Pak. j. sci. ind. res., vol.38, nos.11-12, November-December 1995

LIPID STUDIES OF *SAPODILLA PLUM*

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(Received May, 17 1995; revised August 5, 1996)

The air dried seeds of *Sapodilla plum* contain moisture 6.0%, hulls 51.7%, and kernels 42.3%. The hulls contain oil 0.45%, ash 0.77%, protein 2.05% (in defatted hulls) whereas the kernels contain oils 19.8%, ash 6.50% and protein 16.93% (in defatted kernels). The fatty acid composition of dark yellow coloured hull oil is lauric acid (5.38%), myristic acid (3.30%), palmitic acid (26.14%), stearic acid (9.3%), oleic acid (39.46%), linoleic acid (13.93%), and linolenic acid (2.36%) whereas light yellow coloured kernel oil contains palmitic acid (17.24%), stearic acid (10.14%), oleic acid (60.40%), linoleic acid (9.30%), linolenic acid (2.03%) and an unknown fatty acid (0.89%) respectively. The unsaponifiable matter is determined as sterols (23.7% and 20.1%) alcohols (32.5% and 33.3%) hydrocarbons (43.8% and 46.6%) in hull and kernel oil respectively. The hydrocarbons C_{15} - C_{29} (in kernel oil), alcohols C_{12} - C_{18} (in hull oil) and C_{16} - C_{18} (in kernel oil) respectively have been separated, identified and characterized by the application of thin layer and gas liquid chromatography. Palmityl and oleyl alcholos are found to predominate in both the oils.

Key words: *Sapodilla plum,* Hulls, Kernels

Introduction

Sapodilla plum locally known as "Chiku" of the Sapotaceae family is used as a fruit in India, Pakistan and in some other countries. Previous studies have shown the presence of ascorbic acid and sugars in the fruit [1,2]. The medicinal importance of *Sapodilla plum* fruit is well established for diseases [3-5] like diarrhoea and paludism. Its seeds are known to be aperient and diuretic and the bark is reputed to be tonic and febrifuge. As no detailed lipid studies have been reported, studies of the physical values and fatty acid composition of the hull and kernel oils have been carried out. The alcohols and hydrocarbons present have also been characterized. The physical values and fatty acid composition of the oils support their being edible oils.

Experimental

Extraction of the oil. Air dried dark brown seeds of the *Sapodilla plum* were broken, separated into hulls and kernels, dried in oven at 105°C for 2 hrs and crushed. The kernels (100 gm) were placed in a Soxhlet apparatus and oil extracted with distilled hexane [6]. The hexane extracts were freed from moisture over anhydrous sodium sulphate and filtered. The solvent was removed by distillation and the yellow coloured oil (19.8gm) was kept under nitrogen. The oil (0.45%) from 100 gm hulls was also extracted similarly.

Physico-chemical values of the oil. Physico-chemical values were determined according to the procedure of British Standard Specifications 684 [7]. Protein value was determined by Kjeldahl's method.

Methylation of oil. Methyl esters of the oils were prepared with BF_3 methanol reagent [8] and purified by thin layer chromatography.

Separation of non-saponifiable matter. The non-saponifiable matter (200 mg) was separated [9] by silica gel thin layer chromatograms (20 x 20 cm) of thickness 0.25 mm into hydrocarbons (67 mg), alcohols (40 mg), sterols (93 mg) with the use of hexane/diethyl ether (1:1 v/v) as a solvent system. The locating reagent 2,7-dichlorofluorescein was used which under uv at 254 nm showed purple yellow coloured bands of separated materials. The R_f values of hydrocarbons, alcohols and sterols are 0.65,0.45 and 0.25 respectively. Thus the band of hydrocarbons is near the solvent front, that of alcohols is in the middle and sterols form band near the base. The respective bands were scrapped and taken in separate conical flasks. The hydrocarbons were extracted with hexane (6 x 60 ml), alcohols and sterols were extracted with chloroform (6 x 60 ml) separately. The solvents were then removed by distillation.

Identification of sterols. The saturated solution of antimony trichloride in chloroform was used for the qualitative check-up of sterols as well as sterol esters. By spraying this solution on sterol spot on thin layer chromatogram and by heating it at 100°C for 10 min., the red violet spot developed (R_f value 0.25) confirmed the presence of sterols [10].

Acetylation of alcohols. The alcohols (100 mg) were stirred with acetic anhydride (8 ml), pyridine (24 ml) at room temperature for 18 hrs [11]. The acetates of alcohols were extracted with diethyl ether (3 x 75 ml) after the addition of distilled water (60 ml). The acetates were thoroughly washed with distilled water (4 x 60 ml) to remove pyridine and acetic

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acid (boh being soluble in water) and were freed from moisture over anydrous sodium sulphate prior to their recovery after the distillation of diethyl ether. The alcohol acetates were purified by thin layer chromatography.

Gas liquid chromatography of methyl esters of oil. Methyl esters of the oil were analyzed for the respective fatty acid composition by gas chromatography on a Shimadzu GC 14A apparatus equipped with a capillary column of PEG (25 m x 0.2 mm id). The temperature was programmed at 180°C for 3 min with rise of 2°C/min to 220°C. Injection and detector temperatures were 250° and 300°C respectively with a flow rate of 2 ml/min of nitrogen as the carrier gas. The methyl esters were identified by comparing their retention times with those of authentic methyl esters under the same conditions. The percentage of various fatty acids was determined with Shimadzu C-R4A chromatopac computing integrator and reported in Table 2.

Analysis of hydrocarbons and alcohols as acetates. The composition of the respective hydrocarbons and alcohols were determined by gas chromatography on a Shimadzu GC-14A apparatus equipped with FID and a capillary column of SE-30 (25 mm x 0.2 mm i.d). The temperature programming of the column oven was 150°C for 5 min and then with a rise of 5°C/min to 300°C with nitrogen as the carrier gas and flow rate of 2 ml per min. The injector and detector temperatures were 250 and 300°C respectively. The peaks recorded on Shimadzu C-R4A chromatopac were identified by comparing their retention times with those of the standard samples.

Results and Discussion

The dried and defatted hulls and kernels of *Sapodilla plum* seeds contain protein 2.05, and 16.93%, ash 0.77 and 6.5%, oil 0.45 and 19.8% respectively. The percentage of oil in kernels is reasonably good for economical extraction. Physico-chemical values (Table 1) are determined by British Standard Specification No. 684 and fatty acid composition (Table 2) is found out by the application of thin layer and gas liquid chromatographies. The hull and kernel oils contain saturated fatty acids (44.25 and 27.38%), and unsaturated fatty acids (55.75 and 72.62%) respectively. Both the oils contain the highest percentage of oleic acid (in hull oil 39.46% and in kernel oil 60.40%) than other fatty acids present in respective oils . The separate work on the hull and kernel oils of *Sapodilla plum* seeds is not available in the literature, however, previous workers have claimed the presence of oleic acid as the highest than other fatty acids in the whole seed oil [12] which supports our work. The literature survey [13] raveals that the oil contains non-saponifiable (1.8%) and according to present work non-saponifiable matter is 1.20 and 1.06% in hull and kernel oils respectively. The characterization of non-saponifiable matter has not been done previously. The non-saponifiable matter of both the oils consists of sterols 23.7 and 20.1%, alcohols 32.5 and 33.3% whereas hydrocarbons 43.8 and 46.6% respectively. The infrared spectrophotometry shows the presence of alcohols by absorption at 3600 cm^{-1} stretching whereas alcohols as acetates show absorption at 1240 cm^{-1} (C-O stretch of acetates). The alcohols as acetates after purification are characterized by gas liquid chromatography (Table 3). The sterols are separated by thin layer chromatography and presence of sterols is confirmed by the colour test [4]. The mixture of hydrocarbons (C_{15}-C_{29}) resolved and identified by using Shimadzu C-R4A chromatopac computing integrator detector (Table 4). Among the

TABLE 1. PHYSICO -CHEMICAL CHARACTERIZATION OF THE *SAPODILLA PLUM* SEED OIL.

		Kernels %	Hulls %
1.	Moisture	6.0	7.5
2.	Ash	6.5	0.77
3.	Protein	16.93	2.05
4.	Oil	19.8	0.45
5.	No-saponifiable matter	1.06	1.2
6.	Iodine value	67.0	63.5
7.	Saponification value	173.6	180.0
8.	Ester value	170.15	177.83
9.	Free Fatty acids	1.74	1.09
10.	Specific gravity	0.895	-
11.	Refractive index at 33°C	1.4638	1.4642

TABLE 2. FATTY ACID COMPOSITION OF THE *SPODILLA PLUM* SEED OIL.

	Fatty acids	Hulls %	Kernel %
1	$C_{12:0}$	5.38	-
2.	$C_{14:0}$	3.30	-
3.	$C_{16:0}$	26.14	17.24
4.	$C_{18:0}$	9.43	10.14
5.	$C_{18:1}$	39.46	60.40
6.	$C_{18:2}$	13.39	9.30
7.	$C_{18:3}$	2.36	2.03
8.	Unknown	-	0.89

TABLE 3. FATTY ALCOHOLS OF *SAPODILLA PLUM* SEED OIL.

Alcohol acetates		Hulls%	Kernels%
1.	$C_{12:0}$	6.4	-
2.	$C_{14:0}$	5.3	-
3.	$C_{16:0}$	19.9	18.1
4.	$C_{18:0}$	11.6	13.0
5.	$C_{18:1}$	35.8	54.2
6.	$C_{18:2}$	18.1	12.1
7.	$C_{18:3}$	2.9	2.6

TABLE 4. HYDROCARBONS OF *SAPODILLA PLUM* SEED OIL.

Chain	length	Percentage
1.	$C_{15:0}$	9.4
2.	$C_{19:0}$	4.5
3.	$C_{21:0}$	3.4
4.	$C_{23:0}$	65.8
5.	$C_{24:0}$	3.9
6.	$C_{25:0}$	6.2
7.	$C_{27:0}$	2.5
8.	$C_{29:0}$	4.3

hydrocarbons ranging from C_{15} to $C_{29,}$ the percentage of $C_{23:O}$ (65.8%) is the highest found out. It is usual pattern of hydrocarbon distribution in the vegetable kingdom. The highest percentage of oleyl alcohol (54.2%) and oleic acid (60.4%) having the same chain length C_{18} is supported by the theory of biosynthesis of fatty alcohols and fatty acids in nature [14].

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