Journal of Investigational Biochemistry
www.scopemed.org
DOI: 10.5455/jib.20140504020638

Original Research

GESDAV

Phytosterol and amino acid profiles of palm kernel oil: Possible metabolic implications

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Received: August 12, 2013
Accepted: May 04, 2014
Published: ???

ABSTRACT

Hypothesis and Aim: Compositions of an edible material can influence its metabolic fate. Palm kernel oil (PKO) contains more saturated fat than unsaturated fat, indicating a propensity to elevate serum total cholesterol and low density lipoprotein-cholesterol (LDL-c). Its phytosterol and amino acid (AA) profiles may also have metabolic implications. The aim of the study was to examine PKO for the presence of phytosterols and its AA profile. **Materials and Methods:** Palm kernel oil was examined for the presence of phytosterols, AA compositions, AA groupings and essential AA (EAA) scores, using palm oil (PO) as a reference. **Results:** The PKO did not contain phytosterols and as such may not competitively inhibit uptake of dietary cholesterol. Its total AA content (g/100 g protein) was 6.87 ± 0.49 compared to PO's 49.45 ± 1.36. The total non-EAA (TNEAA) /total EAA (TEAA, with His) ratios of PKO and PO were 1.04 ± 0.01 and 0.98 ± 0.00, respectively; suggesting that PKO contained more TNEAA than TEAA (with His) while the reverse was the case in PO. The PKO did not contain some EAAs such as Ile, Val, His and Thr and as such its EAAs cannot support protein synthesis based on the all-or-none principle of EAAs in respect of protein synthesis, but would rather be used for energy, converted to carbohydrate or fat, and stored as such. **Conclusion:** The PKO lacked phytosterols and some essential AAs and these may contribute to its acclaimed atherogenicity.

KEY WORDS: Amino acid profile, palm kernel oil, phytosterols

INTRODUCTION

Palm kernel oil (PKO) and palm oil (PO) are edible oils extracted from the kernels and ripe fleshy mesocarps, respectively, of the fruits of tropical palm tree (*Elaeis guineensis* Jacq.) [1-3]. Palm kernel oil does not contain the same nutritional advantages as other vegetable oils [4]. These oils have high energy contents [5]. PO is widely used as a vegetable oil and contains 43.5% palmitic acid while PKO contains 47.0% lauric acid [6,7]. On the other hand, PKO is underutilized as edible oil in Nigeria [8]. However, it is a common cooking oil in tropical regions such as Southeast Asia and central Africa; also becoming popular in other areas due to its low manufacturing cost relative to those of other cooking oils [9].

Oils add nutrients to the diets they are used in preparing [6]. They also serve as concentrated sources of energy [10], thereby leading to reductions in feed intakes [5,10]. Food constituents can influence the levels of endogenous metabolites. Foods high in saturated fatty acids (SFAs) increase endogenous cholesterol levels [11]. However, unsaturated and polyunsaturated fatty acids (PUFAs) have the reverse effects [6]. Likewise, endogenous cholesterol levels are reduced by increasing dietary phytosterol intakes [12]. PO has been found to reduce endogenous cholesterol content [13]. It has been reported to contain antioxidants like β-carotene (a pro-vitamin A) and vitamin E (particularly tocotrienols) and has high unsaturated and PUFAs [5,6] which it adds to meals. PO has also been reported to discourage the development of atherosclerosis and thrombosis [14,15]. Consumption of PO as a source of dietary fat does not pose any additional risks for coronary artery disease as it raises plasma cholesterol only when an excess of dietary cholesterol is presented in the diet [5]. On the other hand, PKO has more saturated fat [6]. It does not contain β-carotene and has been reported to contain less mono- and PUFAs than PO [6,7]. It increases serum low density lipoprotein (LDL) levels and serum total cholesterol/high density lipoprotein (HDL) and LDL/HDL ratios; indicating that it is atherogenic [5,16,17]. Although PKO does not contain cholesterol, it is extremely high in artery-clogging saturated fat [4]. PKO-treated diets were reported to have also increased serum aspartate aminotransferase activity, aspartate aminotransferase/alanine aminotransferase ratio, and serum total protein and globulin concentrations, but reduced the serum albumin concentration and albumin/globulin ratio in rats [18]. The atherogenicity of PKO may be principally due to its very high SFA content; reportedly up to 80% [19] and the overconsumption of saturated fat is associated with high serum LDL-c content [6].

While literatures are awash with the lipid profiles of PKO and their metabolic implications, there is little or no report on its phytosterol and amino acid (AA) profiles. The aim of the study

was to examine PKO for the presence of phytosterols, determined its AA compositions, AA groupings and essential AA (EAA) scores using PO as a reference.

MATERIALS AND METHODS

Procurement of Samples

The PO and PKO samples used in the study were purchased from 10 local markets situated in ten states in the southern part of Nigeria. The oils were extracted from the kernels and ripe fleshy mesocarps, respectively, of fruits of the tropical palm tree (*E. guineensis*) sourced from different localities where the industries involved in producing them procure their raw materials.

Test for the Presence of Phytosterols

The test for the presence of phytosterols was carried out by adapting the principles of the Liebermann–Burchard test for cholesterol as described by [20]. Briefly, 1.0 mL of oil was dissolved in 1.0 mL of chloroform and 1.0 mL of acetic anhydride was carefully added followed by 2.0 mL of concentrated H_2SO_4. A color change from red to purple then blue and finally blue-green indicated the presence of phytosterols.

Estimation of Amino Acid Compositions of Samples

The estimations of the AA compositions of the oils were carried out by ion-exchange chromatography using the Technicon Sequential Multi-sample (TSM) AA analyzer (model DNA 0209 of Technicon Instruments Corporation, New York) as described by [21,22]. The oils were defatted, hydrolyzed and evaporated. Briefly, an aliquot (200.0 mL) of the sample was mixed with an equal volume of chloroform/methanol (2:1) in a 500 mL capacity separatory funnel. The layers were allowed to separate and the chloroform (lower) layer bearing the fat discarded. The fat in the sample was extracted thrice using this protocol. More of the sample was defatted until the quantity required for analysis was got. The defatted samples were put together and concentrated using a rotary evaporator at 50°C. The nitrogen contents of the oils were estimated, in duplicates, using the principles of the micro-Kjeldahl method as described by [20,23]. Briefly, 1.00 g of the defatted sample was put into a digestion tube and 20 mL of concentrated H_2SO_4 added. A quantity (3.0 g) of a digestion catalyst (prepared by mixing 10.0 g of anhydrous Na_2SO_4 and 1.0 g of $CuSO_4$) was poured in and four pieces of anti-bumping granules added to make the mixture boil more calmly. The mixture heated until the dirty-brown solution became clear and colorless. The digest was cooled and diluted to 100 mL with distilled water. A Markham distillation apparatus was set up with the tip of the delivery tube dipping below the surface of a 10 mL 2% boric acid solution. The boric acid solution contained two drops of methyl red-methylene blue double indicator and was placed in a calibrated 50.0 mL conical flask that was used as the receiver. An aliquot (30.0 mL) of 40% NaOH was added to 10 mL of the diluted digest that was placed in the distillation flask with the aid of a syringe and needle. Distillation lasted until the volume of liquid in the receiver reached 20 mL. This took 25 min. The outside of the delivery tube was washed with little distilled water. The distillate was titrated with 0.01 N HCl until the first pink tinge appeared and the titer value (*T*) noted. The percentage of nitrogen in the defatted sample was calculated by multiplying the ratio of the product of *T*, a 1.40 factor and the dilution factor to that of the weight of the defatted sample that was digested expressed in milligrams by 100. For the estimation of the AA compositions, 0.50 g of the defatted sample was put into a glass ampoule, and 7 mL of 6 N HCl added. Oxygen was expelled from the ampoule by passing nitrogen gas into it. The ampoule was then sealed with a Bunsen burner flame, put in the oven, and its content hydrolyzed at 105°C for 22 h. It was brought out, cooled and a tip broken open. The hydrolysate was filtered, and its filtrate evaporated to dryness at 50°C *in vacuo*. The hydrolysate was dissolved in 5 mL acetate buffer (pH 2.0). The resin in the cartridge was equilibrated at pH 2.0 using 0.20 N NaOH and 0.20 N sodium citrate buffer (pH 2.0), respectively. Then, 5.0 μL of 0.025 μM of norleucine (Nleu) was added to 10.0 μL of the dissolved hydrolysate and the mixture loaded into the cartridge of the TSM AA analyzer. Nleu was used as the internal standard. The acidic, neutral and basic AAs contained in the hydrolysate were evaluated using C-3 resin type at a flow rate of 0.50 mL/min and temperature of 60°C. The resin bed for acid-neutral column was 23.5 cm long while that for the basic column was 5.0 cm. The assay period lasted for 76 min. A standardizing run was also made using the internal standard and standard AAs. The chromatograms generated by the continuous trace chart recorder were then used to calculate the amounts of the various AAs in the samples [21]. Each peak represented an AA. First, area of the peak (Q) for an AA was calculated by multiplying the net height of the peak by the width at the half-height. Then its Nleu equivalent in the standardizing run (NE_{std}) was calculated as the ratio of the area of Nleu peak in the standardizing run to that of the AA. A standard (S_{std}) for the AA in the standardizing run was calculated as the product of NE_{std}, molecular weight of the desired AA and concentration of the Nleu (in μM). A constant (*C*) was first calculated as the ratio of the product of the dilution factor and a 16 factor and the product of the weight of sample hydrolysed, percentage nitrogen content of the defatted sample, a factor 10 and the volume of hydrolysate loaded into the cartridge. This ratio was then divided by the area of the Nleu peak. The quantity of the AA (g/100 g protein) was eventually calculated as the product of the area of the peak of the desired AA (Q), its S_{std} and the constant (*C*). The assays were run in duplicates. The Trp contents were not determined as it is normally destroyed during acid hydrolysis for the determination of the amino acid compositions [24,25].

Resolution of Glu Content from Glx and Asp Content from Asx

The Glu content was resolved from Glx (which is Glu, plus the Glu group of Gln) and the Asp content resolved from Asx (Asp, plus the Asp group of Asn) content as described by [26]. Asparagine (Asn) and Gln contain amide side-chains which are often hydrolysed in the chemical procedure used for the determination of the AAs of proteins and their Asp and Glu residues added to the Asp and Glu contents [24,25].

However, the average percentage occurrences of Glu, Gln, Asp and Asn in 1150 proteins of known AA sequences are 6.3, 4.2, 5.3, and 4.3, respectively [24]. The ratios of 6.3/4.2 occurrences for Glu and Gln and 5.3/4.3 occurrences for Asp and Asn were then used to resolve their contents from Glx and Asx contents. The Glx and Asx contents (g/100 g protein) of PKO and PO were 2.38 ± 0.04 and 6.78 ± 0.04, respectively, and 0.30 ± 0.03 and 6.29 ± 0.03, respectively, as determined.

Estimation of Amino Acid Profile and Protein Quality

The total acidic AA (TAAA) was calculated by summing their Asp and Glu contents; the total basic AA (TBAA) was calculated by summing their His, Lys, and Arg contents; the total aromatic AA (TArAA) was calculated by summing their Phe and Tyr contents; the TEAA was calculated by summing the Phe, Leu, Ile, Met, Val, His, Lys, Arg, and Thr content. The total nonessential AA (TNEAA) was calculated by summing the Cys, Ala, Gly, Pro, Glu, Gln, Ser, Asn, Asp, and Tyr contents. The total branched-chain AA was calculated by summing Leu, Ile, and Val contents. The total sulfur AA (TSAA) was calculated by summing the Cys and Met contents. Total hydrophobic amino acid (THAA) was calculated by summing the Ile, Leu, Al, Val, Pro, Met, and Phe contents. Percentages of these groupings and some AAs in their proteins and ratios of some of these groupings were computed.

The chemical scores for the EAA in the oils were calculated as the ratio of the amount of each EAA (in mg) to that of an "ideal" amount for that EAA (also in milligram) of a reference food protein [6].

Statistical Analysis

Data were analyzed using the Student's *t*-test of significance. Values were declared significant at $P < 0.05$.

RESULTS

The PKO samples used did not respond to the Liebermann–Burchard test for cholesterol. This indicated that they did not contain phytosterols.

Table 1 shows the AA profiles of the oils. It shows that the quantities of AAs in PKO were significantly ($P < 0.05$) lower than those in PO. PKO samples did not contain Ile, Val, His, Thr, Cys, Pro, Ser, and Tyr.

Table 2 shows the total AAs and the AA groupings of the oils from *E. guineensis*. It shows that PKO contained significantly ($P < 0.05$) lower amounts of all the AA groupings than the PO. The TNEAA/TEAA (with His) ratio of the PKO samples was significantly ($P < 0.05$) higher than that of PO samples.

Table 3 presents the EAA scores of the oils. It shows that the EAA scores of the PKO samples were significantly ($P < 0.05$) lower than those of the PO samples.

Table 1: Amino acid compositions (g/100 g protein) of the palm oils*

Essential amino acid	Sample		Nonessential amino acid	Sample	
	Palm oil	Palm kernel oil		Palm oil	Palm kernel oil
Phe	3.54±0.07[b]	0.36±0.04[a]	Cys	0.56±0.08	0.00±0.00
Leu	3.23±0.09[c]	0.75±0.08[a]	Ala	3.40±0.04[e]	0.38±0.02[a]
Ile	2.50±0.04	0.00±0.00	Gly	2.46±0.04[b]	0.44±0.02[a]
Met	0.57±0.04[b]	0.45±0.03[a]	Pro	1.35±0.07	0.00±0.00
Val	3.61±0.12	0.00±0.00	Glu	6.78±0.04[d]	2.38±0.04[a]
His	2.28±0.02	0.00±0.00	Gln	2.71±0.02[a]	0.95±0.02[a]
Lys	3.08±0.03[b]	0.24±0.01[a]	Ser	1.50±0.06	0.00±0.00
Arg	2.95±0.12[b]	1.57±0.08[a]	Asn	2.82±0.03[d]	0.13±0.01[a]
Thr	3.28±0.07	0.00±0.00	Asp	3.47±0.03[d]	0.17±0.01[a]
Trp	ND	ND	Tyr	2.07±0.00	0.00±0.00

*Values are mean±SD of duplicate determinations. Values on the same row with the same superscript letter a, b, d or e are not significantly different ($P>0.05$). ND: Not determined

Table 2: Total amino acids, amino acid groupings, percentage occurrence of some amino acids, and total hydrophobic amino acid contents of the oils*

Parameter	Palm oil	Palm kernel oil
TAA	49.45±1.36[a]	6.87±0.49[b]
TNEAA	24.41±0.57[a]	3.50±0.27[b]
TEAA		
With His	25.04±0.79[a]	3.37±0.23[b]
Without His	22.76±0.76[a]	3.37±0.23[b]
TNEAA/TEAA (with His) ratio	0.98±0.00[a]	1.04±0.01[b]
TAAA	7.54±0.10[a]	1.60±0.10[b]
TBAA	8.31±0.24[a]	1.81±0.13[b]
TBAA/TAAA ratio	1.10±0.03[a]	1.13±0.02[a]
TSAA	1.13±0.17[a]	0.45±0.04[b]
% Cys in TSAA	49.36±2.60	0.00±0.00
TArAA	5.61±0.10[a]	0.36±0.06[b]
% Tyr in TArAA	36.90±0.80	0.00±0.00
THAA	18.51±0.45[a]	1.94±0.06[b]
% THAA	37.43±1.20[a]	28.24±0.44[b]

*Based on Table 1; values are mean±SD of duplicate determinations. Values on the same row with the same superscript letter a or b are not significantly different ($P>0.05$). TAA: Total amino acid, TNAA: Total nonessential amino acid, TEAA: Total essential amino acid, TAAA: Total acidic amino acid, TSCAA: Total sulfur-containing amino acid, TArAA: Total aromatic amino acid, THAA: Total hydrophobic amino acid, TBAA: Total basic amino acid, SD: Standard deviation

Table 3: Essential amino acid score based on FAO/WHO/UNU [29] provisional amino acid scoring pattern*

Essential amino acid	Palm oil	Palm kernel oil	FAO/WHO/UNU [29] (mg/g)
Ile	0.61±0.02	0.00±0.00	40
Leu	0.46±0.02[a]	0.11±0.02[b]	70
Lys	0.56±0.01[a]	0.05±0.01[b]	55
Met+Cys	0.33±0.05[a]	0.13±0.01[b]	35
Phe+Tyr	0.94±0.02[a]	0.06±0.01[b]	60
Thr	0.82±0.03	0.00±0.00	40
Trp	ND	ND	10
Val	0.73±0.04	0.00±0.00	50
Total	4.45±0.03[a]	0.35±0.02[b]	360

*Values are mean±SD of duplicate determinations. ND: Not determined. Values on the same row with the same superscript letter a or b are not significantly different ($P>0.05$)

DISCUSSION

The PKO samples did not contain phytosterols. This implied that when they are used as vegetable oil, the intestinal uptake of available dietary cholesterol would not be competitively inhibited, thereby further increasing the endogenous cholesterol content. The antioxidants earlier detected in PKO were only tannins and flavonoids while PO contained tannins, flavonoids, β-carotene, and saponins [5]. It was reported that one teaspoonful of PKO and PO contains 0.5 and 3.0 mg of vitamin E, respectively [6]. Tocotrienols (types of vitamin E) appear to reduce serum cholesterol concentrations; with those in PO ostensibly being able to stimulate the synthesis of protective HDL-c and removal of harmful LDL-c [5]. These seem to suggest that PKO contains less types and quantities of antioxidants and may have lower capacities to prevent the oxidation of LDL-c to the atherogenic type (the oxidized LDL-c).

The PKO samples investigated were very poor sources of AAs [Table 1]. Table 2 further explained that the PO samples were better sources of AAs and contained more TEAA (with His) than TNEAA. This suggested that PO would support the synthesis of body proteins. Biochemically, the TBAA/TAAA ratios of the oils [Table 2] suggested that they contained more basic proteins, which may act as bases at physiological pH. However, PKO and PO had been reported to be acidic [5]; with PKO having a pH of 5.85 while PO had a pH of 5.54. Thus, suggesting that the charges on their proteins did not influence the general physiochemical property of the oils. The TNEAA/TEAA (with His) ratios of the oils [Table 2] suggested that the PKO samples contained more TNEAA than TEAA, while the reverse was the case in the PO samples.

The Met contents of the PKO samples may always be used to synthesize Cys since they did not contain it [Table 1]. However, the % Cys content in TSAA for PO samples indicated that their Cys contents may have Met sparing actions [Table 2]. The % Cys contents of the PO samples were higher than the 37.8% reported for *Garcina kola* [27]. They were however lower than the more than 50.5% value expected of plant proteins. Legumes contain substantially more Cys than Met [28]. The oils' % Tyr in TArAA [Table 2] suggested that while almost all the Phe contents of the PKO samples may be used to produce Tyr since they did not contain it, >30% of the Phe contents of the PO samples may be spared by Tyr [Tables 1 and 2]. Phe is an EAA and its depletion could halt protein synthesis. The oils' THAAs were <38% of the TAAs [Table 2]. This may debunk the belief in certain quarters that oils contain more hydrophobic AAs than hydrophilic AAs.

The EAA scores for the PKO samples were poor [Table 3], based on FAO/WHO/UNU [29] provisional AA scoring pattern, because they did not contain Ile, Thr, Val, and His [Table 1]. These AAs could then be termed their limiting AAs (LAAs). These LAAs would not be met by consuming large quantities of the PKO or its protein; their protein content being reportedly 0.90 g/100 mL [5]. On the other hand, the LAAs in the PO samples were Met and Cys [Table 3]. Recall that PO is sometimes used traditionally in processing cassava (*Manihot esculenta*) tubers into *garri* (a fine to coarse fried product of grated, fermented and sieved cassava). These sulfur-containing AAs convert the cyanogenic glycoside and HCN contents of the processed *garri* to SCN^-. The LAAs in the PO samples can be met by consuming 1/0.33 or 3.03 g of their protein. This suggested that their LAAs can be met by consuming 126.25 mL/day. The protein content of PO had earlier been reported to be 2.40 g/100 mL [5]. Comparatively, palm kernel cakes (PKCs) have better AA profile and higher protein contents [30,31]. The PKCs contained all the AAs and had just Lys as the LAA. Their protein contents ranged from 14.5 to 19.6%. This seems to suggest that much of palm kernel protein is lost to the PKC when PKO is extracted.

Since the PKO samples did not contain some EAAs [Table 1], the metabolic implication may be that the residual EAAs would not be available for protein synthesis going by the all-or-none rule for EAAs. This rule stipulates that either all the essential AAs are available for protein synthesis or none would be used for such [6]. This seemed to suggest that the EAAs in the PKO samples may only be available for energy purposes and for the biosynthesis of fat or carbohydrate. The fat or carbohydrate can then be stored in tissues. This may further explain the underlying biochemical mechanism for the atherogenicity of PKO as reported by [5,16,17] and other researchers. The lack of phytosterols and some EAAs in PKO may contribute to other factors such as high SFA contents, low PUFA/SFA ratio, and low antioxidant status that may singly or collectively contribute to the oil's atherogenicity.

CONCLUSION

The PKO did not contain phytosterols and some essential AAs and the absence of these may contribute to the other factors that encourage its atherogenicity.

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Source of Support: Nil, Conflict of Interest: None declared.