Effects of Processing on Physicochemical Properties and Fatty Acid Composition of *Hibiscus sabdariffa* Seed Oil

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ABSTRACT

Expeller pressed crude *Hibiscus sabdariffa* seed oil (mesta oil) is dark and most unappealing. On refining, the appearance of the oil is improved. The unusual fatty acids present in the *H. sabdariffa* seed oil, cyclopropene acids (malvalic $C_{18}H_{32}O_2$ and sterculic $C_{19}H_{34}O_2$) and epoxy oleic acid ($C_{18}H_{32}O_3$), are not reduced by refining. Partial hydrogenation did not reduce the epoxy oleic acid, but it eliminated the cyclopropene acids. Heating for 10 min was sufficient for complete elimination of cyclopropene acids, but heating up to 60 min did not affect the epoxy acid.

INTRODUCTION

The deteriorating edible oil situation and the consequent high price and low per capita consumption in India have stimulated the search to identify new sources of oil seeds and render them safe for human consumption. In this context *H. sabdariffa* seed has attracted the attention of agriculturists and oil technologists. The seeds of species *Hibiscus cannabinus* and *Hibiscus sabdariffa*, of the family Malvales, contain about 18–20 percent oil. The crude oil extracted from the seed is dark in appearance, with high free fatty acid content and unsaponifiable matter (1). The oil contains 3 unusual fatty acids, namely the cyclopropene fatty acids (CPFA), malvalic and sterculic, and epoxy oleic acid (2–6).

The information regarding the effect of alkali refining of crude, partially hydrogenated and heated refined oil is scanty. This study, therefore, gives an understanding of the effect of processing on the physicochemical nature and fatty acid composition of *H. sabdariffa* seed oil in general and of the unusual fatty acids in particular.

MATERIALS AND METHODS

The expeller pressed oil from the AMV-I variety of *Hibiscus sabdariffa* species was used in the study. The crude oil was analyzed for physical characteristics, namely refractive index, specific gravity, color, viscosity and smoke temperature. The chemical characteristics studied were free fatty acids (FFA), iodine value (IV), saponification value (SV), unsaponifiable matter and peroxide value, using official methods of AOCS (7). To detect the presence of the 2 cyclopropene fatty acids in the oil, the Halphen test was used.

The crude oil was then alkali refined by adopting the conditions recommended for cotton seed oil, since both *H. sabdariffa* and cotton seed oils contain cyclopropene fatty acids. The physicochemical nature and the Halphen test response of the refined oil also were determined.

The fatty acid composition of the refined oil was determined by preparing stable derivatives of the fatty acid methyl esters (8, 9). A Varian Model 3700 gas chromatograph, fitted with a flame ionization detector, was used for the analysis of the fatty acids. The stationary phase was 15% DEGS coated on 100–120 mesh Chromosorb 'W' packed in a 6 ft × 3 mm stainless steel column. The temperatures of the column (200 C), the detector (250 C) and the injection port (210 C) were maintained throughout the analysis period. Nitrogen was the carrier gas, with a flow rate of 30 ml per min. The peaks were identified by comparing with a standard fatty acid mixture and *Sterculia foetida* oil methyl esters.

Detection of Epoxy Acid (10): The epoxy acid in the *H. sabdariffa* seed oil was detected by loading the methyl esters of the oil sample on thin layer chromatography plates (20 × 20 cm) coated (250 μ) with a slurry of silica gel-G (ACME). After activation, the methyl esters of the oil samples and the epoxidized fat (*Shorea robusta*) reference were loaded on the plates using glass capillary tubes and were developed for one hour in a solvent system containing petroleum ether, solvent ether and acetic acid (75:25:1). The plates were removed and sprayed with 0.025 M picric acid. Immediately after spraying, the plates were put in a chamber saturated with a second solvent system containing solvent ether, ethanol and acetic acid (80:20:1, v/v/v). After 30 min the plates were removed and exposed to ammonia vapors. The presence of orange spots indicated the epoxy compounds.

Quantitation of Epoxy Acid: The quantitative determination of epoxy oleic acid was carried out according to the microtitrimetric procedure (11).

Partial Hydrogenation of Refined *H. sabdariffa* Seed Oil: Refined *H. sabdariffa* seed oil, about 500 g, was partially hydrogenated in a 1-l stainless steel autoclave fitted with a revolving type stirrer set at 440 rpm, using appropriate amounts of Rufert nickel catalyst (16% Ni). The hydrogen gas pressure of 30 psi at 180 C was maintained throughout. Samples of the oil were collected at 15-min intervals to test the Halphen response of the partially hydrogenated oil after filtration. The reaction was stopped at 1½ hr of hydrogenation, when the Halphen response of the oil was negative.

Heat Treatment: About 500 g of the refined oil was heated to smoke in a deep frying pan, and the smoke temperature was recorded. Just at smoke temperature and after 10, 20, 30, 40 and 60 min of heating, while maintaining the smoke temperature, 50 ml samples of the oil were taken, cooled and analyzed for physicochemical characteristics. The Halphen response of the oil, fatty acid composition and epoxy oleic acid content were determined for each sample.

RESULTS AND DISCUSSION

The physicochemical characteristics and the Halphen response of the crude (expeller pressed) and alkali refined *H. sabdariffa* seed oil are given in Table I.

Alkali refining caused very slight differences in the refractive index and the specific gravity of the oil. The color of the crude oil changed from amber to light yellow. Considerable reduction in the viscosity of the oil was observed on refining. There was a marked increase in the smoke temperature of the soil. The free fatty acids and unsaponifiable matter decreased to a great extent with refining. Both crude and refined oils were positive to the Halphen test, revealing the presence of cyclopropene fatty acids. The process of refining could not modify the Halphen test response, and the same was reported earlier (1). The fatty acid composition and the levels of unusual fatty acids in the refined oil are shown in Table II.

The fatty acid composition of the refined *H. sabdariffa* seed oil was found akin to the fatty acid composition of most commonly used edible oils with a high ratio of unsaturated to saturated fatty acids. The process of refining did not cause any reduction in the levels of unusual fatty

TABLE I

Physicochemical Properties of Crude and Refined Oils

Characteristics	Crude Oil	Refined Oil
Physical		
Refractive index (30 C)	1.4581	1.4684
Specific gravity	0.9280	0.9218
Color (Y + R + B) (Lovibond 1" cell)	20.0 + 5.7 + 3.2	20.0 + 1.1
Viscosity (centipoise)	61	52
Smoke temperature (C)	132	188
Chemical		
Free fatty acids (as oleic acid %)	2.8	0.1
Iodine value (Wijs')	103.8	103.0
Saponification value	N.D.	194
Unsaponifiable matter (%)	1.39	0.96
Peroxide value (meq O_2/kg oil)	N.D.	Nil
Halphen test	+	+

+ = positive. N.D. = not determined.

TABLE II

Fatty Acid Composition of Refined and Heated *H. sabdariffa* Seed Oils (% of Total Methyl Esters)

Fatty Acids	Refined Oil	Heated Oil	
		30 min	60 min
Myristic	Trace	Trace	Trace
Palmitic	15.0	16.0	17.5
Stearic	Trace	Trace	Trace
Oleic	29.6	36.0	38.5
Linoleic	49.0	47.5	43.5
Malvalic	0.8	Nil	Nil
Sterculic	3.5	Nil	Nil
Linolenic	2.0	Nil	Nil
Epoxy oleic acid	2.77	2.78	2.80

acids. The same observation also was recorded earlier (1, 12) for refined *H. sabdariffa* seed oil.

Heat Processing Effects on Physicochemical Characteristics: The effects of heat processing of the oil are indicated in Table III. All measurements also were carried out at 20 and 40 min of heating, and data were equal to or between those presented for 10, 30 and 60 min.

Heating the oil even up to 1 hr did not grossly affect the physical nature of the oil except for a small increase in color and viscosity. The Halphen test was negative after 10 min of heating. Heating the oil to 1 hr resulted in a slight reduction in iodine value and a small increase in peroxide value.

The fatty acid composition of the refined oil heated for 30 and 60 min is indicated in Table II, along with refined *H. sabdariffa* seed oil. The ratio of unsaturated to saturated fatty acids was lowered gradually with the increase in the period of heating. This change is attributed mainly to the total loss of linolenic acid and a reduction in linoleic acid, which was reported earlier for other edible oils (4, 13, 14). The GLC analysis for fatty acid methyl esters of the heated oil did not indicate presence of cyclopropene fatty acids since they are heat labile.

Partial Hydrogenation—Effect on Cyclopropene Fatty Acids (CPFA): Partial hydrogenation of the refined *H. sabdariffa* seed oil at 180 C for 1¼ hr gave a positive Halphen response with a trace of red color, and further heating to 1½ hr gave a negative response, showing the destruction of the cyclopropene groups. The effect of heat on CPFA was reported earlier (6, 15).

It can be concluded that unrefined or crude *H. sabdariffa* seed oil is not acceptable for consumption, due to its dark appearance, high free fatty acid level and viscosity. The process of refining favorably modified the appearance and the physicochemical nature of the oil, similar to many commonly used edible oils. CPFA could be eliminated with heating and partial hydrogenation. Though large quantities of edible oils are used as salad oils in developed countries, in most Indian homes the oils generally are heated before

TABLE III

Physicochemical Characteristics of Heated Oil

Characteristics	At smoke temp. (180 C)	Heating time (min)		
		10	30	60
Physical				
Refractive index (30 C)	1.4684	1.4684	1.4684	1.4684
Specific gravity	0.9218	0.9218	0.9228	0.9228
Color (Y + R) (Lovibond 1" cell)	20 + 1.1	20 + 1.1	20 + 1.2	20 + 1.4
Viscosity (centipoises)	52	52	52	53
Chemical				
FFA (oleic acid %)	0.1	0.1	0.1	0.1
Iodine value (Wijs')	103	103	102	101
Saponification value	194	194	194	194
Peroxide value (meq O_2/kg oil)	Nil	Nil	Nil	1.5
Halphen test	(+)	(−)	(−)	(−)
Epoxy oleic acid (%)	2.77	2.77	2.78	2.80

(+) = Positive. (−) = Negative.

consumption. Hence, CPFA could not be a problem as these are eliminated in the cooking process. But epoxy oleic acid could not be eliminated with any of the processing techniques. It is present in small quantities only, and its level can be reduced further by blending with common cooking oils. Processing of *H. sabdariffa* seed oil thus may render it suitable for human consumption.

ACKNOWLEDGMENTS

The Andhra Pradesh Agricultural University provided the opportunity to do the research and I.C.A.R. provided financial assistance. Part of the work was done in the Regional Research Laboratory and National Institute of Nutrition.

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[Received September 6, 1983]

Determination of Mono- and Diglycerides in Palm Oil, Olein and Stearin

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ABSTRACT

Partial glycerides are important constituents of palm oil and can have significant effects on the physical properties of products containing palm oil or on the fractionation of palm oil. A method is described for their routine determination in palm oil. By analysis of 28 weekly composite samples of crude palm oil the following results were obtained: free fatty acids, mean = 3.76%, range 2.4 to 4.5%; monoglycerides, mean = 0.28%, range 0.21 to 0.34%; diglycerides, mean = 6.30%, range 5.3 to 7.7%. During detergent fractionation of palm oil, diglycerides concentrate in the palm olein, but monoglycerides concentrate in the palm stearin. Palm fatty acid distillate was found to contain approximately 3% each of mono- and diglycerides. Because the refining and fractionation processes are continuous in the refinery, it is not possible to follow a single identifiable batch of crude palm oil through the refinery. To circumvent this problem, crude palm oil, stearin and olein from the refinery were bleached and steam refined in the laboratory and the partial glyceride contents determined at each stage of processing. Except for fractionation, the content of glycerides did not change during processing. For oil, olein and stearin, monoglycerides were reduced significantly both after bleaching and after steam refining.

INTRODUCTION

Partial glycerides are important constituents of oils, especially palm oil, and they can have significant effects on physical properties. The lifetimes of α polymorphs in palm oil (1, 2, 3) and in shea fat (4) were influenced significantly by the level of diglycerides in the oils. Palm oil is unusually rich in partial glycerides (5), and their level is probably commercially important since partial glycerides also affect the solid fat contents at all temperatures. Hernqvist and Anjou (6) have used diglycerides successfully to stabilize the β' polymorph in margarines containing hydrogenated rapeseed and soyabean oils. In a margarine stored at 20 C, development of the β polymorph could be delayed from four to 44 weeks by the addition of 5% diglycerides.

Monoglycerides are used at low levels, typically 0.3%, to stabilize the oil/water emulsion in margarine. Palm oil naturally contains this level of monoglycerides (5). Although refining reduces the content of monoglycerides, the surface active properties of monoglycerides also are important in the detergent fractionation of crude palm oil to yield palm olein and stearin.

In this paper we report the use of a gas liquid chromatographic (GLC) method for the study of partial glycerides in crude, fractionated and refined palm oils. Trimethylsilyl (TMS) derivatives are prepared prior to GLC analysis. Similar analytical methods using various silylating and GLC procedures have been reported previously (7, 8, 9, 10, 11).

EXPERIMENTAL

Samples

All samples of palm oil, palm olein and palm stearin were taken from the storage tanks or directly from the refinery of Kempas Edible Oil Sdn. Bhd., Pasir Gudang, Johore, Malaysia.

Fractionation was by detergent fractionation (Alfa-Laval). Refining was by physical refining comprising degumming (0.04–0.07% phosphoric acid at 85 C), bleaching (1–2% earth at 110 C), and steam refining/deodorization at 270 C (EMI).

Analysis of Monoglycerides, Diglycerides and FFA

Preparation of TMS derivatives. 30 mg of the oil are weighed accurately (±0.1 mg) into a 2 ml screw-capped glass vial fitted with a septum (Supelco, Inc., Bellefonte, Pennsylvania, Catalog No. 3-3113). 400 μL of pyridine are added using a syringe and the vial capped and shaken until the oil dissolves. 100 μL of N-trimethylsilyl imidazole (Sigma Chemical Co., Saint Louis, Missouri) are then added through the septum using a syringe and needle. The vial is then shaken well for one min. Finally, 200 μL of the internal standard solution [250.0 mg n-triacontane (Sigma Chemical Co.) in 25 ml of iso-octane] are added, again