in relation to the total area. The linear relationship between log retention times of the standard monoenoic and dienoic acids and the number of carbon atoms of these compounds was used to characterise 14:1 and 15:1 fatty acids in the lipids studied, as stated by Farag *et al.* (1983).

Determination of total proteins

This was performed using the modified micro Kjeldahl method as described by the AOAC (1980). Protein content was calculated by multiplying the N value by the conversion factor (6·25).

Determination of amino acids

The amino acids were determined in defatted guava seeds, after 6N HCl hydrolysis; this was carried out according to the method of Moore *et al.* (1958) using a Beckman Amino Acid Analyzer (Model 121). Type PA–35 custom spherical resin and type M–82 spherical ion exchange resin were used for basic, neutral and acidic amino acid determinations, respectively.

RESULTS AND DISCUSSION

Physical and chemical properties of the oil extracted with $CHCl_3$: MeOH(2:1) from guava seeds are presented in Table 1. The oil content was 9·1%

The saponification value of guava seed oil was quite similar to that of most vegetable oils whilst Itoh *et al.* (1973) reported a saponification value of 190·7 for soybean oil. The iodine value of guava seed oil was

TABLE 1
Physical and Chemical Properties of the Oil Produced from Guava Seeds Compared with Soybean Oil

Property	*Density (26°C)*	*Refractive index (n = 26°C)*	*Saponification value*	*Acid value*	*Iodine value*	*Unsaponifiables (%)*
Guava seed oil	0·921	1·4763	191	0·54	124	1·37
Soybean oil	0·920	1·4766	192	0·20	132	0·67

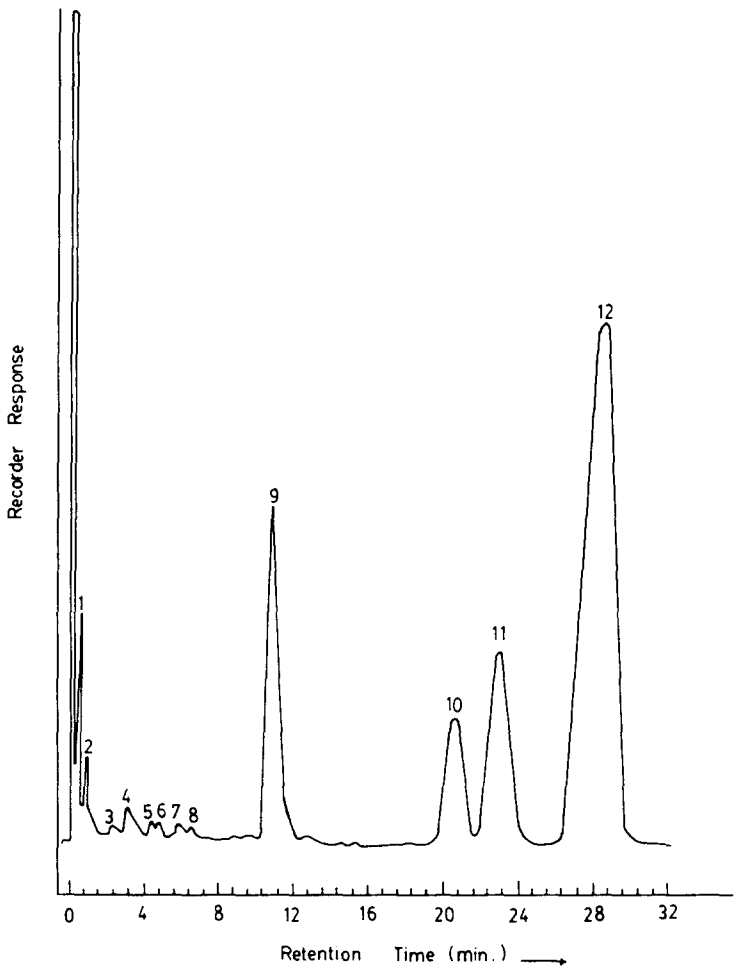

Fig. 1. A GL chromatogram of the fatty acid fractions of guava seed oil.

TABLE 2

The Relative Concentration of Fatty Acids present in Guava Seed Oil compared with Fatty Acids in Cottonseed Oil (GLC separated).

Type of oil / *Fatty acid*	*0:8*	*10:0*	*12:0*	*13:0*	*14:0*	*14:1*	*15:0*	*15:1*	*16:0*	*16:1*	*18:0*	*18:1*	*18:2*	*DU*[a]	*TU/TS*[b]
Guava seed	2·41	1·18	0·76	1·46	0·89	0·85	1·20	0·76	13·3	—	11·1	14·0	52·1	1·20	2·0
Cotton seed	1·24	1·10	0·73	0·93	1·33	—	0·00	0·53	19·0	0·88	7·1	26·0	41·2	1·10	2·1

[a] DU, Degree of unsaturation = 1 (Per cent monoenes/100) + 2 (Per cent dienes/100) as stated by Farag *et al.* (1983).

[b] TU/TS, Ratio between the total unsaturated fatty acids and the total saturated fatty acids.

also quite similar to those of many other vegetable oils. It has been mentioned by Itoh *et al.* (1973) that the iodine value of soybean oil is 134·6, that of wheat germ oil 134·4 and that of sunflower oil 135·3.

Fatty acid composition of guava seed oil

It is important to examine the lipid composition of guava seed. Table 2 and Fig. 1 show the fatty acid composition of oil produced from guava seeds. The relative concentration of various fatty acids can be divided into groups, i.e. trace (< 1 %), minor (> 1 %– < 10 %) and major (> 10%) components.

The fatty acids 12:0, 14:0, 14:1 and 15:1 constituted the trace acid components in guava seed oil. The minor fatty acids in guava seed oil were 15:0, 13:0, 10:0 and 8:0. Palmitic, stearic, oleic and linoleic acids were found in major concentrations. Table 2 also indicates that the pattern of fatty acids of guava seed oil is similar to that of cottonseed oil GLC separated under the same conditions.

Composition of unsaponifiable matter of guava seed oil

Unsaponifiable matter was isolated from guava seed oil with diethyl ether and subjected to GLC analysis. Table 3 and Fig. 2 show that eight

TABLE 3

The Relative Concentrations and Relative Retention Times of Fractions of the Unsaponifiable Matter of Guava Seed Oil

Peak No.	*RT (min)*	*RRT*[a]	*Fraction (%)*	*Identification*	*Itoh* et al. *(1973)*	
					RRT	*Identification*
1	5·6	0·31	24·0	C_{31}		
2	7·5	0·41	14·5	C_{32}		
3	9·3	0·51	3·76	C_{33}		
4	10·9	0·60	3·99	Cholesterol		
5	12·5	0·69	13·4	—	0·69	Brassicasterol
6	15·9	0·88	5·03	—	0·88	Stigmasterol
7	18·0	1·00	30·7	β-Sitosterol		
8	21·6	1·20	4·96	—	1·18	Δ⁷-Stigmasterol

[a] RRT indicates the relative retention times of fractions. The relative retention time of β-sitosterol (18·0 min) is given a value of 1·00.

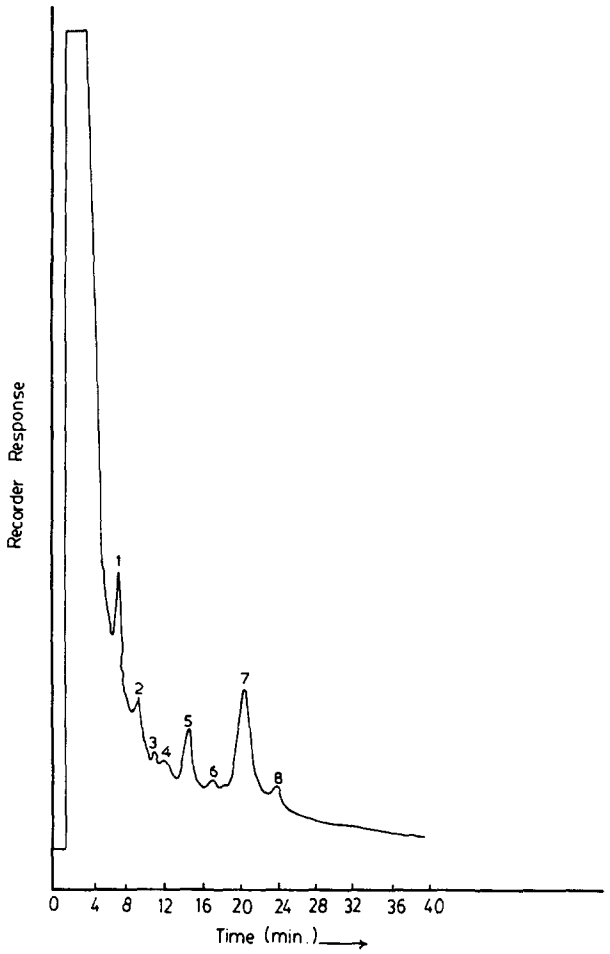

Fig. 2. A GL chromatogram of unsaponifiable matter extracted from guava seed oil with petroleum ether. Oil was extracted with chloroform–methanol(2:1).

fractions were detected at different relative retention times (RRT) and concentrations in the unsaponifiables (UNS). Five fractions were identified by comparing their RRTs with those of standard materials GL chromatographed under the same conditions. They are C_{31}, C_{32}, C_{33}, cholesterol and β-sitosterol and existed in relative concentrations of 24·0%, 14·5%, 3·76%, 3·99% and 30·7%, respectively. The other three fractions were primarily designated brassicasterol (peak 5), stigmasterol (peak 6) and Δ^7-stigmasterol (peak 8) by comparing their RRTs with those of the corresponding ones reported by Itoh *et al.* (1973).

TABLE 4
Amino Acid Composition of Guava Seed Protein

Amino acid	*Arg.*	*Glu.*	*Asp.*	*Gly.*	*Leu.*	*Val.*	*Ser.*	*Tyr.*	*Phe.*	*Ala.*	*His.*	*Isoleucine*	*Pro.*	*Thr.*	*Lys.*	*Total*
mg/16 g N	2060	1770	800	700	590	340	340	330	330	290	290	280	240	190	180	8730
Per cent of total amino acids	23·6	20·3	9·16	8·01	6·75	3·89	3·89	3·78	3·78	3·32	3·32	3·20	2·75	2·17	2·06	99·9

These fractions were determined in percentages of 13·4, 5·03 and 4·96 in the same order as mentioned above. Hydrocarbons ($C_{31} + C_{32} + C_{33}$) represent 42·2% of total UNS fractions. β-sitosterol constituted 30·7% of the total fraction and more than half of the sterols present. It should be mentioned that cholesterol, which is recognised as the characteristic sterol of animal fats, is present in considerable amounts (3·99%). Cholesterol has also been reported in many other vegetable oils, e.g. coconut oil, palm oil, linseed oil (1%), palm kernel oil (3%), cocoa butter (2%) and as a trace in several other vegetable oils, as indicated by Itoh *et al.* (1973). Farag *et al.* (1981) reported cholesterol in two varieties of Egyptian onion oils at concentrations of 4·43–5·78%. El-Tahawi *et al.* (1982*a*, *b*; 1983) indicated the presence of cholesterol in soybean oil (3·83%), corn oil (1·69%) and peach seed kernel oil (2·78%).

These results indicate that the unsaponifiable matter composition of oil produced from guava seeds follows almost the same pattern as that of many other vegetable oils.

Amino acid composition of guava seed protein

The total protein content of guava seed was found to be 9·73% of the dry weight and 10·7% in defatted seeds. The protein was acid hydrolysed and the hydrolysate subjected to analysis using an amino acid analyser. The data obtained are recorded in Table 4. These revealed the presence of fifteen amino acids in the protein of guava seeds. These are arranged in decreasing order as follows: arginine, glutamic acid, aspartic acid, glycine, leucine, valine, serine, tyrosine, phenylalanine, alanine, histidine, isoleucine, proline, threonine and lysine. The major amino acids in guava protein were arginine, glutamic acid, aspartic acid, glycine and leucine. These major acids constituted about 67% of the total amino acids present in the protein of guava seed.

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