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Evaluation of the chemical composition of *Pentadesma butyracea* butter and defatted kernels

Fidèle Paul Tchobo^{1,3*}, Alitonou Guy Alain¹, Noudogbessi Jean-Pierre¹, Laguerre Mickael³, Barea Bruno³, Georges Piombo³, Natta K. Armand², Villeneuve Pierre³, Soumanou Mohamed Mansourou¹, Souhounhloue K. C. Dominique¹

¹Laboratoire d'Etude et de Recherche en Chimie Appliquée. Ecole Polytechnique d'Abomey-Calavi, Université d'Abomey-Calavi, Bénin, 01 BP 2009 République du Benin ²Laboratoire d'études et de recherches Forestières (LERF), Faculté d'agronomie, Université de Parakou, BP 123 Parakou, Bénin ³Unité mixte de recherche ingénierie des agropolymères et technologies émergentes (IATE) CIRAD, INRA, UM II, Montpellier Supagro, Place Pierre Viala, Bat 31 34060 Montpellier Cedex 1, France

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Abstract

Studies were carried out on *P. butyracea* kernels to determine their nutritional and antinutritional composition. The kernels contained 41.9% of butter. The physicochemical properties of the butter are as follows: saponification value, 192.5 mg KOH/g of oil; refractive index (20°C), 1.462; unsaponifiable matter, 1.8%; acidity, 0.41%; peroxide value, 17.3 meq O_2 /kg of butter. The butter contains high levels of oleic acid (47.3%) and stearic acid (47.2). The composition of triacylglycerols (TAGs) was characterized by two components SOO and SOS. The DSC melting curves of butter revealed two melting points of 20.4°C and 39.3°C and melting enthalpy values of 30.6 J/g and 78.2 J/g. Stigmasterol (56.3%) and campesterol (27.6%) were the abundant sterols. Protein content (7.3 % dry weight) was recorded in the kernel containing eighteen amino acids in varying proportions. Three sugars (glucose, fructose, sucrose) were identified. An ash content of (4.1% dry weight) was contained calcium, magnesium potassium, nitrogen and phosphorus. The result of the anti-nutritional factors showed that levels of phytate (0.42%), total phenol (0.14%) and oxalate (1.06%).

* Corresponding Author: Fidèle Paul Tchobo 🖂 fideletchobo@yahoo.fr, fidele.tchobo@epac.uac.bj

Introduction

In the Sudanese area of West Africa, the Forests Galleries constitute small islands of vegetation, which are more or less dense and broad along the rivers. They have some ecological, economic, social and cultural impacts on the populations (Natta, 2007; Ceperley *et al.*, 2010).

In Benin, the forest galleries shelter approximately one thousand species of plants, which represent approximately the third of the flora of the country (Natta, 2003). This phytodiversity includes a varied range of typical vegetable species of various vegetable formations and of multipurpose tree species (Natta et al., 2011). Among these multipurpose plants, one can name P. butyracea Sabine. This species is recognized for its economic, nutritional, medical, social, cultural, cosmetic, and pharmaceutical utilities. (Dencausse et al. 1995; Sinsin and Sinadouwirou, 2003; Tchobo et al., 2007; Avocevou-Ayisso et al., 2009, Natta et al., 2010). Indeed, several studies showed the biological activities of the extracts of various parts of the P. butyracea. In Gabon and Côte d'Ivoire, the marcerated bark is utilized in lotions for traitement of the parasitic diseases of the skin and as antidiarrhetic (Raponda-walker and Sillans, 1961). In Ghana, the decoction of the roots is used to fight intestinal worms (Abbiw, 1990). Recent work on the biological activities of P. butyracea Sabine showed that the xanthone isolated from their roots and stem bark present some antiproliferative, Cytotoxic and Antiplasmodial activities (Zelefack et al., 2009, Wabo et al., 2010).

The kernels are rich in edible butter similar to shea butter and consumed like kola when they are fresh (Sinsin and Sinadouwirou, 2003). Studies carried out by Adomako (1997), Kouadio et *al.*, (1990), Dencausse et *al.*, (1995) et Tchobo et *al.*, (2007), allowed to determine some physical and physicochemical characteristics of the *P. butyracea* butter. Kouadio et *al.*, (1990), with an interest in the nutritional potential of *P. butyracea* seed, showed that the *P. butyracea* kernels contain 1.82 % (dm) of ash, 4.5 % (dm) of protein 5.6 % (dm) of cellulose and 44.84% (dm) of carbohydrate.

This study was carried out in order to complete the chemical composition of *P. butyracea* butter and to investigate the nutrients, minerals and anti-nutritional components of defatted kernels.

Material and methods

Collection and treatment of samples

P. butyacea kernels were collected on the ground in Penessoulou village (Benin). Before analysis, kernels were washed and dried at 50°C for 48 h. Samples were pounded to powder with a mechanical millstone.

Oil analysis

Samples of ground kernels were weighed (50 g) and extracted four times with hexane (7 h) using a continuous Soxhlet extractor. The fat content was gravimetrically measured after removal of the solvent by rotary evaporation under vacuum. Extraction was run in triplicate (three times on 50 g samples each) on the original iso kernels. For determination of acid, peroxide, and saponification values, standard IUPAC methods were used.

Unsaponifiable Content: The unsaponifiable content with was determined in accordance the corresponding AFNOR method NF T 60.205-2. Samples (5 g) were saponified with 50 mL alcoholic potassium hydroxide (1N) under reflux for 1 h. The unsaponifiable components were separated from the soap in a separator funnel with hexane and washed with ethanol (10%). The solvent was evaporated off and residue was dried in an oven at 103 ± 2 °C for 15 min and weighed. Fatty acids and sterols compositions, tryaclglycerols profiles were determined by the methods described by Tchobo et al., (2007).

Pentadesma butter was subjected to DSC analysis using a DSC 2920 Modulated DSC de TA Instruments (New Castle, DE). The samples (5–15 mg) were weighed into aluminum pans, which were then hermetically sealed with a "cold welder." An empty pan was used as the reference. The samples were held for 5 min at -15°C and thereafter subjected to a heating rate of 5°C/min from -15 to 60°C.

Defatted kernels analysis

Defatted kernel was subjected to analysis:

Moisture, lipids and ash contents were determined following the standard AOAC methods. Fiber contents were obtained by Van Soest method (AFNOR, NF V 18-122, 11).

The minerals in *P. butyracea* were analysed from solutions obtained by first dry-ashing the seed flour at 550° C. The ash obtained was boiled with 15mL of 20% hydrochloric acid in a beaker, filtered into a 100mL standard flask and made up to the mark with distilled water. Minerals were analysed from solution of dry ash as follows: 5 g of the sample were placed on a dish and heated in a Bunsen flame for 10 min and transferred into a muffle furnace at 525° C for 5 h. The ash was dissolved in 10mL of 0.1 N aqueous HNO₃ and made up to the mark with distilled deionised water; the metals were analyzed using inductively coupled argon plasma equipped with a CCD detector (Varian Vista).

Proteins in defatted cake were hydrolysed by treatment with 6N HCl in a sealed tube for 24 hours at 110°C. Proteins in the extract were precipitated with 1mL 0.1M lead ethanoate followed by setting the pH to 7.0 with 0.5M NaHCO₃. Precipitated proteins were centrifuged at 3000 rpm for 10 min, while amino acid composition was determined in an automatic amino acid analyser (Spectra-Physics AS3000). Three different samples were analysed in triplicate.

The sugar composition was determined by a HPLC (Shimadzu, Tokyo, Japan) fitted with a refractive index detector (HPLC-RI) at 30°C. The sugar was extracted to defatted cake with ethanol (80%) by ASE 200 in 35 minutes. Extraction temperature and pressure were respectively 80°C and 100 bars

The total phenolic was determined using Folin-Ciocalteu reagent according to the method describes by Marigo (1973). Phenolic compounds were extracted with methanol (70%) to defatted cake. A mixture of 0.4 mL of the methanolic extract, 2.4mL of distilled water and 0.4mL of Folin-Ciocalteu reagent was homogenized. After 3 minutes 0.8 mL of sodium carbonate (20%, p/v) was added and the mixture was incubated at 40°C for 20 minutes. The absorbance was measured at 760 nm by ANTHELIE ADVANCED 2 spectrophotometer. The same operation was performed after absorption of phenolic compounds on polyvinylpyrrolidone.

The total oxalate was determined according to Day and Underwood (1986) procedure. To 1 g of the ground powder, 75 ml of 15 N H_2SO_4 was added. The solution was carefully stirred intermittently with a magnetic stirrer for 1 h and filtered using Whatman No 1 filter paper. 25 ml of the filtrate was then collected and titrated against 0.1 N KMnO₄ solutions untill a faint pink colour appeared that persisted for 30 s.

Phytate was determined using Reddy and Love (1999) method. 4 g of the ground sample was soaked in 100 ml of 2% HCl for 5 h and filtered. To 25 ml of the filtered, 5 ml 0.3% ammonium thiocyanate solution was added. The mixture was then titrated with Iron (III) chloride solution until a brownish-yellow color that persisted for 5 min was obtained.

Result and discussion

Table 1 shows the physico-chemical characteristics of *P. butyracea* butter .The *P. butyracea* kernels have an 41.9 wt% of oil content of dry matter, that is close to those reported by Tchobo *et al.*, (2009), Dencause *et al.*, (1995) Kouadio et *al.*, (1990). The knowledge of acidity allows a quantification of the free fatty acids in oil. Their presence constitutes a factor of deterioration of oil. The acidity of the sample is low in our study. The peroxide value permits to evaluate the oxidation degree of oil. For this study, the peroxide value is higher than the acceptable limit fixed for traded vegetable (10 meq O₂ kg⁻¹). This is

probably due to exposure to air. The peroxide value is high than those reported by Aissi *et al.*, (2011). The saponification number is 192.5 and compatible with a preponderance of C18 fatty acids. It is similar to the result of Aissi *et al.*, (2011) and in agreement with the data for current food oils used in Benin (Djenontin *et al.*, 2012). The Unsaponifiable content is 1.8 wt%.

Table 1. Physico-chemical characteristics of P.butvracea Butter.

Property	<i>P. butyracea</i> Butter		
Oil content	41.9 ± 1.7		
Oleic acidity (wt%),	1.5 ± 0.1		
Peroxide value (meq O_2 /kg ⁻¹)	17.3 ± 0.4		
Saponification number	192.5 ± 2.3		
Unsaponifiable (wt%)	1.8 ± 0.1		
Refractive index	1.462 ± 0.001		
Physical state at room temperature	solid		
Temperature de fusion	20.4 ±	39.3 ± 0.2	
(°C)	0.1		
Energie de fusion (J/g)	30.6 ±	78.2 ± 1.2	
	2.3		

The thermal analysis of the Pentadesma butter shows two major peaks forming the DSC melting curves of Pentadesma butter (Figure 1). The first one located between $10.9 - 22.3^{\circ}$ C and the other between 37.5 - 42.3°C. From this result, two fusion points were obtained at 20.4 and 39.3°C. The latter melting point of the Pentadesma butter is higher than that of the cocoa butter (33°C). Pentadesma butter is subject to polymorphism depending on its Triacylglycerols profiles. These two melting points require respectively 30.6 J/g and 78.2 J/g like energy for fusion. Table 2 gives the FAs, TAGs and sterols profiles. FAs composition of Pentadesma butter is characterized by 4 FAs principally (C16:0; C18:0; C18:1 and C18:2). However FAs C18:0 and C18:1 constitutes nearly 95% (wt) of FAs content. The two high content of FAs (C18:0 and C18:1) characterizes the TAG profiles. The TAG profiles are constituted principally by OOO (5.2%), POO (1.5%), SOO (41.3%), POS (3.8%), and SOS (46.3%). This profile is confirmed by the literature (Tchobo et al.,

2007). However, they are observed some minors TAGs (SLO, POP, OOE). Moreover, the TAG analysis reveals the presence of TAGs hydrolysis products (AGL+MAG+DAG (2,7%)). The presence of two TAGs majority (SOO and SOS) could explain the two peaks observed in the DSC thermograms (Fig. 1). The sterols are generally the major components of unsaponifiable matter. In this study, The *P. butyracea* butter is characterized by seven sterols that are Brassicasterol (6.2%), Campesterol (27.6%), stigmasterol (56.3), β -sitosterol (6.2%), spinasterol (1.6%), Δ 7-stigmastérol (1.3%) and Δ 7-Avenastérol (0.7%). The sterol composition was identic to that mentioned by Tchobo *et al.*, (2007), and Dencause *et al.*, (1995).



Fig. 1. DSC melting curves of *Pentadesma butyracea* butter.

Table 3 shows the approximate composition of *P*. butyracea defatted kernels investigated. The moisture content, oil content, ash, protein are 5.3%, 2.1%, 4.1%, 7.3%, respectively. These results are comparable to those reported by Kouadio et al., (1990). Fiber analysis shows that the NDF, ADF and ADL contents are in order of 39.3%, 29.7% and 9.0%, respectively. The deduction of hemicellulose, cellulose and lignin contents gives 11.4%, 18.4% and 4.9%, respectively. Cellulose content is higher than that reported by Kouadio et al., (1990) in P. butyracea harvested in Ivory Cost and close to those reported for Canarium (Agbo N'zi et al., 1992) and Shea nuts. Ash content is comparable with that in groundnut, soya, cotton and Azadirachta indica seeds (Lusas and Hernandez, 1997; Djenontin et al., 2012). The mineral fraction of *P. butyracea* contains Nitrogen (1.6%), phosphorus (0.21%), potassium (0.14%), calcium (1.33%) and magnesium (0.35%) (Table 3). The potassium is the more abundant element with 50 to 75% of the total identified minerals. The calcium and phosphorus contents are negligible compared to that contained in colza, sunflower and soya cakes (Evrard, 2005). *P.* *butyracea* kernels contain 7.3% of proteins. This proteins content is lower than that contains in groundnut (26-42%), soya cakes (36-53%), cotton seeds (28-50%) (Lucas and Hernandez, 1997), colza (34%) and sunflower (30-35%) (Evrard, 2005), but similar to that in *Canarium cakes* (Agbo zi *et al*, 1992).

FAs (%)		TAG (wt	%)	Sterols (v	vt%)
Palmitic acid C16:0	$3,3 \pm 0,1$	AGL+MAG+DAG	$2,7 \pm 0,7$	Brassicastérol	$6,2 \pm 2.1$
Stearic acid C18:0	$47,2 \pm 2,9$	000	$5,2 \pm 1,1$	Campestérol	$27,6\pm0.6$
Oleic acid C18:1	$47,3\pm2,5$	SLO	$0,9 \pm 0,5$	Stigmastérol	$56,3 \pm 2.1$
Linoleic acid C18:2	$1,4 \pm 0,5$	POO	$1,5 \pm 0,9$	β- Sistostérol	$6,2 \pm 0.3$
linolenic acid C18:3	$0,1\pm0,0$	POP	$0,5\pm0,1$	Spinastérol	$1,6 \pm 0.5$
Arachidic acid C20:0	$0,2\pm0,1$	SOO	$41,3 \pm 3,1$	Δ 7-stigmastérol	$1,3 \pm 0.2$
		POS	$3,8\pm0,5$	Δ 7-Avenastérol	$0,7\pm0.2$
		OOE	$0,2\pm0,1$		
		SOS	$46,3 \pm 3,0$		

Table 2. FAs, TAGs and sterols profiles of *P. butyracea* butter.

Table 3. Proximate composition of P. butyracea defatted kernels.

Parameters (wt%)	This work	
Moisture	5.3 ± 0.9	
Oil content	2.1 ± 0.4	
Ash	4.1 ± 0.4	
Proteins	7.3 ± 0.2	
NDF (dm)	39.3 ± 3.5	
ADF (dm)	29.7 ± 4.9	
ADL (dm)	9.0 ± 2.1	
Hemicellulose (NDF - ADF)	11.4 ± 4.0	
Cellulose (ADF - ADL)	18.9 ± 2.3	
Lignin (ADL-Ash)	4.9 ± 1.6	
Nitrogen	1.65 ± 0.04	
Phosphorus	0.21 ± 0.01	
Calcium	0.14 ± 0.01	
Potassium	1.33 ± 0.02	
Magnesium	0.35 ± 0.01	

Furthermore, the quality of proteins is evaluated through the composition in amino acids (Table 4). In *P. butyracea* kernels, eighteen amino acids were identified. We note the absence of glutamine and asparagine. The lysin content is 0.32% of the dry

matter and 4.4% of proteins. Sulphur amino acid accounts were 0.31 % (Methionine, 0.16% (2.2% of proteins) and cysteine 0.15% (2.1% of proteins)). The threonine content is greater than the lysine content. It should be mentioned that the main amino acid is the glutamic acid. These results compared with those in the literature (Lusas and Hernandez, 1997; Evrard, 2005) show that the oil cakes of *P. butyracea* kernels are very poor in proteins and particularly in essential amino acids. The results of sugar composition show the presence of glucose, fructose and sucrose (Table 4). The sample analyzed contains 0.02 % of various sugars. The presence of antinutritional factors is one of the major drawbacks limiting the nutritional and food qualities of the Seeds. The negative nutritional effect of phytic acid in biological system may chelate divalent metals like calcium, magnesium or blocks the absorption of essential minerals in the intestinal tract, and consequently decreasing their bio-availability (Onibon *et al.*, 2007). Oxalate can remove calcium in form of calcium oxalate in the blood and this may result to kidney damage. The antinutrient content of *P. butyracea* kernels is presented in table 4. Phytate, oxalate and total phenolic contents are determined. The result gives 0.14% of phenol, 0.42% of phytate and 1.06% of oxalate.

Parameters		content (wt%)
	Tryptophane	0.11 ± 0.01
Amino acids -	Cystéine	0.15 ± 0.01
-	Acide aspartique	0.68 ± 0.02
-	Méthionine	0.16 ± 0.01
-	Thréonine	0.36 ± 0.01
-	Sérine	0.34 ± 0.01
-	Acide glutamique	1.13 ± 0.03
-	Glycine	0.61 ± 0.02
-	Alanine	0.38 ± 0.01
-	Valine	0.45 ± 0.01
-	Isoleucine	0.32 ± 0.01
-	Leucine	0.50 ± 0.01
-	Tyrosine	0.29 ± 0.01
-	Phénylalanine	0.43 ± 0.01
-	Histidine	0.18 ± 0.01
-	Lysine	0.32 ± 0.01
-	Arginine	0.62 ± 0.02
-	Proline	0.26 ± 0.01
-	Total	$\textbf{7.29} \pm \textbf{0.21}$
	Glucose	0.023 ± 0.001
Sugar	fructose	0.019 ± 0.001
	saccharose	0.022 ± 0.002
Antinutrients	Total phenol	0.14 ± 0.02
	Phytate	0.42 ± 0.01
	oxalate	1.06 ± 0.01

Table 4. Amino acids, simple sugars and antinutrients composition of	<i>P. butyracea</i> seeds.
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These study show that *P. butyracea* are a good source of oil, cellulose and potassium, but a poor source of protein, calcium, sodium and phosphorus. The kernels contain antinutritional factor such as phytate, oxalate and phenolic compounds.

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