



## CHEMICAL COMPOSITION OF TRADITIONALLY PROCESSED *PENTADESMA BUTYRACEA* SABINE SEEDS AND BUTTER

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### ABSTRACT

Traditionally, production of *Pentadesma butyracea* butter starts by a pretreatment of the seeds which can be stored after. Thus, the seeds are untreated or heated by roasting or boiling and then sun-dried. This study compared the end products derived by these three pretreatments. The fresh seeds were divided into three batches and each of them was pretreated as described above and processed into butter. Seeds were analyzed for proximate composition and inorganic ions and butter for fatty acids composition and triglycerides profile. Neither treatment influenced the fat content of *P. butyracea* seeds (48%). There was significant ( $P < 0.05$ ) variation in the crude protein and ash content of untreated seeds. The applied pretreatments didn't lead to significant reduction ( $P > 0.05$ ) in most of the inorganic ions. However, a significant decrease ( $P < 0.05$ ) was observed in zinc and sulfate contents. On the other hand, the seeds pretreatment significantly ( $P < 0.05$ ) influenced the fatty acids and triacylglycerol profiles of the extracted butter. As observed, boiling of seeds appeared as a process allowing a high level of unsaturated fatty acids in the butter and can be recommended to the *P. butyracea* seeds processors in rural area.

**Keywords:** *Butter tree*, seeds, composition, post-harvest, heat pretreatment

### INTRODUCTION

Various tropical seeds and fruits from Africa are veritable sources of fats. They provide many natural components with suitable properties for cosmetic, pharmaceutical and food industries. Among these raw materials, are *Pentadesma butyracea* Sabine seeds. *Pentadesma butyracea* is a tree from the Clusiaceae family, with an area distribution spanning from Sierra Leone to the Congo (Bamps, 1971). In Benin, *Pentadesma* trees are found in the gallery forests and in river banks of the Sudanian phytocorion. This species is known for the edible butter produced from its seeds which is similar to that of the shea butter tree (*Vitellaria paradoxa*) (Sinsin and Sinadouwirou, 2003). According to Ayegnon et al. (2015), *P. butyracea* butter produced by cottage enterprises in Benin exhibited quality characteristics which are better than that of shea butter.

Half of the *P. butyracea* applications belong to the butter derived from the seeds (Natta et al., 2010). Seeds utilization is also indicated for various uses for ill-defined symptoms treatment, digestive system disorders treatment, muscular-skeletal system disorders, respiratory system disorders and inflammations (Avocèvou et al., 2008).

The traditional production of *Pentadesma* butter requires a pretreatment of the seeds. Thus, the seeds are submitted to a heat pretreatment and then sun-dried to reduce the moisture content of the seeds. Two heat treatments, namely boiling in a pot and roasting in a traditional furnace are applied to *P. butyracea* seeds. The type of the pretreatment applied to *P. butyracea* seeds varies with socio-cultural groups in production areas (Aissi et al., 2015).

Many investigations were performed on the physicochemical characteristics and the composition of *Pentadesma* butter (Dencausse et al., 1995). *Pentadesma* butter is particularly rich in stigmaterol, a rare sterol encountered in nature (Dencausse et al., 1995). Over the years, a full study based on the characterization of the *P. butyracea* butters of different production regions in Benin was done by Tchobo et al. (2007). Moreover, Tchobo et al. (2009) showed the way to valorize this butter via the synthesis of an equivalent of cocoa butter through enzymatic transesterification. The effect of the seeds post-harvest pretreatments on the physicochemical characteristics, the aqueous extraction yield and the quality of the *Pentadesma* butter was studied by Aissi et al. (2011). Chemical changes of lipids occurring during processing are numerous and can be desirable or undesirable (Shahidi et al., 1997). While fatty acid composition of

*Pentadesma* butter was completely determined, no attention was paid on the triacylglycerol profiling of the extracted butter from the pretreated seeds. Yet, the composition and fine structure of triacylglycerols determine to a large extent the functionality of fats and oils as food ingredients and the physiological effects of fats and oils as component of the human diet (Buchgraber et al., 2004). Previous endeavors on *Pentadesma* butters' nutritional contents have yielded some interesting results. For instance, on dry matter basis, *Pentadesma* seeds are mainly oleaginous with a high level of carbohydrate (44.84%) and a low level of protein (4.5%) (Kouadio et al., 1990).

Raw materials should be analyzed in terms of their composition, secondary metabolites contents and probable toxic constituents. *Pentadesma butyracea* Sabine natural stands are limited to riparian forests (Natta et al., 2002). These soils and climatic conditions could influence the presence of inorganic elements in the seeds. Many studies highlighted the risk of their presence in food chain and their potential toxicity (Pennington, 1998). Some of them as fluoride, chloride, bromide, nitrate, nitrite, sulphate, or phosphate are naturally present in the crude matter and may also be introduced during industrial manipulations (Lopez-Ruiz, 2000). The analysis of inorganic anions in food samples is important from the nutritional, toxicological and technological point of view (Dugo et al., 2007).

To our knowledge, there is no available study on the levels of some inorganic ions in the *P. butyracea* Sabine seeds. Moreover, no attention has been paid to the effect of the seeds traditional post-harvest treatments on their chemical composition. This study aimed to fill that gap with respect to the influence that the different heat treatments applied in production areas might have on the chemical composition of the seeds and the fatty acids and triglycerides profiles of the butter extracted from these seeds.

### MATERIAL AND METHODS

#### Seeds pretreatment

Three types of pretreatments were performed in collaboration with a female butter production group in a cottage industry located in Natitingou (North-Western of Benin). Fresh *P. butyracea* seeds obtained from fruits collected and depulped were divided into three portions. Each portion was treated differently before being sun-dried for two weeks. One portion was just sun-dried without a

treatment. The second was sun-dried after roasting in a traditional tall oven by two steps: 220-270 °C for 20 minutes and 100-125 °C for one hour. The third portion was boiled in a pot at (94-98 °C) for one hour and sun-dried.

### Butter extraction

The butter was extracted from the three differently pretreated *P. butyracea* seeds by the processors according to the rural aqueous extraction method described by Aissi et al. (2011).

### Preparation of samples

Pretreated seeds samples (5 kg) were stored in plastic bags at ambient temperature (25-30 °C) until grinding. The pretreated seeds were grounded in a steel mortar with a pestle and then thoroughly using a laboratory mill. The grounded seeds were then screened through a 600 µm test sieve and used for chemical analyses. The seeds powder was stored in plastic bags at 4 °C until analyses were performed. The butter after production was cooled and packaged in plastic tubes of 250 mL. The butter was dissolved in the appropriate solvent and analyzed.

### Determination of proximate composition of seeds

The proximate analysis of the seeds for the moisture content, lipid content, crude protein and ash was done according to the standards and the methods described by the Association Française de Normalisation (AFNOR, 1993). The moisture content was determined in triplicate according to the standard NF V 03-921. Dry matter was then calculated from the moisture content. The total fat content was determined in triplicate according to standard NF V 03-908. For the protein content, the nitrogen content was first determined in triplicate by the Kjeldahl method according to standard NFV 18-100. It was then converted to protein content by using the conversion factor 6.25. Ash content was determined in duplicate according to standard NF V 03-922. The crude fiber was determined in duplicate by the method of Weende (Wolff, 1968). Briefly, 1 g of sample was boiled in 50 mL of sulphuric acid 0.25 N for 30 min and then in 50 mL of sodium hydroxide 0.31 N for 30 min. The obtained residue was dried at 105 °C for 8 hours and then incinerated at 550 °C for 3 hours. The crude fiber was obtained by difference.

### Determination of inorganic ions of seeds

The cations (Sodium, Ammonium, Potassium, Magnesium and Calcium) and anions (Fluoride, Chloride, Nitrate, Phosphate and Sulfate) were determined in triplicate by Ion Chromatography using a Dionex ICS-1000 System (Thermo Fisher Scientific, France) managed by the Chromeleon software after samples treatment. The samples were incinerated and dissolved using distilled deionized water. The sample solution was filtered through a Whatman paper N° 1. The filtration was made up to volume in a 100 mL standard flask and centrifuged at 700 rpm for 30 min. One mL of solution was then filtered through a nylon filter 0.45 µm and inject in the chromatograph Dionex ICS-1000. Iron and zinc were determined in triplicate using an atomic absorption spectrometer (Varian Spectra AA 110) managed by the Varian Spectra Software version 5.5 after wet ashing by concentrated nitric acid and perchloric acid (1:1, v/v). The mineral and inorganic ions contents of the samples were quantified against standard solutions of known concentrations which were analyzed concurrently.

### Fatty acids analysis of butter

Fatty acids analysis of butter was done as described by Aissi et al. (2011). Fatty acids methyl esters were prepared according to NF T60-233. In a 25-mL round bottom flask, 10 mg of oil samples were added to 3 mL of sodium methylate solution containing phenolphthalein. The mixture was refluxed for 10 min, and 3 mL of methanolic HCl was added until phenolphthalein discoloration occurred. The mixture was refluxed again for 10 min and cooled to room temperature. Eight mL of hexane and 10 mL of water were added and the organic phase recovered, dried over anhydrous sodium sulfate and filtered for subsequent gas chromatography analysis. Fatty acids methyl esters were directly injected into the GC (Agilent 6890 series) equipped with a flame ionization detector and a SUPELCOWAX 10 capillary column (L x I.D 30 m x 0.32 mm,  $d_f = 0.25 \mu\text{m}$ ). The gas carrier was helium with a flow rate of 1 mL/min, and a splitting ratio of 1/80. The injector temperature was 250 °C and that of the flame ionization detector was 270 °C. The temperature settings were as follows: 150–225°C at 5°C/min, and then held at 225 °C for 2 min. Fatty acids were identified by comparing them with the standards.

### Triacylglycerol profiling of butter

Triacylglycerol profiles were determined on a HPLC system (Thermo Fisher Scientific, France) equipped with a pump (SpectraSYSTEM P1000xR), an automatic sampler (SpectraSYSTEM AS1000) and a detector (DDL, Model

ALLTECH, 500 ELSD) as described by Tchobo et al. (2009). The separations were performed on two C18 columns QS LICHROSPHER 5 ODS 2 (5 µm, 250 x 4.6 mm) in series from INTERCHIM. The eluent used was a gradient of mixture acetone/acetonitrile (50:50 v/v (A)) and chloroform (B). Elution was carried out at a solvent flow rate of 1 mL/min with a linear gradient as follows: 0 min 100% A, 60 min 80% A, 80 min 80% A, returning to the initial conditions within 15 min and holding at these conditions for 15 min. The effluent was monitored with an ELSD detector, with the following settings: evaporator temperature 40 °C, air pressure 42 psi. The triacylglycerols were identified by comparing retention times to pure standards. For the analyses, the butter was dissolved in acetone (5 mg/ mL). The injected volume was 20 µL.

### Statistical analyses

The results in all tables are presented as means ± standard error. Data were analyzed by one way analysis of variance (ANOVA) using SPSS 18.0. The means were compared using Duncan's multiple range test. Probability was set at  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Chemical composition of *P. butyracea* seeds

Proximate composition of untreated or treated by two heat treatments and sun-dried *P. butyracea* seeds are presented in Table 1.

**Table 1** Proximate composition of *P. butyracea* seeds untreated, boiled or roasted followed by sun drying

	Seeds pretreatment		
	Untreated and sun-dried	Boiled and sun-dried	Roasted and sun-dried
Dry matter (dm)	92.59 ± 0.01a	92.65 ± 0.02b	92.97 ± 0.04c
Lipid (% of dm)	48.86 ± 2.43a	48.58 ± 2.89a	48.21 ± 1.94a
Protein (% of dm)	6.18 ± 0.30a	4.48 ± 0.17b	4.19 ± 0.10b
Fiber (% of dm)	1.26 ± 0.04a	1.14 ± 0.01b	1.13 ± 0.01b
Ash (% of dm)	4.26 ± 0.07a	3.93 ± 0.08b	3.71 ± 0.07b

Mean values ± standard error within a row not sharing the same letter are significantly different at  $P < 0.05$ .

There was a significant increase ( $P < 0.05$ ) in the dry matter content of the dried treated seeds compared with the control (untreated and sun-dried seeds). The dry matter content was more than 92% in the untreated, boiled or roasted and sun-dried seeds (Table 1). Therefore, it can be concluded that the moisture content of the dried seeds was lower than 8% and favorable to a good preservation for a long time without spoilage. Indeed, higher moisture content can lead to food spoilage through increasing microbial action (Onyeike et al., 1995) and insect infestation. This observation is in agreement with the moisture content under 8% obtained by Aissi et al. (2011) for sun-dried *P. butyracea* seeds. Similar values have also been obtained for other oilseeds, sources of solid vegetable fats. Thus, Zaidul et al. (2006) found the moisture content of dehulled ground palm kernel to be 4.87%. Using a direct solar dryer, Hii et al. (2006) dried fermented cocoa beans below 7.5% moisture content.

The fat content of all the untreated or treated seeds was also more than 48% (Table 1). No significant difference in the lipid content ( $P > 0.05$ ) was found among the three types of treated seeds. Boiling and roasting did not have significant effect on the fat content of *P. butyracea* seeds. Nevertheless, this effect could become significant according to the preservation duration. The obtained fat content in this study is similar to the 50%, 43% and 40 – 50% reported by Kouadio et al. (1990) and Tchobo et al. (2007) respectively.

The ash content is a measure of the total mineral content in the seeds. The untreated seeds had significantly higher protein and ash contents than roasted and boiled seeds. No significant ( $P > 0.05$ ) differences in protein and ash contents were observed between heat pre-treatments of *P. butyracea* seeds. The protein content of untreated seeds analysed in this work (5.87 to 6.47%) is higher than that found by Kouadio et al. (1990). The *P. butyracea* seeds pretreatment lead to a little protein degradation as indicated by the slightly brown outer colour of the treated seeds. The ash content of untreated or heated seeds is higher than the 1.82% reported by Kouadio et al. (1990). These decreases of the protein and ash contents might be attributed to the uncontrolled application of heat during household boiling or roasting of seeds on the one hand and also to their diffusion into cooking water for the boiled seeds particularly.

The fiber content did not vary significantly ( $P > 0.05$ ) between untreated and treated seeds. The level of crude fiber obtained in this study is lower than the previously reported level of fibers by Kouadio et al. (1990) for *P. butyracea* seeds from Côte d'Ivoire. The difference obtained in fiber content may be explained by the origin of the seeds and also by the pretreatment applied to the seeds. These treatments reduce the fiber content of the seeds by removing a part of their outer coat.

Traditionally, inorganic compounds are considered to be of a mineral origin. Minerals are of interest due to their pro-oxidant activity and health benefits (Alphan et al., 1996). The inorganic compositions of the untreated or treated *P. butyracea* seeds are presented in Table 2.

**Table 2** Inorganic composition of *P. butyracea* seeds untreated, boiled or roasted followed by sun drying

Mineral (mg/100 g dm)	Seeds pretreatment		
	Untreated and sun-dried	Boiled and sun-dried	Roasted and sun-dried
Potassium	1061.16 ± 33.21a	941.62 ± 3.36b	908.81 ± 20.87b
Calcium	36.83 ± 3.99a	27.71 ± 2.40a	28.76 ± 2.02a
Magnesium	99.45 ± 2.10a	87.60 ± 7.08a	98.59 ± 5.80a
Sodium	20.93 ± 4.78a	18.19 ± 2.03a	4.64 ± 1.46b
Iron	0.055 ± 0.007a	0.055 ± 0.013a	0.0560 ± 0.027a
Zinc	1.86 ± 0.23a	1.49 ± 0.17b	1.15 ± 0.053c
Chloride	138.09 ± 15.48a	153.30 ± 3.23a	132.78 ± 2.69a
Phosphate	4.71 ± 0.63a	6.17 ± 0.43a	6.02 ± 0.57a
Nitrate	2.04 ± 0.29a	2.65 ± 0.37a	2.20 ± 0.56a
Sulfate	164.59 ± 1.46a	134.34 ± 4.87b	124.78 ± 9.66b

Mean values ± standard error within a row not sharing the same letter are significantly different at P < 0.05.

Overall, boiling and roasting of the seeds didn't lead to a significant reduction (P > 0.05) in most of the minerals, especially phosphorus, calcium, potassium, iron and magnesium and in some inorganic anions especially chloride and nitrate. However, there was significant decrease (P < 0.05) in zinc and sulfate contents after boiling or roasting and in sodium content after roasting only. Potassium and magnesium were the major minerals. Calcium, sodium, phosphate and zinc contents were also present in significant amounts while iron was in trivial amounts. Our results are in accordance with the previous study based on the major mineral elements of the *P. butyracea* seeds done by Kouadio et al. (1990). Compared to the selected Nigerian oil seeds (castor seeds, coconut seeds, dikanut seeds, groundnut seeds, melon seeds, oil bean seeds and palm kernel seeds) investigated by Onyeike and Acheru (2002), the *P. butyracea* seeds had the highest concentrations of potassium, sulphate and sodium but the lowest concentrations of phosphate.

The presence of sulfate, chloride and nitrate at a high level may be related to the composition of soil and the climatic conditions. Indeed, the *P. butyracea* tree is found in gallery forests and in banks of rivers. Ačkurt et al. (1999) also found that variety, location, composition of soil, uses of fertilizer and irrigation affect the mineral composition of hazelnuts cultivated in Turkey. Nitrate was the scarcest of all detected inorganic anions in the *P. butyracea* seeds. Of all inorganic anions studied, bromide nitrite and nitrate were the most important in term of food chain contamination because of their potential toxicity (Dugo et al., 2007). Therefore, knowing that the *P. butyracea* seeds are according to Avocéyou et al. (2008) used against the digestive diseases, there is a need to pay attention to their utilization because of the presence of nitrate revealed in them. Traditionally, the residue after butter extraction is thrown. Based on the chemical analysis performed in this study, the *P. butyracea* seeds and therefore their by-products are good sources of minerals, especially potassium, magnesium, sodium, calcium, sulphate and chloride. The waste products of the butter aqueous

extraction can also be used as a source of potassium for traditional soap manufacture and as natural fertilizers in cultivation.

**Chemical composition of *P. butyracea* butter**

*Pentadesma butyracea* butter was characterized by seven saturated and unsaturated fatty acids, although in greatly different percentages related to the seeds pretreatment (Table 3).

**Table 3** Fatty acids composition (weight %) of butter from *P. butyracea* seeds untreated, boiled or roasted followed by sun drying

Fatty acids (Weight %)	Butter origins		
	Untreated and sun-dried seeds	Boiled and sun-dried seeds	Roasted and sun-dried seeds
Palmitic (C16:0)	3.05 ± 0.12a	2.88 ± 0.11b	2.95 ± 0.12ab
Palmitoleic (C16:1)	0.13 ± 0.00a	0.14 ± 0.01b	0.15 ± 0.01c
Stearic C18:0	44.66 ± 1.74a	40.89 ± 1.59b	47.02 ± 1.59c
Oleic (C18:1)	51.23 ± 2.00a	55.04 ± 2.15b	49.07 ± 2.15c
Linoleic (C18:2)	0.72 ± 0.03a	0.75 ± 0.03b	0.62 ± 0.03c
Linolenic (C18:3)	0.10 ± 0.00a	0.14 ± 0.01b	0.11 ± 0.01c
Arachidic (C20:0)	0.12 ± 0.00a	0.11 ± 0.00b	0.09 ± 0.00c

Mean values ± standard error within a row not sharing the same letter are significantly different at P < 0.05.

This fatty acids composition of the butter agrees with those obtained by Tchobo et al. (2007) and Dencausse et al. (1995). The pretreatments caused significant differences in the percentages of fatty acids which varied in heated and untreated *P. butyracea* seeds although the fatty acids were mainly stearic and oleic acids. The boiled seeds were richer in unsaturated fatty acids and less rich in saturated fatty acids than untreated seeds and roasted seeds. In spite of variations in the essential fatty acids concentrations, this result was similar to that obtained by Aissi et al. (2011) who reported the fatty acids compositions of extracted butter from *P. butyracea* pretreated seeds using the traditional extraction technology and butter from the untreated seeds extracted using the solvent extraction by Soxhlet apparatus. However, this and the mentioned studies undoubtedly revealed that the seeds pretreatment influenced their fatty acids composition. The changes caused by heating are complex and are chemical and physicochemical in nature. Regarding the fatty acids composition of the extracted oils from sunflower seeds, microwave heating increased oleic acid 16–42% and decreased linoleic acid 17–19%, but palmitic and stearic acid contents were not affected significantly (P < 0.05) (Anjum et al., 2006). By studying the effects of heat processing on soya bean fatty acids content, Žilić et al. (2010) observed that high temperatures caused changes in unsaturated fatty acids with 18 carbon atoms resulting in relative increase of the stearic acid content. This was observed particularly in *P. butyracea* seeds roasted. Likewise, a comparison of the volatile profile of 16 different shea butters from four African countries showed that processing steps including drying of kernels before to produce the fat and additional roasting procedures influence shea butter composition significantly (Bail et al., 2009).

The variations in physicochemical composition of vegetable oils have often been attributed to environmental factors such as rainfall, soil fertility, maturation period, agronomic practices and genetic substitution (Maranz et al., 2004; Sanou et al., 2006). Thus, the genetic or environmental influences on chemical properties of *P. butyracea* seeds and butter needs to be investigated.

Table 4 showed the triacylglycerols profile of *P. butyracea* butter from seeds untreated, boiled or roasted followed by the sun drying.

**Table 4** Distribution of triacylglycerols (weight %) of butter from *P. butyracea* seeds untreated, boiled or roasted followed by sun drying

Triacylglycerols (Weight %)	Butter origins		
	Untreated and sun-dried seeds	Boiled and sun-dried seeds	Roasted and sun-dried seeds
Triolein (OOO)	2.40 ± 0.10a	3.88 ± 0.16b	7.90 ± 0.33c
1(3) palmitoyl-2 oleoyl-3(1) oleoyl-sn-glycerol (POO)	0.47 ± 0.02a	0.77 ± 0.03b	1.14 ± 0.05c
1(3) stearoyl-2 oleoyl-3(1) oleoyl-sn-glycerol (SOO)	33.38 ± 1.40a	36.56 ± 1.54b	43.40 ± 1.59c
1(3) palmitoyl-2 oleoyl-3(1) stearoyl-sn-glycerol (POS)	2.37 ± 0.10a	3.67 ± 0.15b	2.56 ± 0.11c
1(3) stearoyl-2 oleoyl-3(1) stearoyl-sn-glycerol (SOS)	61.39 ± 2.58a	55.12 ± 2.32b	45.01 ± 1.89c

Mean values ± standard error within a row not sharing the same letter are significantly different at P < 0.05.

The five major triacylglycerols were SOS, SOO, OOO, POS and POO in the butter extracted from seeds untreated, boiled or roasted followed by sun drying. This result is in agreement with the one obtained by Tchobo et al. (2007) who observed that SOO and SOS were the predominant triacylglycerols in *P. butyracea* butters. These authors also mentioned the absence of tristearin (SSS) and a low content of triolein (OOO) despite the high content of stearic and oleic acids. SOS was highest in the butter from untreated seeds followed by the butter from boiled seeds and lowest in the butter from roasted seeds. However, SOO the second main triacylglycerol was highest in the butter from roasted seeds followed by the butter from boiled seeds and lowest in butter from untreated seeds. The OOO and POO levels were particularly high in the butter from roasted seeds. The

triglyceride profile of *P. butyracea* butter is almost closed to those of shea butter but with the highest amount of SOS and SOO largely superior to the maximum found (45.9 and 30.7 for SOS and SOO respectively) by Maranz et al. (2004) in the shea butter. This observation justifies the fact that transformer usually mix shea kernel with *P. butyracea* seeds for processing into butter.

**CONCLUSION**

Currently, there is increasing interest in the valorization of traditional techniques of food processing and preservation. The results of this study illustrated high content of fat and minerals in *P. butyracea* seeds and the slight effect of the rural

boiling and roasting to their chemical composition. Therefore these pretreatments in general and the boiling particularly could be encouraged as a post-harvest heat treatment for the preservation of similar seeds in rural areas. However these heat pretreatments need to be optimized so that the desired beneficial effects are promoted and the undesirable effects are counteracted as much as possible. The genetic or environmental influences on the chemical composition of *P. butyracea* seeds and butter need also to be investigated.

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