TABLE III

Elemental Analysis of Acylurea Derivatives

Compound	RNHCONHR'								
			% Carbon		% Hydrogen		% Nitrogen		% Chlorine	
	R	R'	Theory	Found	Theory	Found	Theory	Found	Theory	Found
1	Myristoyl	Phenyl	72.79	73.05	9.89	9.75	8.08	8.12		
2	Decanoyl	3,4-Dichlorophenyl	56.83	56.65	6.73	6.92	7.80	7.81	19.73	19.54
3	Stearoyl	3-Nitrophenyl	67.08	67.09	9.23	9.11	9.39	9.43		
4	Lauroyl	4-Chlorophenyl	64.66	64.88	8.28	8.44	10.05	10.01	7.94	8.05
5	Palmitoyl	2-Nitro-4-chlorophenyl	60.85	60.80	7.99	8.22	7.81	7.65	9.25	9.45
6	Stearoyl	Butyl	72.20	72.41	12.12	12.02	7.32	7.32		
7	Palmitoyl	3-Chlorophenyl	67.54	67.73	9.12	9.38	6.85	5.97	8.67	8.52
8	Benzoyl	Butyl	65.43	65.23	7.32	7.57	12.72	12.81		
9	Lauroyl	4-Nitrophenyl	62.79	63.04	8.04	8.34	11.56	11.32		

ACKNOWLEDGMENT

Elemental analyses were obtained from Micro-Analysis, Inc., Wilmington, DE.

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⁂Mafura Nut Oil and Meal: Processing and Purification

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ABSTRACT

The seeds of the tropical tree known as mafura (*Trichilia emetica*) contain significant amounts of oil and protein. So far, commercial use of the mafura seeds has been prevented by the presence of a strong bitter and emetic taste, as well as a brownish color in both oil and meal. A refining procedure which gives edible oil with acceptable organoleptic properties has been developed. The raw meal has been extracted with aqueous alcohol to give a debittered meal with a protein content of about 36%. The amino acid composition of the meal was found to be comparable to that of other vegetable food proteins, except for a relatively low content of sulfur-containing amino acids. The brown color has been shown to result from browning reactions taking place during storage and processing. Most of the color may be removed by repeated treatments with dilute sodium hydroxide, followed by bleaching with earth and activated carbon.

INTRODUCTION

Mafura oil is obtained from the seeds of the mafura tree (*Trichilia emetica*) which is found in Tanzania and several other parts of East Africa as well as in some West African countries. The seeds are in the size range of 0.35-1.0 g and consist of roughly 23% of an oily shell-like husk and 77% kernel. The kernels contain 55-65% of a brownish fat (mp 41 C) and 13% protein (N × 6.25). The mafura nuts represent a potential source of nutrients which would be welcome in many areas. Unfortunately, the use of mafura oil and meal for edible purposes is prohibited by their bitter taste and by emetic properties.

An exception is the oil obtained by a method devised by certain natives in Tanzania. By boiling ripe, whole nuts for 10-15 min, drying in the sun and then agitating in water, a clear yellow oil of satisfactory quality is obtained. This oil has been shown to originate from the husk, which is known to contain 35-45% of a light-yellow oil.

In Tanzania, commercial use of mafura nuts has so far been restricted to a limited production of low-grade, inferior quality soaps. Accordingly, the price obtained by local farmers for their mafura nut crops has been low. Thus, growth and harvesting of mafura trees is discouraged. If proper refining procedures existed, the mafura oil could be a substitute for part of the sizable tallow and edible oil import while at the same time providing local employment.

The meal obtained by extraction of mafura kernels contains about 36% protein and its composition is comparable to that of other vegetable meals (1). The mafura meal can neither be used for human consumption nor for animal feed because of its bitter, emetic taste. Successful refining of the meal not only would furnish a valuable protein supply, but might also improve the economics of mafura nut processing.

The papers published on mafura nut processing during the last 80 years are relatively few (1-9). Most of the reports are analytical in nature. The only comprehensive investigation of various aspects of mafura nut oil and meal processing and purification is that of Fupi (1). Engelter

and Wehmayer (9) have reported the fatty acid composition of mafura oil to be 38.8% palmitic, 2.2% stearic, 48.5% oleic, 10.4% linoleic and 1.0% linolenic acid. The brown color has been noted by several authors (3,4,8) to be very difficult to bleach. Paris and Mignon (6) reported the presence of a bitter, non-nitrogenous unsaturated principle in "the mafura plant," whereas Henry and Grindley (7) state that steam distillation and treatment with animal charcoal at 100 C had no apparent effect in removing the bitter taste.

This paper summarizes some of the results obtained in a study (1) aimed at exploring the possibilities of developing a mafura nut processing industry.

EXPERIMENTAL PROCEDURES

Analysis

AOCS official methods (10) were used for determination of oil content (Ab 3-49), melting points (Cc 4-25), free fatty acids (FFA) (Ca 5a-50) and unsaponifiables (Ca 6a-40). Nitrogen, ash, fiber, calcium and phosphorus also were determined (11).

Extraction of Mafura Nuts

The kernels of the mafura nuts were separated from the shells by hand and ground in an ordinary, home meat-grinding machine (Electrolux, Sweden). About 3 kg of ground kernels (oil content 62%) were extracted in a Soxhlet (Quickfit and Quartz Ltd.) with 13 L of hexane for 25-30 hr. About 60-70% of the solvent was evaporated during solvent recovery and the rest was removed by a vacuum rotary evaporator at 40 C. The resulting oil was stored in brown bottles at -15 C.

Refining Apparatus

Degumming, neutralization and bleaching were done in an all-glass apparatus as illustrated in Figure 1. The double-wall, 3-L reaction vessel was equipped with a stainless steel stirrer (speed range 20-260 rpm) with twisted arms. Heating was accomplished by circulating hot water or glycerol between the double walls. The separating funnel was used for slow addition of degumming agent, lye and washing water. When used for bleaching, the separating funnel was replaced by a powder funnel through which bleaching earth could be added.

Degumming procedure. Raw mafura fat was melted on a water bath at 50 C in a stream of purified nitrogen (99.99%). About 2-2.5 kg oil was added to the reaction vessel followed by evacuation at 10-20 mm Hg. The pressure was then restored with nitrogen and the oil was heated to 60 C with gentle stirring (65 rpm). After temperature equilibration, the stirrer speed was increased to 260 rpm and 3% water, prewarmed to 60 C, was added over a period of 2 min. Stirring was continued for 30 min followed by 30 min settling. The gums were separated and the oil was washed 3 times with 20% water (60 C) added over a period of 2-3 min under vigorous stirring (260 rpm). The degummed oil was dried at 95 C in vacuum. When either acetic anhydride (analytical grade) or phosphoric acid (85%, analytical grade) were used for degumming, 0.1% of the degumming agent was added and 260 rpm stirring was applied for 15 min before addition of water, according to the procedure already outlined.

Neutralization. The degummed oil was neutralized at 92 C under stirring (65 rpm). After evacuation and nitrogen purging of the degummed oil, the calculated amount plus 10% excess of 1 N NaOH containing 4 g/L NaCl was added

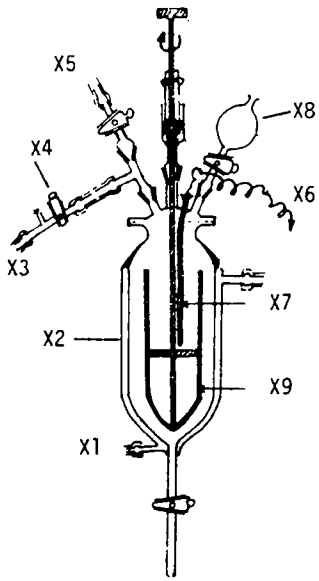

FIG. 1. Apparatus for degumming, neutralization and bleaching. Heating medium inlet (X1), double-wall reaction vessel (X2), vacuum connection (X3), connection to manometer (X4), nitrogen supply line (X5), connection to temperature recorder (X6), thermo-element pocket (X7), separating funnel (X8) and stainless steel stirrer (X9).

over a period of 3-4 min. After removal of the soapstock (settling time 30 min), the oil was washed twice with 15% of 0.25 N NaOH containing 1% NaCl, then with 15% of a 1% NaCl solution, and finally twice with 15% water. Each 15% portion was added in the course of 5 min. Separation time was 15 min. The stirring speed was 65 rpm in the first 3 additions and 120 rpm in the last two. Depending on the quality and color of the oil, the washing procedure was repeated 1 or 2 times.

Bleaching. The neutralized fat was evacuated under heating and gentle stirring. When the temperature reached 50 C, the pressure was restored with purified nitrogen and the temperature was increased to 95 C. A slow stream of nitrogen was maintained while the required amount of bleaching earth (Tonsil OFF) was added slowly through the powder funnel at a stirring speed of 260 rpm. After 15 min, 10 g Celite (Johns-Hanville, Hyllo super-cel) was added. After another 5 min, the oil was filtered through a layer of 20 g Celite on a 9-cm glass sinter filter (G3). During filtration, the oil was protected by a nitrogen blanket.

Deodorization Apparatus and Procedure

The degummed, neutralized and bleached oil was deodorized at a pressure of 1-2 mm Hg in the all-glass apparatus shown in Figure 2. The oil was heated to 220-245 C by a heating mantle. The evaporating (steam producing) chamber was filled with 200 mL distilled water. The rate of evaporation could be controlled by the position of the IR lamp. During a typical deodorization experiment, the steam produced corresponded to about 40 g water evaporated/kg oil/hr. Deodorization was terminated after 3-4 hr. The pressure was slowly restored with purified nitrogen and the oil flask was disconnected; 0.02% of a 10% ascorbic acid solution was added and the oil was dried in vacuum. Samples for taste scoring were immediately cooled to -15 C.

Debittering of Mafura Meal

About 500 g of the bitter mafura meal obtained by extraction of the nuts was ground to pass through a 2-mm sieve and then treated with 2.5-3 L aqueous ethyl alcohol (96,

85 or 55%) in a Soxhlet extractor (Quickfit Ex 5/105) at 60 C. The organoleptic quality of the meal was checked at regular intervals after withdrawing samples of the meal from the extraction thimble and drying in vacuum at 60 C.

RESULTS AND DISCUSSION

Mafura Oil

Various batches of mafura nuts, harvested and stored at different times and conditions, were used in this work. For this reason, the FFA content of the raw oils varied from 5 to 12% and the mp from 34 to 41 C. The phosphatide content (% P×30) was found to be within the range of 0.07-0.08%. The raw oils obtained by extraction of mafura nut kernels were pale brown to brown with bitter taste and distinct emetic properties. In contrast, the oil extracted from mafura nut shells (oil content 45%) was pale yellow with no bitterness or emetic properties.

Removal of Bitter Taste and Emetic Properties

The various processing steps usually applied in edible oil refining were explored at different experimental conditions to improve the organoleptic properties of the mafura oil. These were judged by a taste panel who gave flavor scores on a scale ranging from 1-7 (1 = extremely bad, 5.5 = acceptable, 7 = excellent). Some selected procedures to illustrate the progressive improvement in the quality of the product are given below.

Crude mafura oil was degummed by 3% water and neutralized with 1 N NaOH. The bitter taste and the emetic property persisted. It was found that the isolated unsaponifiable matter had a strong bitter taste. It appears that the undesirable organoleptic properties of the oil are associated with the unsaponifiable matter, which was also suggested by Henry and Grindley (7).

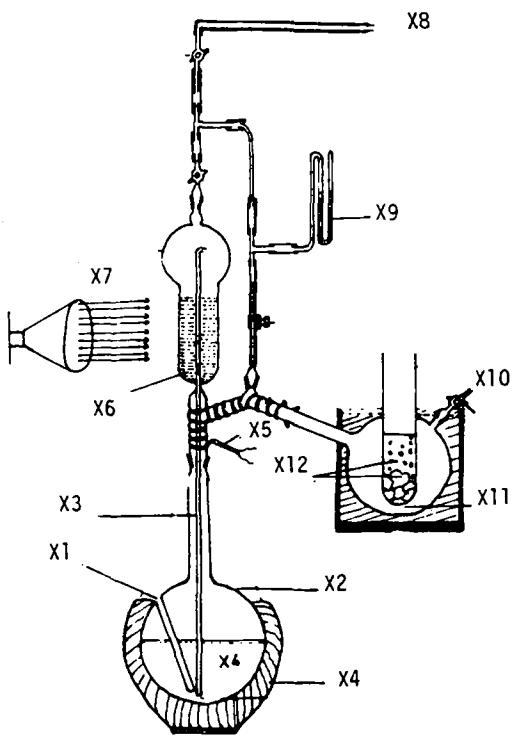

FIG. 2. Deodorization apparatus. Thermometer pocket (X1), oil flask (X2), steam injector (X3), heating mantle (X4), heating tape (X5), evaporation chamber (X6), infrared lamp (X7), nitrogen inlet (X8), manometer (X9), connection to vacuum (X10), cooling trap (X11) and dry ice/acetone mixture (X12).

The degummed and neutralized oil was bleached with 2-3% Tonsil OFF and deodorized at 220 C and 1-2 mm Hg for 3-4 hr. The resulting oils received flavor scores below 5.5, i.e., unacceptable.

The refining procedure was then modified by treating the raw oil with 0.1% acetic anhydride for 15 min before degumming with 3% water. The use of acetic anhydride had a notable effect, as the flavor score of the deodorized oil improved to 5.5.

Further improvement of the oil was obtained by including 0.3% active carbon (Norit) in the bleaching process. Flavor scores of 6.0-6.5 were then obtained.

Mafura oil thus can be satisfactorily processed for acceptable flavor by 4 consecutive steps: (a) degumming with 0.1% acetic anhydride and 3% water; (b) neutralization with 1 N NaOH + 10% excess followed by repeated washings with 0.25 N NaOH, brine and water; (c) bleaching with 1.5-3.0% Tonsil OFF + 0.3% active carbon; (d) deodorization at 220-240 C and 1-2 mm Hg for 3 hr.

Mafura Meal

Debittering time and protein content. The meal obtained by extraction of the mafura nut kernels had a sufficiently strong bitter and emetic taste to prevent its acceptance by animals. According to native Tanzanians, even rats and birds refuse to eat it.

Qualitative experiments showed that the bitter principle is more or less soluble in a number of solvents, including ethyl alcohol and water. Water would, of course, be an ideal solvent for extracting the bitter principle from the mafura meal. However, an appreciable amount of valuable components such as proteins, carbohydrates, and micronutrients are lost due to their water solubilities. Ethyl alcohol keeps the loss of proteins low, but requires very long extraction times. As a compromise, extraction was done with various aqueous alcohol mixtures. At regular intervals, samples were withdrawn, dried at 60 C in vacuum and tasted for bitterness. The debittering times given in Table I refer to the extraction times after which no bitter taste could be detected.

The meals obtained by the 96 and 85% alcohol treatment were pale brown like the original meal, whereas the 55% alcohol product was somewhat darker brown with a characteristic cocoa-like smell.

Mafura nut shells were also processed to a meal containing 15% protein and 22% fiber. This meal, like the oil obtained from the shells, had no bitter or emetic taste.

Amino acid content. Amino acid analysis of the meal debittered with 85% ethyl alcohol was done according to Moore and Stein (12) on a Beckman amino acid analyzer Model 120 C. The results are presented in Table II. The amino acid distribution in the mafura meal is satisfactory, except for the comparatively low content of sulfur-containing acids.

Fiber, ash and mineral content. Table III gives further information regarding the constituents of mafura meal debittered with 85% ethyl alcohol. For comparison, some typical values for soybean meal are included.

BROWN COLOR

Origin and Properties

When mafura nuts are extracted with hexane at room temperature, a pale yellow oil is obtained. Apparently, the brown color observed in commercial mafura oil results from a browning reaction taking place during processing and storage. The color of the cold, extracted oil is 55Y, 6R in terms of Lovibond units, compared to 35Y, 11R-15R for

TABLE I

Debittering Time and Protein Content of Meal Extracted with Various Water/Ethyl Alcohol Mixtures

Solvent	Debittering time (hr)	Protein content (%)
96% Alcohol	25	37.2
85% ↓	22	35.8
55%	16	34.1

TABLE II

Amino Acid Distribution in Debittered Mafura Meal Protein (%)

Alanine	4.0	Tyrosine	4.8
Asparagine	14.0	Isoleucine	7.2
Arginine	8.1	Leucine	9.5
Cystine	1.0	Lysine	5.3
Glutamic acid	14.8	Methionine	0.5
Glycine	5.2	Phenylalanine	5.3
Histidine	2.8	Threonine	5.0
Proline	5.1	Tryptophan	ND[a]
Serine	5.4	Valine	8.0

[a]Not determined.

TABLE III

Fiber, Ash and Mineral Content of Mafura Meal Compared to Soybean Meal (dry basis)

	Fiber (%)	Ash (%)	Calcium (g/kg)	Phosphorus (g/kg)
Mafura	18.9	10.5	9.2	8.6
Soybean[a]	6.0	6.7	2.4	5.5

[a]Data from ref. 13.

oils obtained by Soxhlet extraction.

The brown pigment was insoluble in hexane and in cold water, slightly soluble in aqueous alcohol, warm water, and concentrated NaOH, and soluble in dilute NaOH. Consequently, the neutralized oil was washed repeatedly with 0.25 N NaOH in order to remove most of the color. The number of washes (usually 4-8) needed to obtain an acceptable color would depend on the particular batch of oil.

The brown pigment is not easily removed by bleaching. Brown color pigment was actually formed during both bleaching and deodorization. Thus, when the degummed and neutralized oil was bleached with only 1% Tonsil OFF, the color increased by about 6-7 Lovibond red units. Bleaching with 2% Tonsil OFF + 0.3% active carbon gave oils with Lovibond red values from about 3.0 to 7.0, depending on oil quality. To reduce the color after bleaching to values below 3R, quantities of about 3% Tonsil OFF + 3% active carbon were needed. When cold, extracted oil was kept at 100 C for 3 hr in air and in an evacuated vessel, respectively, almost the same darkening effect (20R) was observed. Thus, the browning reactions in mafura oil may be independent of oxygen.

Hydrogenation did not affect the brown color. The color could be almost completely removed by passing a 20% (v/v) miscella (oil in hexane) through an activated aluminum oxide column (14) at 55 C. Unfortunately, the low yield (51%) resulting from the adsorption of oil on the column prevents the practical application of this procedure.

In contrast to pigments like the carotenoids, which are unstable to heat, the brown pigment in mafura oil is rather stable. In fact, an increase in color corresponding to 1-5 Lovibond red units is observed during deodorization at 240 C.

The brown pigment was precipitated from 85% ethyl alcohol solution by addition of cold water. After washing with ethyl ether, various physicochemical properties such as solubility, reducing power, fluorescence, UV spectra and molecular weight were observed. The results were qualitatively in accordance with the properties of a "standard melanoidin" obtained by reacting proteins with carbohydrates (Maillard reaction). It was also observed that the browning reaction is accompanied by the formation of carbon dioxide, another characteristic of the Maillard reaction (15,16).

On the basis of these observations, the brown color which develops during storage and processing of mafura nuts is due to a brown-black, high molecular weight pigment formed by a nonoxidative reaction between carbohydrates and amino acids (Maillard reaction). It is also likely that, in addition to the Maillard reaction, degradation of sugars (glucose and fructose) with formation of brown products occurs. Fructose, which has been shown to be present in mafura nuts (1), may easily undergo such degradation reactions (17,18).

Also, at elevated temperatures, foods containing nonreducing sugars, e.g., sucrose, may undergo Maillard reactions, presumably as a result of the splitting of glycosidic bonds in the sucrose molecule to give glucose and fructose (19,20).

Inhibition of the Browning Reaction

It was found that the browning reaction occurring in the still during extraction of the nuts or meal could be inhibited by the addition of 1.5% $Na_2S_2O_5$ to the extracting solvent. Sulfites block the carbonyl groups of the free reducing carbohydrates involved in the carbonyl-amino acid reaction (21). Ethyl mercaptan was also found to inhibit the browning reaction to some extent.

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Analysis of Phosphatidylcholine in Soy Lecithins by HPLC

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ABSTRACT

A simple and rapid high pressure liquid chromatographic method with RI detector was developed to determine the content of phosphatidylcholine in soy lecithins.

INTRODUCTION

During the course of our recent study on soy lecithin production by solvent extraction (1), we needed a rapid method of analysis for phosphatidylcholine (PC). Analysis of phospholipids using high pressure liquid chromatography (HPLC) has been extensively studied by several researchers (2-6). However, these methods were based on either UV detector in the range of 203-214 nm (2-4) or FID (5,6). Recently, Nasner and Kraus (7) reported a very sensitive HPLC method for determination of PC in soy lecithins by using a UV detector at 206 nm.

EXPERIMENTAL

Materials

Crude soy lecithin was obtained from a local company (Cheil Sugar Co. Seoul). Acetone-insoluble fraction and 2-propanol-soluble fraction were made according to the methods of Weenink and Tulloch (8) and Liebing (9), respectively. PC, phosphatidylethanolamine (PE), phosphatidylinositol (PI), sphingomyelin (Sph), triolein and cardiolipin were purchased from Sigma (St. Louis, MO). Solvents of chromatographic grade were purchased from Burdick & Jackson (Muskegon, MI). All other reagents and chemicals were of technical grade.

HPLC Equipment and Conditions

HPLC was performed with a Waters Associates' ALC/GPC-244 (Milford, MA) equipped with R401 RI detector and the column used was a μ-Porasil column (Waters Associates). The operating conditions were: flow rate, 2 mL/min; solvent, chloroform/methanol/acetate/water (14/14/1/1, by vol); sample size, 6 mg; attenuation, 32X; chart speed, 0.5 cm/min. Integration of peak area was done by multiplying the height of the peak by the width at half-height (10).

RESULTS AND DISCUSSION

Retention times of triolein, PE, PI, cardiolipin, PC, and Sph were 1.6, 2.0, 2.2, 2.3, 6.4 and 10.3 min, respectively.

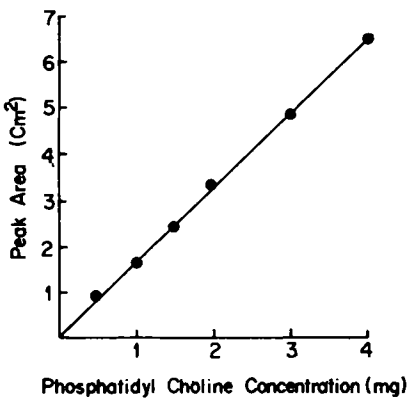

FIG. 1. Quantitative analysis of phosphatidylcholine standard by HPLC.

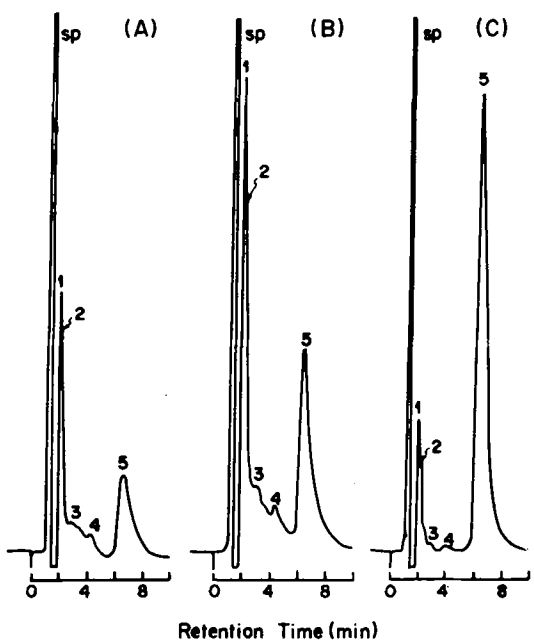

FIG. 2. (A) HPLC of crude lecithin, (B) acetone-insoluble fraction, and (C) 2-propanol-soluble fraction. SP, solvent peak; 1, phosphatidylethanolamine; 2, phosphatidylinositol; 3 and 4, unknowns; 5, phosphatidylcholine. Operating conditions: flow rate, 2 mL/min; solvent, chloroform/methanol/acetate/water (14/14/1/1, by vol); sample size, 6 mg; attenuation, 32X; chart speed, 0.5 cm/min.

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