



**African Journal of
Biochemistry Research**

Volume 10 Number 4, May 2016

ISSN 1996-0778



*Academic
Journals*

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ARTICLE

Oil palm fruit calyx as a resource for phospholipids extraction

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Gold I. L., Oseni N. T. and Imoisi O. B.

Full Length Research Paper

Oil palm fruit calyx as a resource for phospholipids extraction

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Received 16 February, 2016; Accepted 5 May, 2016

Oil palm calyx is a solid waste in the oil palm industry which is yet to find economic usage. This study aimed to determine the feasibility of recovering phospholipids from the oil palm calyx. Preliminary functional property of calyx phospholipids was determined using emulsion stability. Results indicated that oil palm calyx had 14% total lipids when extracted with methanol and 62.3% of lipids were acetone insoluble lipids (phospholipids). Methanol extracted phospholipids showed that the highest concentration of total phospholipids (1971 mg/L) with phosphatidylcholine (PC) have been the predominant phospholipid. The calyx phospholipids stabilized oil in water emulsion at 2% concentration. The study serves as a foundation on the positive usage of oil palm calyx in producing industrial phospholipids.

Key words: Oil palm, lecithin, phospholipids, waste, palm calyx oil.

INTRODUCTION

Oil palm (*Elaeis guineensis*) is one of the Nigerian economic crop (Olagunju, 2008). It is used to produce palm oil and palm kernel oil. Several by products have also been produced from its leaves, empty bunches, shells and press cake, such as, palm kernel cake, activated charcoal, furniture, bio fertilizers, biogas, etc. In Nigeria, 80% of palm oil production is carried out in small or medium scale levels (Poku, 2002). The key unit operation in small scale production of palm oil is threshing, picking, sieving, sterilization, extraction and clarification. Palm calices are usually generated during the sieving of loose fresh fruits. The oil palm calyx is a set of sepals of the palm fruit blossom that remain on the fruit

after harvesting. In most oil palm plantation in Nigeria, sieving of loose fruits is carried out using a mechanical sieve. At present, the oil palm calices generated are disposed as solid waste in the environment.

The emphasis on zero waste technologies has been the advocacy for sustainable development. In the palm oil industry, most of the waste produced have found economic usage such as the bunch refuse used for energy generation and palm oil mill effluent (POME) used in soap making and biogas production (Sumathi et al., 2008; Tay, 1991). One tonne of oil palm fresh fruit bunches (*Tenera* variety) contains 600 to 750 kg of loose fruits, 300 to 320 kg of bunch refuse and 90 to 110 kg of

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calyx. Based on the 2013 Food and Agriculture Organization statistics of oil palm fruit production in Nigeria (<http://faostat3.fao.org>), Nigeria produces between 450,000 and 590,000 tonnes of oil palm calices annually which are discarded into the environment as waste. The use of this waste in the production of industrial phospholipids will go a long way to increase the income generated by the oil palm industry as well as serve in implementing zero waste technology advocacy.

Several studies have found out that palm-pressed fiber are excellent source of extracting phospholipids (Choo et al., 2004; Chua et al., 2008). Since in Nigeria the calices are isolated from the remaining pressed fiber, extraction of phospholipids from calices will further boost the income of these small scale farmers, because phospholipids are highly sourced ingredients for food, cosmetics and pharmaceutical industries. Phospholipids mostly consist of diglyceride and a phosphate group attached to the end, hence, it has a hydrophobic tail and hydrophilic head, with the ability to bind water and oil. This property of phospholipids has made it an important ingredient in the food industry, such as, emulsifying agent, freeze-thaw stability, release agent, anti-sticking and viscosity enhancer (Hinton et al., 1970; Szuhaj, 1989). Phospholipids are also key ingredients in biomedical and pharmaceutical industries (Kang and Row, 2002). The current study aims at determining the potential of using oil palm fruit calyx waste for the production of functional phospholipids.

MATERIALS AND METHODS

Oil palm calyx used for the experiment was donated by the Agricultural Engineering mill, Nigerian Institute for Oil palm Research (NIFOR). Oil palm calyx was air dried for 48 h and packed in airtight nylon bags.

Extraction of calyx oil

Prior to extraction, oil palm calyx was blended using a waring blender. Three solvents were employed in the extraction of calyx oil (methanol, ethanol, and hexane).

Cold extraction was used in the extraction of calyx oil according to Choo et al. (2004). Extraction involved soaking oil palm calyx with hot solvent in a sealed container for 48 h at room temperature. Solvent was collected using cheese cloth and subsequently filtered under pressure (Whatman Filter paper no. 1). Calyx oil was recovered from the solvent via rotary evaporation at 40°C under vacuum. Calyx oil was refrigerated at 5°C prior to further analysis.

Oil yield

The oil yield from each solvent used was determined according to the AOCS Official Procedure Am 5-04 (American Oil Chemists Society, 2005).

Oil yield (%) = Weight of oil/Weight of oil palm calyx × 100

Determination of total phospholipids

Total phospholipids content of the calyx oil samples were

determined using Nephelometric Method (AOCS Ca 19-86) (Firestone, 2009). To determine the equation curve for calculation of total phospholipids from turbidity, 0.33 g (12 replicate) of calyx oil was used to determine turbidity. Phosphorus was also determined from each sample using AOCS Ca 12-55 (Firestone, 2009). The equation curve derived was used for further determination of total phospholipids.

Fatty acid composition

Calyx oil from methanol extraction was methylated according to Sigma Aldrich (1997) method with slight modification to allow for sufficient volatility of fatty acid methyl esters (FAMES) by the gas chromatography-flame ionization detector (GC-FID). Oil (0.1 g) was added to a screw cap tube and dissolved in 5 ml of toluene and 10 ml BCl₃-Methanol. Mixture was flushed with N₂ gas and heated to 60°C for 10 min in a water bath. FAMES were extracted twice using 20 ml of hexane. In each case, the upper hexane layer containing FAMES was carefully separated from the water layer. The FAMES mixture was washed five times with water to remove any trace of BCl₃. Anhydrous sodium sulphate was used to dry the samples. Finally, samples were transferred to 100 ml volumetric flasks and filled to volume with hexane.

The fatty acids from the FAMES were analysed according to Coorey et al. (2012) using gas chromatography (Perkin Elmer model, AutosystemXL USA), equipped with a SGE forte BPX 70 capillary column 30 m × 0.32 mm × 0.25 μm (SGE Analytical Science Pty. Ltd., Ringwood Victoria Australia). The oven temperature was initially set at 80°C for the first 2 min, and subsequently increased to 130°C at a rate of 45°C min⁻¹, where it remained for 10 min. It was then increased at a rate of 2°C / minute to 172°C, for the final 6 min. The FID detector ports and injector were set at 250 and 200°C, respectively using Helium as the carrier gas set at 20 ml/min. One microliter of each sample was injected using an auto sampler and each peak was identified using a Sigma-Aldrich pure methyl ester standard.

Fatty acid (%) = (FA concentration × Dilution factor × Extraction volume) / Sample weight × 100

Determination of phospholipid composition

Determination of phospholipids composition was according to AOCS ja 7b 91 (Firestone, 2009) with slight modification. Sample (0.05 g) was dissolved in 10 ml of the test mobile phase. Mobile phase was made up of hexane, 2-propanol and acetate buffer in the ratio of 80:19.5:0.5 v/v and initially degassed with Fisher band ultrasonic bath FB15056. Test sample (10 μl) was injected into the column at a flow rate of 1 ml/min using HPLC mobile phase hexane/propanol (80:20 v/v). The instrument used was HPLC-Shimadzu LC-6A coupled with a UV detector at wavelength of 206 nm. Each peak was identified using software Azur version 5.0.

Phospholipids (%) = Individual phospholipids area/Total phospholipids area × 100

Emulsion stability

Calyx oil was de-oiled by hydroxylation with 2% water and washed twice with chilled acetone. Emulsion preparation was according to Rydhag and Wilton (1981). De-oiled calyx oil (2 and 5%) in varying oil in water emulsion ratio (0.2, 0.4, 0.6 and 0.8) was hand mixed and placed into measuring cylinder. Visual inspection of continuous phase measured in milliliters was used to determine stability of emulsion immediately after mixing and time interval of 10, 20, 30

Table 1. Palm calyx oil from different solvent extract.

Solvent	Yield (%)	Total phospholipids ^a (mg/L)
Ethanol	10.33 ± 0.37	1151 ± 2.08
Methanol	14.24 ± 0.23	1971 ± 1.15
Hexane	3.80 ± 0.01	992 ± 1.53
P value	< 0.001	0.003

Mean (n = 3), ^aEquation Curve: $y = 4 \times \text{NTU} + 56$.

Table 2. Fatty acid composition of palm calyx oil in comparison to palm oil.

Fatty acid	Palm fruit calyx oil (Composition (%))	Palm oil (Composition (%))
Lauric (C _{12:0})	3.00	0.3
Myristic (C _{14:0})	1.24	1.0
Palmitic (C _{16:0})	39.65	42
Palmitoleic (C _{16:1})	0.41	0.2
Stearic (C _{18:0})	6.07	4.2
Oleic (C _{18:1})	35.79	38.0
Linoleic (C _{18:1})	11.16	9.9
Arachidonic (C _{20:0})	0.25	0.3

Table 3. Phospholipid composition of calyx oil and its acetone insoluble compared to soy oil.

Sample	Phospholipid Composition (%)				
	PE	PI	PC	PA	PS
Calyx oil	16.78	3	76.63	0.036	3.55
Calyx oil acetone insoluble	3.61	12.98	82.26	0.52	-
Soy oil	81.38	1.36	11.84	2.36	3.07
Soy oil acetone insoluble	47.95	10.99	34.43	3.68	2.96

and 40 min or when first separation was observed or 24 h later. Visual inspection was also used to determine if foaming occurred after mixing.

Statistical analysis

Analysis of variance (ANOVA) with turkey test using IBM SPSS version 22 software (IBM Corp., NY, USA) was used to compare mean. Significant difference was determined at $p < 0.05$.

RESULTS AND DISCUSSION

From Table 1, it can be seen that methanol is the best solvent to be employed in extracting the lipid content of oil palm calyx. Gober et al. (1993) reported that phospholipids were more soluble in polar solvent. Methanol has the highest polarity among the solvent used. This indicates that the lipid content of oil palm calyx mostly consist of polar lipids. The total phospholipids content of calyx oil from the different solvents also

correspond with oil yield (Table 1). Methanol extracted oil also gave the highest total phospholipids content which was statistically different at 0.05. This indicates that methanol extraction optimizes both the quantity of oil and the concentration of total phospholipids from the waste.

Table 2 shows the fatty acid profile of calyx oil in comparison with that of palm oil. Although, the medium chain fatty acids of calyx oil were slightly higher than that of palm oil, most of the prominent fatty acids were similar with both oils. According to De Leonardis and Macciola (2012) and Gunstone (2011), the fatty acid profile of oil plays a significant role in the biochemical and physiochemical properties of oil. Since palm oil is known to have a good relative stability to oxidation due to its high percentage of saturated fatty acids (De Leonardis and Macciola, 2012), it can also be extrapolated that calyx oil will also be stable to oxidation.

The phospholipid composition of calyx oil in comparison with soy oil is given in Table 3. Calyx oil had a significantly high amount of phosphatidylcholine (PC)

Table 4. Emulsion separation of oil in water emulsion without calyx oil.

Time	A (16:4) (ml)	B (12:8) (ml)	C (8:12) (ml)	D (4:16) (ml)
10 min	16	12.25	6.5	3
20 min	16	12.25	6.5	3
30 min	16	12.25	6.5	3
40 min	16	12.25	6.5	3
24 h	16	12.25	6.5	3

Table 5. Emulsion separation of oil in water emulsion containing 2% calyx oil.

Time	A (16:4) (ml)	B (12:8) (ml)	C (8:12) (ml)	D (4:16) (ml)
10 min	4.5	2.5	0	0
20 min	11.5	7	0	0
30 min	11.5	7.25	0	0
40 min	11.5	8	0	0
24 h	11.5	8	0	0

Table 6. Emulsion separation of oil in water emulsion containing 5% calyx oil.

Time	A (16:4) (ml)	B (12:8) (ml)	C (8:12) (ml)	D (4:16) (ml)
10 min	7.75	0	0	0
20 min	8.5	0	0	0
30 min	9	0	0	0
40 min	9	0	0	0
24 h	9	0	0	0

which increased from 76.63 to 82.26% when phospholipids were concentrated with acetone to remove neutral fats. Soy oil had PC of 11.84% which increased to 34.43% when concentrated with acetone. Higher value of PC in soy oil was reported by Rydhag and Wilton (1981), however, a decrease in the composition of PC was observed by Mounts and Nash (1990) on stressed soy beans stored for 28 days. Pre-harvest conditions and post-harvest stress may lead to variation in phospholipid composition of soy beans (Mounts et al., 1996; Mounts and Nash, 1990). The phosphatidylethanolamine (PE) of calyx oil decreased from 16.78 to 3.61% when concentrated with acetone, while phosphatidylinositol (PI) increased from 3 to 12.98%. Since the concentrated fraction of phospholipids, that is, without neutral oil is what is used for most industrial application (Van Nieuwenhuyzen, 1976), it is of advantage that the PI is increased. Rydhag and Wilton (1981) reported that for good emulsifying properties of phospholipids, a relative high concentration of negatively charged phospholipids (PI and Phosphatidic acid) is required. This is due to higher solubility of charged phospholipids in water compared to electrically neutral phospholipids (PE and

PC) resulting to easier diffusion of these negatively charged phospholipids to the oil-water interface.

The emulsifying property of calyx oil was used to determine its functional property. The emulsifying property of acetone precipitated calyx oil extracted from methanol (62.3% w/w) of the total lipid (table not shown) is shown in Tables 4, 5 and 6. Oil in water emulsion with acetone precipitated calyx oil (Tables 5 and 6) gave the higher emulsion stability compared to emulsion without calyx oil. Also, as the concentration of calyx oil increased from 2 (Table 5) to 5% (Table 6), the emulsion stability also increased. This indicates that calyx oil played a role in improving the emulsion stability of oil in water emulsion. Overall, the separation of emulsion reduced as oil concentration increased. At 8:12, oil in water emulsion was stable for 24 h even at 2% concentration of calyx oil. Nikovska (2010) reported that emulsion stability is influenced by concentration of emulsifier and oil phase. Soy phospholipids have been shown to serve as emulsifiers in oil-water emulsion (Rydhag and Wilton, 1981). According to the study, emulsion stability is largely influenced by the types of phospholipids present.

Phospholipids with approximately 100% PC stabilized

oil in water emulsion at high oil in water ratio (above 4:6). As negative charged phospholipids (PA and PI) increased, emulsion was stable at low oil in water ratio, with separation at 6:4 and above. This study showed a more stable emulsion at higher oil in water concentration. The reason for this phenomenon could be as result of higher PC in acetone insoluble calyx oil used (82.26%) as shown in Table 3 which is similar to what was observed by Rydhag and Wilton (1981). However, a lower concentration (that is, 0.5%) was required to stabilize the emulsion in Rydhag and Wilton study as compared to 2% used in this study. It is understandable as modified phospholipids were used. The need to conduct further studies on the modification of oil palm calyx phospholipids is necessary to optimize functionality.

CONCLUSIONS AND RECOMMENDATION

Oil palm calyx shows high potential in the production of industrial phospholipids. The use of this waste as industrial source of phospholipids will increase income of oil palm industry as well as further add value to the existing food chain. Also, calyx phospholipids will also complement the high demand of soy and egg phospholipids. Future work on the effect of maturation of oil palm bunches and post harvest stress on the phospholipid properties of calyx oil is required. Detailed characterization, separation and modification of calyx phospholipids are also necessary to optimize the competitiveness of calyx phospholipids with other phospholipid sources.

Conflict of Interests

The authors have not declared any conflict of interests.

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