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Full Length Research Paper

Comparative physico-chemical evaluation of kombo kernel fat produced by three different processes

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Characterization of fats and oils is important in assessing their potential economic uses. This study was carried out to determine and compare the physicochemical characteristics of a pressed crude fat (PCF), solvent extracted fat (SEF) and the refined pressed fat (RPF) from the kernels of *Pycnanthus angolensis* (*Pycnanthus Kombo*). The stability of the Kombo fat over a period of 21weeks was also investigated. Solvent extraction recorded a higher yield of 74.13 (±2.21)% compared to 41.08(±0.56)% by mechanical pressing. A comparison of the physicochemical characteristics of the three fat samples showed significant ($p < 0.05$) differences. The high saponification value suggests that the Kombo fat, preferably the refined fat is suitable for soap production. The low iodine value indicates a high degree of saturation of the fat as well as its non-drying nature. Stability of the crude fat under ambient conditions over the period of study was partly due to the presence of a natural antioxidant. Thus the fat is a good source of natural antioxidant in the stabilisation of human and animal foods against rancidity, colour and odour development.

Keywords: *Pycnanthus angolensis,* Kombo fat, physicochemical characteristics

INTRODUCTION

Pycnanthus angolensis, also known as *Pycnanthus Kombo* belongs to the myristicaceae family. Its common names include African nutmeg and wild nutmeg. The trees grow widely in West and Central Africa (Eckey, 1954). They are widely distributed locally in most forest zones in the southern part of Ghana (Acquaye, 1999). Parts of the plant are reported to have several medical applications (Agyare *et al*., 2009 ; Akendengue and Louis, 1994). The bark exudates, twigs, leaf juice, and/or seed fat are used as mouthwash to treat oral thrush in children; the seed fat is applied topically to treat fungal skin infections (US Patent No: 5,674,900). In Ghana, a hot water extraction of the root is taken orally as an antihelmintic (Ayensu, 1969). The leaf and bark are used to treat toothache and the sap of the plant is applied topically to arrest bleeding (Abbiw, 1990). The kernel or seed fat commonly known as kombo butter is reported to be effective for the treatment of arthritis and to combat inflammations in joints (US Patent No. 7,371,413). The fat is also reported to contain kombic acid, previously identified as 16(2',5'-dihydroxy-3'-methylphenyl)-2,6,10,14-tetramethyl-2,6,10,14-hexadecatetraenoic acid, which exhibits antioxidising properties in the stabilization of human and animal foods (Lok *et al*., 1983). Eckey (1954), Irvine (1961), and Mapongmetsem (2007) have reported the solvent extract of the Kombo kernels as 54-62%, 54%, and 45-70%, respectively. The two most widely used methods for the extraction of fats and oils from plant seeds are solvent extraction and mechanical expression. Whilst solvent extraction generally gives a higher yield, mechanical expression is employed for high oil content seeds for economic reasons. The analytical characteristics and fatty acid composition of fats besides their value for reference purposes, serve as useful guides to oil and fat analysts in the determination of the components of unknown mixtures and for checking the specifications of supplies and products (Cocks and Rede van ., 1966). Research work on *Pycnanthus Kombo* in recent times is focussed on medicinal applications of the

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stem bark, leaves, roots and kernel fat. In spite of the high fat content of kombo nuts coupled with the abundant distribution of the trees in Ghana, with an estimate of over 282 metric tonnes of Kombo nuts in five selected regions in Ghana (Acquaye, 1999), the nuts are mostly left to rot except in minor herbal therapies. This research is therefore geared towards expanding the knowledge base and economic applications of Kombo fat by determining and comparing the physico-chemical characteristics of the fat obtained using three different extraction processes.

MATERIALS AND METHODS

Source of Raw materials

Kombo nuts used for this study were collected between January and February from the Central region of Ghana. The nuts were obtained from fully ripe fruits which split-open and fell to the ground for collection.

Sample Preparations

Kombo Kernels Samples

The nuts were sun-dried for about two weeks and shelled to obtain the kernels. The kernels were sorted by hand-picking to get rid of defective (spoilt/cracked) ones. The quartering sampling method was then used to select about 4.8kg kernels sample (from a bulk of about 20kg kernels). About 3.5kg kernels were used for mechanical expression whilst 1.3kg kernels were used for solvent extraction. Kernels were stored under refrigerated conditions for two weeks prior to use.

Test Samples

The samples were obtained as follows and stored under refrigerated conditions (below 0^{o}C) prior to performing of tests.

Pressed Crude Fat

Four hundred grams (400g) portions of milled kombo kernels were placed in linen cloth-bags and heated in a thermostatically controlled oven at 80^{o}C for 2 hrs. The oil was then expressed using a low pressure (40kg/cm^{2}) manual press (Obeng *et al*, 2010).

Solvent Extracted Fat

One Hundred gram (100g) portions of milled kombo kernels were placed in muslin thimbles and the fat extracted using a soxhlet extractor with petroleum ether (B.P 40-60^{o}C) as solvent. The flask containing the fat and residual solvent was placed on a water bath to evaporate the solvent, followed by a further drying in a hot - air oven at 103°C for 30 minutes (AOCA Method 948.22, 1990).

Refined Pressed Fat

The refined fat samples were prepared by taking potions of the pressed crude fat and treated as follows:
Melted fat was filtered through a funnel fitted with a filtering cloth to remove impurities. The volume of 3.0N NaOH necessary to neutralize the pressed crude fat was found by titrating 2g of the melted fat dissolved in ethanol with 3.0N NaOH using phenolphthalein indicator solution. 10% excess of the volume of NaOH required (Volume in ml of 3.0N NaOH/gramme weight of fat = 0.231) was then used to ensure complete neutralization. The required volume of NaOH was then mixed with a weighed amount of the fat and agitated at 100rpm for 10minutes at 60°C. The oil was then allowed to settle for 1 hour at 60°C and the clear oil was decanted, 10% (based on the weight of the unrefined oil) volume of water was then added to the decanted oil and stirred for 15minutes. The mixture was then allowed to settle and the clear oil was separated using a syringe. The oil was dried in a hot - air oven at 65°C for 6 hours (Gunstone *et al*., 1986; Devine and Williams, 1960).

Determination of physicochemical properties of Kombo kernel fat

Refractive index

The fat sample was melted at 60°C and several drops placed on the lower prism of an Abbe refractometer (which was also adjusted to the same temperature as the sample. The prisms were closed and tightened firmly with the screw head, ensuring that the sample came to the same temperature of the instrument. The instrument was adjusted until the most distinct reading possible was obtained and the refractive index noted.

Melting range

The fat sample was melted and drawn into a capillary tube .The tube containing the sample was then placed in a refrigerator between 5°C–10°C for 16hr for the oil to solidify. The sample was then placed in a melting point apparatus and observed until it began to melt. The temperature range at which the melting occurred was noted and recorded as the melting range (AOCS, Capillary tube method Cc1-25, 1993).

Specific Gravity

The specific gravity was determined using the specific gravity bottle. The bottle was then placed in a water bath maintained at 25°C and filled with distilled water. It was removed, wiped dry and weighed. The bottle was emptied, dried and placed in a water bath at 60°C and allowed to attain a temperature of 60°C for about 30minutes. The bottle was then refilled with the melted fat sample. It was then cleaned and wiped completely dry and weighed. The specific gravity was calculated using the formula below (AOCS method Cc 10a-25, 1993).

$$\text{Specific Gravity at 60/25°C} = \frac{\text{(weight of bottle + oil) – (weight of bottle)}}{w\,[1 + 0.000025\,(t - 25)]}$$

w = weight of water at 25°C, t = temperature in °C = 60°C

Calorific value

The calorific value was determined using the bomb calorimeter. An empty crucible was weighed and 0.7g of oil was added. The crucible with the oil was then positioned in the seating in the bomb cover and a piece of ignition wire was attached across the terminals, but touching the oil surface. 15ml of distilled water was poured into the base of the bomb and the bomb was then assembled carefully. It was then charged with oxygen to 25 atmospheres. 2400ml of water was added to the calorimeter vessel. The calorimeter vessel was then positioned in a water filled jacket, which provided a constant temperature environment; the bomb was set in place in the calorimeter vessel. The stirrer and Beckmann thermometer were put in place. The electrical circuit was connected and the stirring gear set in motion. The thermometer readings at 1 minute intervals were observed for a preliminary period of five minutes. The firing circuit was then completed and the thermometer readings continued to be noted until the maximum temperature was attained. A graph of Beckmann readings was plotted against time and the rise in temperature was obtained from the plot. The calorific value was then calculated using the formula below (Eastop and McConkey, 1993)

$$Caloric\ value, Q = (W_w + W_a)(\Delta t) \times 4.2 / W_f$$

Q in KJ/kg, W_f = weight of fat in kg, W_w = weight in kg of water

Wa = weight in kg of the “water equivalent” of the apparatus (0.52kg), Δt = temperature rise in °C

Viscosity of the oil

The viscosity was determined using the Redwood No.1 viscometer. The viscosity of the pressed crude oil was determined at various temperatures (ranging from 50°C to 100°C) in order to study the effect of temperature on the viscosity of oil. The viscosity of the pressed crude fat, refined fat and solvent extracted fat were also determined at 60°C for the purpose of comparison. In each case the fat was melted and sufficient quantity of it was introduced into the oil cup while the ball valve was closed. The temperature was then adjusted to the desired value by means of the water bath. The ball valve was then opened and the time taken to discharge 50ml of the oil through the capillary tube situated in the base of the oil cup was noted. The values obtained as “tsec Redwood at T°F” were then converted to cP (Holman, 1994; Tapley, 1990).

Unsaponifiable value

Five grams (5g) of a well-mixed melted Kombo fat was weighed into a soxhlet flask. Thirty millilitres (30ml) of 95% ethyl alcohol and 5ml of 50% KOH solution were then added. The mixture was boiled gently but steadily, under reflux for 1 hour and then transferred to an extraction cylinder and washed with 95% ethyl alcohol to the 40ml mark. Warm and then cold distilled water were used to complete the transfer to a total volume of 80ml. The flask was then washed with 5ml of petroleum ether and added into the cylinder. The mixture then cooled to room temperature and then 50ml of petroleum ether added to it. The stopper was inserted and the content was vigorously shaken for at least 1 minute and allowed to settle until both layers were clear. A separation funnel was used to separate the upper layer (petroleum fraction) portion. The extraction process was repeated six times, using 50ml portions of petroleum ether each time. The petroleum ether fractions were combined in a 500ml separatory funnel and washed with 25ml portions of 10% ethyl alcohol in distilled water, shaking vigorously and drawing off the aqueous alcohol layer after each extraction. The washing was repeated with 10% ethyl alcohol solution until the wash solution no longer gave a pink colour after the addition of one drop of phenolphthalein indicator solution. The petroleum ether extract was then transferred to a tarred beaker and evaporated to dryness on a water bath, the extract (residue) dried to constant weight in an oven dryer and weighed as A. The reagent blank was corrected for by conducting the unsaponifiable matter procedure without any fat present, the blank determined by this procedure as “B”. The unsaponifiable matter was calculated using the formula below (AOCS Method Ca 6a-40, 1993).

$$\%Unsaponifiable\ matter = \frac{A - B}{W} \times 100$$

A = weight of residue in sample, B = weight of residue in blank, W = weight of sample.

Impurities

Two grams (2g) of the melted fat was weighed into a 250ml conical flask and 20ml of 1:1 solvent mixture

(petroleum ether + diethyl ether) was added. The flask was then shaken vigorously, allowed to stand for 30mins at 32°C. The liquid was then filtered through a dried and weighed Whatman number 1 filter paper. The filter paper was carefully washed with about 10ml of the solvent mixture. It was then dried to constant weight in an oven at 103°C. The increase in weight represented the weight of impurities and was expressed as a percentage of the initial sample (Cocks and Rede van,1966)

Free Fatty Acids (FFA)

The fat was melted at 50°C, well mixed and 1.4g of it was weighed into a flask containing 15ml of hot neutralized alcohol and 0.4ml of phenolphthalein indicator was then added. The content was titrated with 0.5N NaOH. The free fatty acid value was calculated (as oleic acid) using the formula below (AOCS Method Ca 5a-40, 1993)

$$FFA\ (as\ oleic\ (\%)) = \frac{V \times N \times 28.2}{W}$$

N = normality of NaOH solution, v = volume (ml) of NaOH solution, W = weight of oil sample.

Acid Value

Five grams (5g) of the melted fat was dissolved in a well mixed neutral solvent consisting of 25ml of diethyl ether and 25ml 95% ethanol. It was then titrated with aqueous 0.1N NaOH using 1ml of phenolphthalein indicator solution and shaking constantly until a pink colour that persisted for at least 15s was obtained. The acid value was then calculated using the formula below (Kirk and Sawyer, 1991).

$$Acid\ value\ mg\ KOH\ /\ g\ of\ sample = \frac{56.1N \times V}{W}$$

N = normality of NaOH solution, v = volume (ml) of NaOH solution, W = weight of oil sample.

Peroxide Value

Five grams (5g) of the fat was weighed into a 250ml-conical flask with a glass stopper. 30mL of 3:2 v/v glacial acetic acid-chloroform solvent was added and swirled to dissolve the sample. 0.5mL of saturated KI solution was added. The solution was left in the dark with occasional shaking for exactly 1min, and 30mL of distilled water added immediately. The mixture was titrated with 0.1N sodium thiosulphate using 0.5mL of starch indicator solution. A blank was also performed at the same time. The test was done in triplicate. The peroxide value was calculated using the formula below (AOCS Method Cd 8-53, 1993)

$$\text{Peroxide value} = \frac{1000(V_1 - V_2) \times N}{W}$$

N = normality of $Na_2S_2O_3$ solution, v_1 = volume (ml) of $Na_2S_2O_3$ solution used in test,
v_2 = volume (ml) of $Na_2S_2O_3$ solution used in blank, W = weight of oil sample.

p-Anisidine value

Two and a half grams ($2\frac{1}{2}$g) of the dry melted oil was weighed into a 50ml volumetric flask. The sample was then dissolved and diluted to the mark with n-hexane. The absorbance (A1) of the solution at 350nm in a 10 mm cell against a blank n-hexane was measured. 5ml of the sample solution was then pipetted into a 10ml stoppered test tube and exactly 1ml of p-anisidine value solution (2.5g/L in glacial acetic acid) was added. After exactly 10min the absorbance (A2) as against the reagent blank was measured as before. The p-anisidine value is calculated using the formula below (AOCS Method Cd 8-80, 1993).
p-Anisidine Value = $25(1.2A_s - A_b)/W$
A_s = absorbance of fat solution after reaction with the p-anisidine, A_b = absorbance of the fat solution, W = weight of sample
The totox value was calculated using the formula (2x peroxide value) + (p-anisidine value).

Iodine value

The fat was melted at 70°C and 0.2g of the oil was weighed into a 500ml flask and dissolved in 15ml of carbon tetrachloride. 25ml of Wijs solution was then dispensed using a pipette into the flask containing the sample. The flask was stoppered, swirled to ensure complete mixing and left in the dark for 30minutes at room temperature. After which 20ml of 10%KI solution was added, followed by 150ml of distilled water. The mixture was titrated with 0.1N $Na_2S_2O_3$ solution using 1.5ml of starch indicator solution. A blank determination was conducted simultaneously. The iodine value was calculated using the formula below (AOCS Method Cd 1-25, 1993).

$$Iodine\ \ value = \frac{12.69(V_2 - V_1) \times N}{W}$$

where, N = normality of thiosulphate solution , V_1 = volume (ml) of thiosulphate solution used in test, V_2 = volume (ml) of thiosulphate solution used in blank, W = weight of sample.

Saponification Value

Two grams (2g) of the melted was dissolved in 25ml of alcoholic KOH and refluxed for 45mins for complete saponification. The mixture was cooled but not sufficiently to form a gel, 1ml of phenolphthalein indicator was added and the hot solution titrated with 0.5M HCl. A blank determination was simultaneously conducted. The

saponification value was calculated using the formula below (AOCS Method cd 3-25, 1993).

$$Saponification \quad value = 56.1N\frac{(V_2 - V_1)}{W}$$

N = normality of HCl , V_1 = volume (ml) of HCl used in test, V_2 = volume (ml) of HCl used in blank, W = weight of sample.

Shelf life study

The crude fat, refined fat, and solvent extract samples were kept in transparent sealed plastic containers and stored at ambient conditions. Samples were then taken periodically for analyses. Determination of FFA, peroxide value and Kreis test (rancidity index) were carried out daily for the first one week, every other day for the second week and weekly for the rest of the study period. The results are illustrated in figures 2 and 3.

Rancidity index *(Kreis* test*)*

Five millilitres (5ml) of the melted fat was shaken vigorously with 5ml of 0.1% phloroglucinol solution in ether and 5ml of conc. HCl for 20 seconds. When a pink colour occurred, indicating rancidity, the test was confirmed by diluting the oil 1 in 20 with heptane and repeating the experiment. The absence of a pink colour however indicates no incipient rancidity (Kirk and Sawyer, 1991).

Statistical analyses

The physical and chemical properties obtained were statistically analysed using ANOVA, Genstat software version 7.0

RESULTS

Composition of Kombo fruit and nut

The weight per seed was found to range from 1.15-1.21g. The mean composition of seeds and shell of the nut were 82.9% and 17.1% respectively. The nut was observed to constitute a small portion of the fruit.

Yield of Extraction

The fat content of 74.13(±2.21)% determined by solvent extraction was comparatively higher than a yield of 41.08(±0.56)% obtained by mechanical pressing.

Physicochemical properties

Physical and chemical properties of the samples are shown in the table 1. The melting ranges of the SEF, PCF and RPF were above the average room temperature of 26°C (in Ghana), with RPF recording the highest and PCF, the least. The specific gravities of the three samples were significantly different ($p< 0.05$). PCF had the highest value (0.927) whilst the least value (0.916) was observed with RPF. The iodine values as well as the refractive indices of the PCF, SEF, and RPF were significantly ($p< 0.05$) different and correlated strongly ($r = 0.963$, $p< 0.05$) .

The FFA value of the PCF was significantly higher than SEF.The value for RPFwas the least as expected. The mean peroxide values (PV) of the PCF, SEF and RPF, although considerable high, were nevertheless within the range of Codex Standards (PV < 10mEq/ kg fat or oil) for edible oils. The calorific values of the samples were significantly ($p< 0.05$) different with RPF liberating the highest energy per gram of fat and PCF, the least..

The saponification values of the samples differed significantly within the range of 238-249.2mgKOH/g fat, with RPF saponifying more than the rest. The converse was true for the unsaponifiable matter levels of the samples. There were slight increases in both p-anisidine values and totox values of the samples after storage.

Fat stability

Temperature effect

Figure1 shows the effect of high heat (temperature) on the deterioration of Kombo fat. There was a positive correlation ($r = 0.977$, $p< 0.01$) between the oil temperature and rate of deterioration of the oil. When the fat was heated from 50°C to 230°C, the FFA value was observed to increase from 17.63% to 20.90% as shown in figure 1 whilst the peroxide value drastically increased from 8.8 to 14.0mEq/kg.

Time/storage effect

Figure 2 and 3 show the variation of peroxide values and %FFA of the pressed crude fat, solvent extract and refined fat over a period of 21weeks. There were weak correlations ($r < 0.349$, $p< 0.05$) between peroxides/free fatty acids formation in the three samples and time indicating slight occurrence of oxidative or hydrolytic rancidity over the period.. The peroxide values of the PCF, SEF, and RPF ranged between 8.80-9.00, 7.95-8.00, and 9.93-10.00mEq/kg respectively. The FFA levels of PCF, SEF, and RPF were observed to vary very slightly, between 17.81 and 18.01%, 11.73 and 12.14%, and 0.5 and 0.55% respectively as shown in figure 3.

DISCUSSION

Physicochemical properties

The relatively high melting range of the Kombo fat is probably due to the presence of high levels of saturated

Table 1. Physical and Chemical properties of Kombo fat

Property	*Sample			Literature values CF (Eckey,1954)		LSD	SED
	SEF	PCF	RPF				
Melting range(^{o}C)	41-44	39.5-43	42-45	38-50		-	-
Refractive index@ 60^{o}C	1.461^{a}	1.466^{b}	1.451^{c}	-		0.003	0.001
Specific gravity 60^{o}C/25^{o}C	0.922^{a}	0.927^{b}	0.916^{c}	0.887 (100^{o}C/15^{o}C)		0.003	0.001
%FFA (calcula-ted as oleic acid)	11.78^{a}	17.84^{b}	0.50^{c}	18.7		0.047	0.019
Acid value	24.31^{a}	36.57^{b}	1.26^{c}	19-27		0.003	0.001
Peroxide value	8.00^{a}	8.93^{b}	9.90^{c}	-		0.133	0.054
p-Anisidine value (fresh fat)	0.732^{a}	0.876^{b}	0.961^{c}	-		0.012	0.005
p-Anisidine value (after 21wks)	0.785^{a}	0.930^{b}	1.031^{c}	-		0.011	0.005
Totox value (fresh)	16.73	18.73	20.76	-		-	-
Totox value (after 21wks)	16.79	18.93	21.03	-		-	-
Saponification value	238.0^{a}	234.5^{b}	249.2^{c}	224-255		2.664	1.089
Ester value	213.69	197.25	247.94	-		-	-
Unsaponifiable matter (%)	1.027^{a}	1.254 ^{b}	0.773^{c}	1.2	0.7^{HH}	0.1085	0.044
Calorific Value (kcal/g)	9.511^{a}	9.302^{b}	9.928^{c}	-		0.030	0.012
Viscosity (cP)	22.91^{a}	45.42^{b}	17.11^{c}	-		0.116	0.048
Iodine value	64.9^{a}	70.6^{b}	30.6^{c}	65-67	32.7^{HH}	2.621	1.135
Moisture and volatile matter at 105^{o}C	0.55^{a}	0.14^{b}	0.16^{c}	-		0.047	0.019
%MIU	2.340	2.354	1.78	-		-	-
Colour	Yello-wish brown	Reddish brown	Pale yellow	Yellow or brown		-	-
Taste	bitter	Bitter	Bland	Bitter		-	-

Values were obtained in triplicates and the average values determined using ANOVA, Genstat software version 7.0
∗Values with different superscripts (a, b, c, d) in the same row are significantly different (at $p<0.05$). LSD: Least significant differences of means.
SED: Standard errors of differences of means.
SEF: Solvent extract, PCF: Pressed crude fat, RPF: Refined fat, CF: crude fat
S: significance
MIU: (Moisture, Insoluble, and Unsaponifiable matter)

fatty acids. RPF possibly contained more saturated fatty acids, which molecular structure allows more close stacking together of fatty acids. Resulting in its relatively higher melting range than the rest.

The significant ($p < 0.05$) difference in specific gravity of PCF, SEF and RPF may be due to the presence of higher levels of high molecular weight non-fatty resin acid in the PCF than in the SEF. This resin acid was however removed in the refined fat (Artherton and Meara, 1939).

Both iodine value (IV) and refractive index are important characteristics which determine the degree of saturation or unsaturation of fats and oils. The significantly low ($p < 0.05$) refractive index of the RPF compared to PCF and SEF could be attributed to the removal of the highly unsaturated resin acid in RPF. This confirmed a previous report that the neutralisation process possibly removed the highly unsaturated resin acids (Atherton and Meara, 1939). The relatively low iodine values (less than 100) of the samples indicate a high degree of saturation of the Kombo fat, classifying it as non-drying and hence unsuitable for production of paints, vanishes and surface coating. .

The FFA value of the PCF compared favourably with the reported value of 18.7% (Artherton and Meara 1939). The acid value of the SEF also compared favourably with reported solvent extract values of 19-27 (Eckey, 1954) . The significantly ($p< 0.05$) high %FFA and acid values of the PCF compared to SEF is probably due to the generation of more free fatty acids in PCF due to hydrolysis during extraction. As high temperatures were observed to increase FFA values during fat stability study. The significantly ($p< 0.05$) lower %FFA and acid value of the RPF compared to PCF and SEF could be attributed to the removal of the inherent free non-fatty

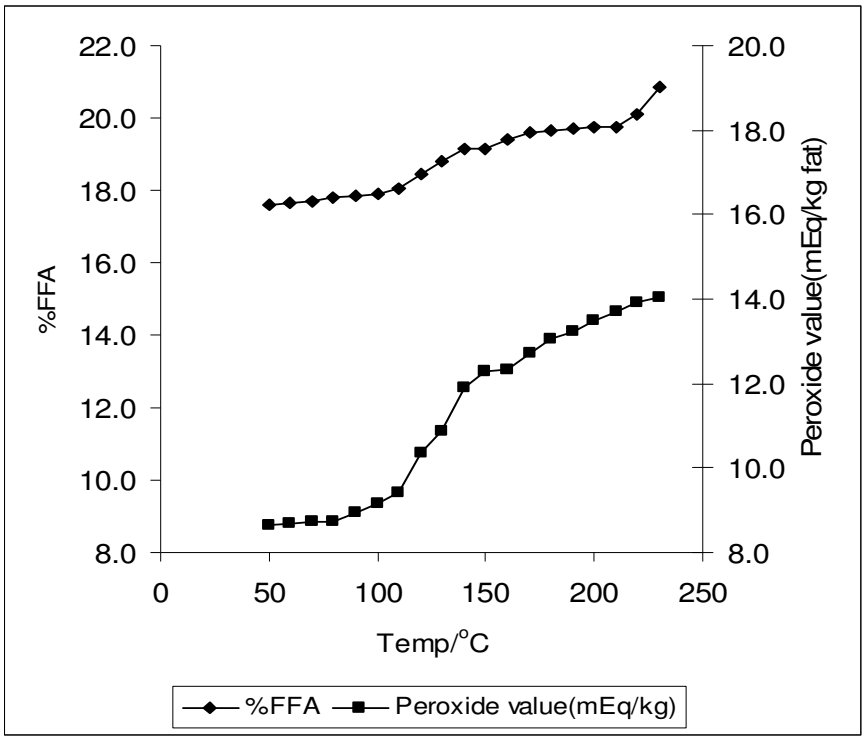

Figure 1. Effects of temperature on free fatty acids and peroxides formation of Kombo fat

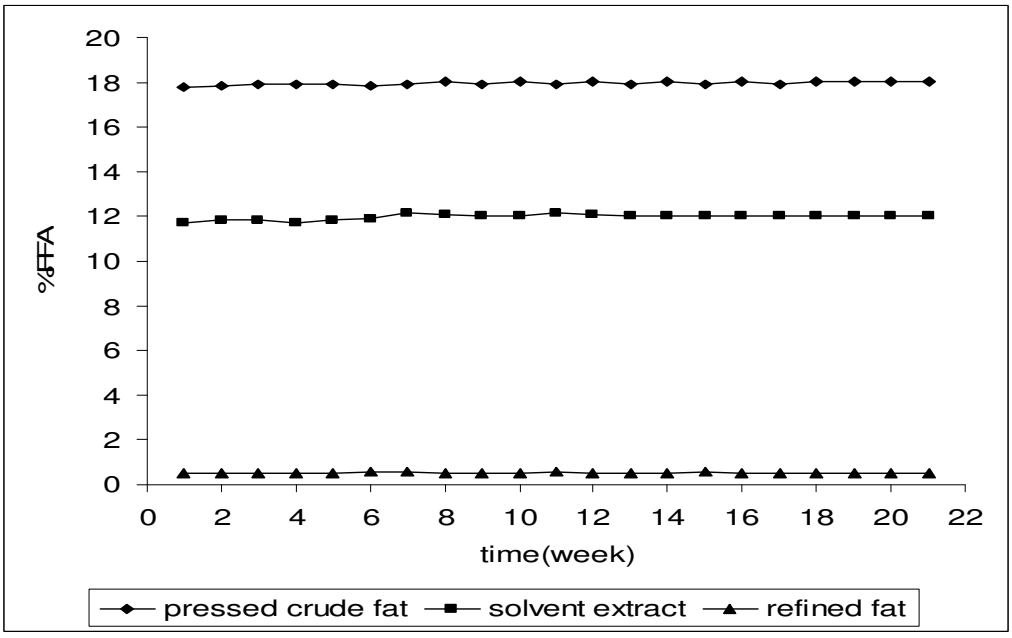

Figure 2. Effect of ambient storage time on free fatty acids formation in Kombo fat

resin acids as well as the free fatty acids generated from hydrolysis during extraction or storage of kernels. Both PCF and SEF are not suitable for direct consumption since their acid values are greater than the maximum

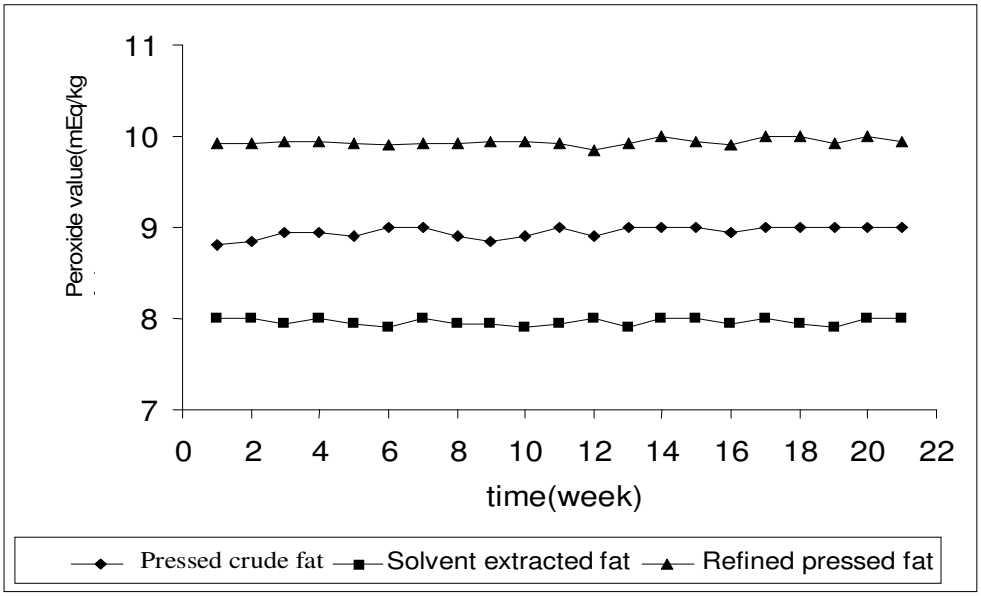

Figure 3. Effect of ambient storage time on peroxide formation in Kombo fat.

permissible acid levels of 4mgKOH/g fat or oil required for edible virgin fats and oils (Codex Alimentarius, 1992).

The PCF and SEF were observed to have significantly ($p< 0.05$) lower peroxide values than the refined fat because of the presence a natural antioxidant (kombic acid) which has been reported (Lok *et al.*, 1983) in the crude Kombo fat. Thus, the crude Kombo fat could be a good source of antioxidant as indicated in previous reports (US Patent No., 6,489,494).

High saponification values of fats and oils are due to the predominantly high proportion of shorter carbon chain lengths of the fatty acids (Kirk and Sawyer, 1991). Low molecular weight (short and medium chain) fatty acids have more glyceride molecules per gram of fat than high molecular weight acids. Each glyceride molecule requires three KOH molecules for saponification, hence the more the glyceride molecules the greater the saponication value (Aurand *et al.*, 1987). Kombo fat have been reported to consist of predominantly high levels of myristic and myristoleic acids which are basically medium chain fatty acids and this accounted for the high saponification values of the fat samples. The saponification value of the RPF and was significantly ($p< 0.05$) higher than the rest apparently due to the removal of free non-fatty resin acids. Consequently, increasing its glyceride content compared to the rest. The saponification value of the SEF was also significantly ($p< 0.05$) higher than that of the PCF probably due its lower level of insolubles and unsaponifiable matter. The values were comparable to reported values of 1.2% (for crude fat) and 0.7% (for the refined fat) (Eckey, 1954). The low values of unsaponifiable matter suggest that the samples contained low level of sterols, paraffin hydrocarbons, alcohols and mineral oil and contaminants such as heavy metals (Boekenoogen, 1964). RPF had the least value indicating a reduction in unsapofiable matter during the neutralization process.

The PCF was observed to have a much higher viscosity at 60^{o}C than SEF and RPF. The low value for RPF may be due to the removal of gums during refining. Gums, which basically consist of phospholipids and proteins, are reported to exhibit high viscosity (Kirk and Sawyer, 1994). The significantly ($p< 0.05$) high viscosity of PCF compared to SEF was probably due to the presence of more gums (hydratable and non-hydratable) in PCF than SEF which apparently contained mainly non-hydratable gums since it SEF was extracted using a non-polar organic solvent. The slight change in p-anisidine or totox value indicated a slight increase in both primary and secondary oxidation in the fat samples This, implies that the Kombo fat is fairly stable under ambient storage condition and this behavior may be attributed to the presence of the natural antioxidant (kombic acid). The values were significantly ($p<0.05$) lower than the maximum value (p-AV=10) recommended for edible purposes. The totox values were also within the range of Codex standards for edible fats).

The significantly ($p< 0.05$) higher calorific value RPF compared to the rest was probably due to its low level of unsaturation as a result of the removal of the highly unsaturated resin acid, impurities and volatile matter. The slightly higher level of impurity of the pressed crude fat may also have accounted for its significantly ($p<0.05$) lower calorific value than the solvent extract.

Fat stability

An increase in temperature promotes lipid oxidation and this was observed to have a direct effect on the oxidative rancidity of Kombo fat, This observation agrees with previous report by Remanna and Sen (1983) that a positive correlation existed between high heat and rate of deterioration of oils. The high stability of the Kombo fat may be attributed to the presence of the natural antioxidant as well as its low degree of unsaturation. Since a low level of unsaturation in a fat makes it less susceptible to rancidification.The induction period of peroxide formation of Kombo fat was probably greater than 21 weeks since there was no significant increase in the peroxide value to values beyond 20mEq/kg over the period of study. A rancid taste is often noticeable in many fats when the peroxide value is between 20 and 40mEq/kg (Kirk and Sawyer, 1991). Thus, the Kombo fat, if stored in sealed plastic containers under ambient conditions can remain wholesome for at least 5 months without spoilage. The rancidity (Kreis) test carried out on each of the sample over the period of 21weeks was negative, confirming the stability of the fat during the period of study.

CONCLUSION

The relatively high fat content of the Kombo kernel suggests commercial extraction of the fat for industrial applications. The physico-chemical properties of the PCF, SEF, and RPF were signicantly ($p < 0.05$) different indicating an effect of processing methods on the quality of the Kombo fat. The refined fat could be most suitable for soap making compared to the rest. The crude pressed fat and solvent extracted fat were unsuitable for direct human consumption considering their high levels of acidity. However, a toxicity test is necessary to establish the edibility of the refined fat. Kombo fat is a non-drying fat and hence unsuitable for production of paints, vanishes and surface coating since its iodine value is less than 100. The high stability of the fat indicates that the Kombo fat produced by solvent extraction, mechanical pressing or in refined state can be stored under ambient conditions for least 21weeks without undergoing deterioration. The crude fat is probably a good source of a natural antioxidant in the stabilisation of human and animal foods against rancidity, colour and odour development as reported in previous study. The fat could also be good for biofuel considering its high calorific value.

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REFERENCES

Abbiw DK (1990) Useful Plants of Ghana. Intermediate Technology Publication & The Royal Botanical Gardens, Kew, London. 178,179.

Acquaye D (1999). Feasibility Report on Kombo butter. Prepared for Technoserve, Ghana.

Agyare C, Asase A, Lechtenberg M, Niehues M, Deters A, Hensen A (2009) An Ethnopharmacological use of medicinal plants used for wound healing in Bosomtwi-Atwima-Kwanwoma area, Ghana. A Journal of Ethnopharmacology 125:393-403

Akendengue B, Louis AM, (1994) Medicinal plants used by the Masango people in Gabon. Journal of Ethnopharmacology 41:193-200

AOAC (1990). Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, AOAC Pub., Virginia, USA.

AOCS (1994). Official Methods and Recommended Practices of the American Oil Chemists' Society, AOCS Press, Washington DC.

Atherton D, Meara ML (1939). Fatty Acids and Glycerides of Some Myristica Fats. J. Soc. Chem Ind, London. 353-357

Aurand LW, Wood AE, Wells MR (1987) Food Composition and Analysis. Van Nostrand Reinhold, New York. 20-33, 181-220.

Ayensu ES (1978). Medicinal plants of West Africa; Reference Publications: Algonac, Michigan, U.S.A. 196.

Boekenoogen, HA (1964). Analysis and Characterisation of Oils, Fats and Product. Vol1. Interscience Publisherss, London, 10.

Cocks LV, Rede van C (1966) Laboratory Handbook for Oil and Fat analysis. Academic Press Inc., London and New York. 80-129.

Codex Alimentarius (1992) Fats, Oils and Related products. Joint FAO/WHO. Food Standards Programme Codex Alimentarius Commission.Vol. 8 2nd ed, Rome.

Devine J, Williams PN (1960) The chemistry and technology of Edible Oils and Fats: Proceedings of a conference arranged by Unilever Limited at Research Department, Port Sunlight, London, March 10-12th 1959,. Pergamon Press, London. p.34-38.Eckey EW (1954) Vegetable Fats and Oil, Reinhold Publishing Corp. 407-426.

Eastop TD, McConkey A (1993). Applied Thermodynamics for Engineering Technologists, 5th ed. Longman Scientific & Technical, England, p. 22-24

Gunstone DF, Hardwood LJ, Padley BF, (1986) The Lipid Hand Book. Champman and Hill, New York, p181-265.

Holman JP (1994). Experimental methods for Engineers, 6th edition. McGraw-Hill, Inc. New York. p 400-406, 411-414.

Irvine FR (1961) Woody Plants of Ghana with special references to their uses. Oxford University Press, London. 28- 30.

Kirk RS, Sawyer R (1991). Pearson's composition and Analysis of Foods, 9th edition, Addison Wesley Longman Ltd., England. 9-29, 608-640.

Lok CM, Groenewegen A, Stroink BA, Ward JP (1983) Phytochemistry Pergamon Press Ltd, Great Britain. Vol.22, No 9: 1973-1976.

Mapongmetsem PM (2007). Pynanthus angolensis (Welw.) Warb. In: van der Vossen, H. A.M & Mkamilo, G.S.(Editors). PROTA 14: Vegetable oils/Oleagineux. PROTA, Wageningen, Netherlands.

Obeng GY, Adjaloo MK, Donkor P (2010) Effect of Temperature, Moisture Content, Particle Size and Roasting on Shea Butter

Extraction Efficiency. International Journal of Food Engineering: Vol.6: Iss. 2, Article 13.

Ramanna BR, Sen DP (1983) Influence of water on the changes of oil during heating. Journal of Food Science and Technology. 20:146-149.

.Tapley BD (1990). Eshbach's Handbook of Engineering Fundamentals 4th ed. John Wiley and Sons, New York, p5.68-5.70.

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