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Shea Press Cake, an Organic Resource of Bioactive Molecules: Biochemical and Phytochemical Profiles of Alcoholic Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MRM and KAB designed the study, wrote the protocol. Authors KAB, DT, KAT and AALR anchored the field study, gathered the initial data and performed preliminary data analysis. While authors KAB, MRM, DGG and NLS managed the literature searches, interpreted the data and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

Shea press cake is a subproduct of shea butter production. It generally serves as animal food or as fuel, in shea areas. This study demonstrated its ability as edible organic bank of bioactive molecules useful for human. Therefore, the hydroalcoholic extract was screened through GC-MS analysis, and antinutritional compounds were quantified. Chromatogram revealed a wide range of molecules belonging to various famillies. Hence, many amino acids involving EAA (Threonine, Valin and pre-tryptophan) were detected. Peaks related to organic acids like quinic, lactic, malic,

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citric, gluconic, galactaric, succinic and phosphoric acids were also identified. These acids would be widely exploited in either food or cosmeto-pharmaceutical, or in both industries. Also appeared on the chromatogram, peaks of oses (glucose, fructose and sucrose) and phenolic acids. Phenolic acids consisted in various catechins and gallic acids which would have antioxidant, antimicrobial, antitumor, anticancer powers. Some other benefic molecules like glycerol and myo-inositol counted among the identified molecules. Above all, shea press cake contents in oxalates (564.66±49.60 mg/100 g DM) and phytates (148.45±0.03 mg/100 g DM) were at far, lower than those of many therapeutic teas. Thus, shea press cake might be considered as a valuable edible bank of bioactive molecules. It could be involved in cosmetics, in drugs and be recommended to consumption as teas leaves, coffee, cinnamon, etc. in prevention to diseases related to metabolic disturbances and oxidative stress (tumor, cancer and degenerative diseases).

Keywords: Shea press cake; bioactive molecules; biochemical and phytochemical profiles; hydroalcoholic extract.

1. INTRODUCTION

The increasing interest for shea butter and consequently the great amount of shea butter produced in order to satisfy demand, lead to producing rejects increasing. These rejects consisted mostly in nuts hulls and press cake. If the first material is used as fuel, the second one is generally given to animals [1,2]. Nevertheless, in areas where these previous approaches are not integrated in people habit, both hull and press cake constitute an environment pollutant during shea butter producing periods. Many structures teach producer about how to use these subproducts, but for lot of producers, shea butter would just be a secondary hobby, so the shorter time devoted to shea butter would be, better it would be. If in many shea producing country shea kernels are just exploited for butter producing, abroad, studies like that performed by of Kitamura et al. [3] reported the high ability of shea kernel pigment as food colorant. These authors linked this pigment to phenolic compounds of shea kernel. It is worth precising here, that shea kernels are ordinary consumed in producing areas, as supplementary dietary [4,5] and would provide vigor to filles workers. Literature presents shea kernels nutritive characteristics but work about shea press cake has to be carry out, specially as far as its nutritive and phytochemical profile are concerned. Hence, the present study aimed to demonstrate shea press cake ability as edible organic bank of useful bioactive molecules, in order to contribute to food safety and to contribute to the fight against degenerative diseases and those oxalate metabolism disturbances and oxidative stress. Therefore, antinutritive compounds (oxalates and phytates) were dosed in shea press cake, and its hydroalcoholic extract was screened using GC-MS analysis for the molecular component's identification.

2. MATERIALS AND METHODS

2.1 Materials

The present study was performed on shea press cake resulting from Megnanou et al. [6] process. Press cakes reduced into powder, was kindly provided by cited authors themselves. Material also consisted in analytical products and reagents for methanolic extraction, GC- MS analysis and for antinutritional compounds quantification.

About GC-MS, it consisted in a PerkinElmer Clarus 680GC 600C MS (connected to a computer) with a 30 m long Restek Rtx-5ms column with an internal diameter of 0.25mm and a stationary phase film thickness of 0.25µm. Helium was used as a carrier gas with a fixed flow rate of 1 ml/min. The oven temperature program was 80 °C for 2 min, then a gradient of 5°C/min was applied up to 300 °C. The latter temperature was maintained for 14 min for a total analysis time of 60 min. The temperature of the injector was set at 300 °C. The injection was carried out in split mode with a ratio of 1:50. The mass spectrometer was set up in electron impact mode with an ionization source temperature of 200 °C, an electron energy of 70 eV, a scanning speed of 200 scans/min and a scanning range between 50 and 800 m/z.

For chromatograms analysis, peaks were identified using pure standards and by analyzing (computer matching) of their spectrum with those of the standard mass spectral. The relative percentages of the different constituents were calculated by the area method (integrator model C-R4A) without taking into account possible differences in their response coefficients methods.

2.2 Methods

2.2.1 Oxalates and phytates quantification in shea press cake

Oxalates and phytates contents in shea press cake were determined following Day and Underwood [7] and Latta and Eskin [8] methods, respectively.

Oxalate quantification consisted essentially in shea press cake powder (2 g) dissolution in 25 mL of sulfuric acid (3 M) for one hour, and the titration of the resulting mineralized with $KMnO_4$, after filtration.

As for phytates, they were quantified first by digesting under agitation, shea press cake powder (1 g) with chloridric acid (0,65 N) during 12 H (28 ± 3 °C) ; digestate was centrifuged at 12000 rpm for 40 min, and then the absorbance of the supernatant was checked at 490 nm with a spectrophotometer.

2.2.2 Hydromethanolic extraction

Guede-Guina et al. [9] method was used to get the hydro methanolic extract of shea press cake; it consisted in macerating shea press cake powder in a hydro methanolic mix (30/70 %: water/methanol).

Hence, a suspension of 100 g of shea press cake powder in 1 L the hydro methanolic mix, was kept under magnetic agitation for 48 hours. The suspension was filtered on hydrophilic cotton and Whatman paper n°2, and submitted to evaporation at 40°C (rotavaporizing) until a dried macerate powder was obtained. The resulting macerate powder was stored in sterile vials at 4 °C for future analysis.

2.2.3 GC-MS analysis of shea press cake macerate

Shea press cake macerate was prepared for injection in CG-MS, by derivatization. This method is generally, performed in GPC analysis in order to transform compounds into derivatives ones with a close chemical structure, but with reduced polarity so that they would be volatile and more stable. Hence, 50 µg of shea press cake powder was derivatized by addition of 250 µL of N,O-Bis (trimethylsilyl)trifluoroacetamide, Trimethylchlorosilane (BSTFA + TMCS, 99:1) and 250 µL of pyridine. The resulting mixture was vortexed for 2 min and then heated to 70 °C in an oven for 30 min. 1 µL of the previous solution was injected into the GC-MS for analysis.

3. RESULTS AND DISCUSSION

3.1 Biochemical Components of Shea Press Cake Hydromethanolic Macerates

The chromatogram resulting from the GC-MS analysis of shea press cake macerate presented a wide range of compounds belonging to several families of biochemical molecules (Fig. 1). Indeed, peaks corresponding to carbohydrates, proteins and organic acids were identified (Table 1).

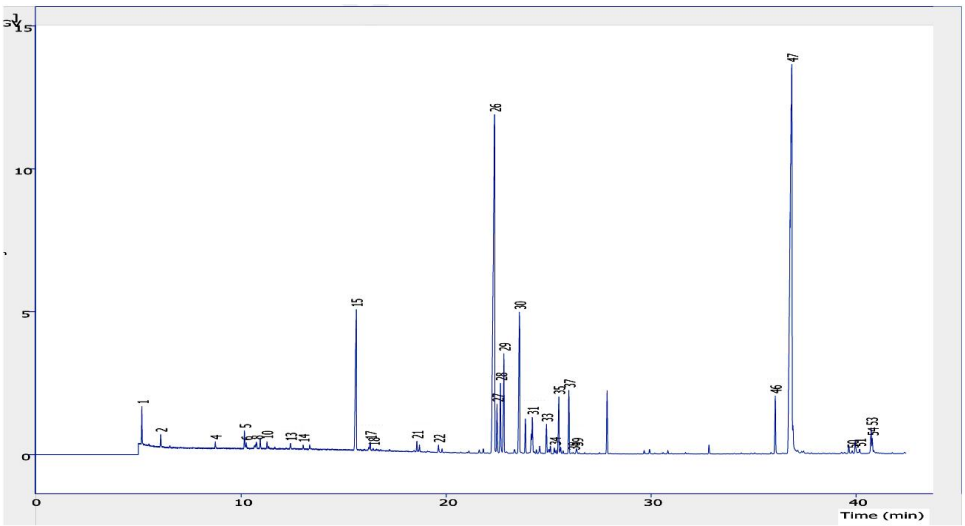

Fig. 1. GC-MS profile of shea press cake macerates

Table 1. Molecules identified in press cake macerate

Amino acids		
L-Alanine : 1.7%	L-Valine : 0.6%	L-Proline : 0.3%
L-Threonine : 0.2%	L-Aspartic acid : 0.4%	L-Glutamic acid : 0.5%
L-Asparagine : 0.3%	γ -Aminobutyric acid : 0.1%	Serine : 0.3%
Organic acids		
Lactic Acid : 1.7%	Succinic acid : 0.4%	Malic acid : 6.3
Citric acid : 4.5%	Quinic acid : 6.3%	D-Gluconic acid : 0.1
Galactaric acid : 0.2%		
Oses		
D-(-)-Fructofuranose 5.3%	β-D-glucose : 1.6%	Sucrose : 17.4%
Others		
Myo-Inositol : 15.5%	Glycerol : 0.3%	Phosphoric acid : 0.8
Glucaric acid-1,4-lactone : 0.3%		

The presence of carbohydrates and protein in shea press cake would be due to shea kernels (seed) intrinsic composition [5,9]. The lack of lipid as for it would suggest an integral extraction of shea kernels fat, during shea butter processing. Various amino acids including essential ones (EAA) such as Valin and threonine were identified. Concerning carbohydrates, molecules like fructofuranose, glucose and sucrose (Table 1), which are simple oses (sugar). They could result from some macromolecules degradation or be free available in the press cake. Relatively to macromolecules, it is to note that demonstrated the presence of saponins and tannins in oilseeds press cake, whereas these compounds would chemically be constituted of various oses. However, the presence of these oses and amino acids in shea press cake macerate, would suppose its good digestibility (in vivo), and would then suggest its consumption like kernels are consumed in shea producing areas [5]. Indeed, according to these authors, shea kernel is eaten as supplementary food during welding periods. Hence, based on its valuable amino acids and its oses, and considering shea kernels empiric consumption by peoples, shea press cake consumption might not constitute nutritional problem. Hence, in order to support the suggestion to shea press cake consumption by human, its oxalates and phytates were quantified (Table 2). Values were 564.66 and 148.45 mg/100g DM, respectively). Despite the relative high contents of these antinutritional compounds, they are at far lower than those of many teas leaves contents in oxalates as reporter by Charrier et al. [10], Hönow et al. [11] and Yagin et al. [12]. Hence, consumers would not be obliged to eat the whole matrix of shea press cakes; they could drink its infused or digestates, like teas, cocoa or coffee. Moreover, macerate powder could be wrapped into edible gelules in adequat amount and be proposed as supplementary food. This suggestion support that of Arrutia et al. [13] about oilseeds (soya, rapeseed/canola and sunflower) press cake as valuable source of proteins. Moreover, food supplementation with shea press cake macerate, seems to be the best option to benefice of the potentiality of its whole components. Indeed, about macerate components, many other benefic molecules such as organic acids (quinic, lactic, malic, citric, gluconic, galactaric and succinic acids) have been identified on the chromatogram of shea press cake. Each of these acids would represents bioactive molecule with pharmacologic virtues [14,15,16]. Quinic acid for instance, would nutritionally support the synthesis of tryptophan and nicotinamide in the gastrointestinal tract; this in turn, leads to DNA repair enhancement linked to the increase of nicotinamide and tryptophan production [17]. It is worth recalling here, that tryptophan is an EAA and nicotinamide is a vitamin (vitamin B3) or pre-NAD/NADP. NAD and NADP are involved in biological oxidoreduction reactions. Hence, when consuming shea press cake (with its quinic acid), that would suppose future tryptophan and vitamin B3 production. Succinic acid as for it, would be involved in supplements for symptoms related to menopause such as hot flashes and irritability. It would also be applied to the skin for arthritis and joint pain. In fact, succinic acid (organic or Bio-succinic acid) is an Ecocert-authorized ingredient, non-GMO and a safe, sustainable and multifunctional alternative to other widely used acids in cosmetics. It would be an antimicrobial, anti-acne, anti-psoriasis, antioxidant, and slimming agent. Hence it allows the development a large range of cosmetics and toiletries.

About malic and galactaric acid, both would actually be considered as an alpha-hydroxy acid (AHA), and would therefore often be included in skin care products for their skin-rejuvenating

abilities. Lactic acid would also be an alpha hydroxy acid, and the most widely used in cosmetics. It is involved in over-the-counter skin care products and professional treatments where it is exploited as anti-aging ingredient. It also serves to exfoliate the skin, lighten dark spots, and improve the look of fine lines and wrinkles [18,19]. Citric acid would have similar abilities as previous acids. Moreover, it would be a powerful antioxidant with antitumerous activity [20]. Concerning gluconic acid, it is widely used in food, pharmaceutical, and other industries (chemical, textile, etc.). For example, it is used in the food industry as acidulant. It also serves as calcium and iron gluconates in medical infusion preparations for the treatment of calcium or iron deficiencies [21].

With all these organic acids known as bioactive compounds, shea press cake would be suggested not only as a benefic edible matrix, but also as a widely exploitable organic (natural) resource for cosmetics with pharmaceutical virtues.

Telling about bioactive molecules, some others like myo-inositol, phosphoric acid, glycerol, etc. which were also detected in shea press cake macerate (Table 1), really confirm its cosmeto-pharmaceutical potentials.

Indeed, myo-inositol for instance, has been established as an important growth-promoting factor of mammalian cells and animals [22]. Inositol's are generally linked to phosphoric acid and glycerides (diglycerides) to form phosphatidyl-inositol, a glycerophosphate. This family of molecules belong to lipid group. Küllenberg et al. [23] widely reporter the therapeutic effects of dietary phospholipids on several diseases. Phosphoric acid and glycerol presence in shea press cake macerate, could result from glycerophosphate hydrolysis; why not glycerophosphatidyl-inositol hydrolysis? Furthermore, study could give more precision on these molecules. However, both phosphoric acid and glycerol are widely exploited in cosmetics setting.

3.2 Phytochemical Profile of Shea Press Cakes Macerate

The GC-MS chromatograph also revealed the presence of phenolic bioactive compounds which consisted in flavonoids and non-flavonoids molecules (Table 3).

Namely, there were gallic acid, catechin, epigallocatechin, arbutin, and catechin like, for peaks which were identified (Table 3). It is important to precise that tea leaves contain so high amount of catechins and derivatives, that these latest would be very important in teas discrimination based on the taste (bitter, astringent, and slight sweet tastes) [24]. Catechins also possess health benefits in the prevention of disease caused by oxidative stress. Indeed, once ingested, catechins work as antioxidants for example by helping prevent lipid peroxidation [25]. Such information would support the suggestion of shea press cake infusion and digestate drinking in order to benefice of its whole active molecules. This consumption would not only contribute to good heath preservation, but also provide juvence to skin. About skin juvence, anti-aging cosmetics are nowdays supplemented with antioxidant compounds for this purpose [26,27]. Another phenolic acid detected in shea press cake macerate, gallic acid, is reported to have several health-promoting effects such as antioxidant, antitumor anticancer power [28]. These authors also reported that gallic acid and its derivatives can inhibit the oxidation and rancidity of oils and fats ascribed to their free radical scavenging and antioxidant nature. Hence shea press cake could valuably be used as additives in the food industry. About this ability, it is worth to recall that Kitamura et al. [3] have demonstrated the non-toxicity of shea kernel pigment (brown), and suggested it as food colorant. Based on this suggestion, macerate of shea press cake could serve as food colorant, and also for shelf-life improvement. Other bioactivities of gallic acid, have been reported by several authors. Hence, Kim [29] underlined its antineoplastic, bacteriostatic and antimelanogenic properties, while its anticancer properties in prostate carcinoma cells was demonstrated by Kaur et al. [30].

Table 2. Phytate and oxalate content of shea cakes

	Value (mg/100 g DM)
Phytates	148.45 ± 0.03
Oxalates	564.66 ± 49.60

DM: Dry matter

Table 3. Phenolic compounds (g/100g DM) identified in shea press cake

Gallic acid	Arbutin	Catechins			
		A_1	A_2	A_3	A_4
2.6	2.6	0.1	1.2	0.2	0.7

Catechins isomers : (1) : (-)-catechin C ; (2) : (-)-epigallocatechin EGC; (3) : (-)-epicatechin EC; (4) : (-)-epigallocatechin gallate EGCG; DM : dry matter

4. CONCLUSION

The screening of shea press cake hydroalcoholic macerate revealed valuable biochemical and phytochemical profiles. It contains amino acids including essential ones (Valin, threonine and pre-tryptophan or quinic acid), organic acids which are currently uses as bioactive molecules for both food and cosmeto-pharmacological sectors. It also contains glucose, fructose and sucrose, which would be easy-assimilable carbohydrates. These components would suggest shea press cake as an organic matrix for dietetical and pharmacological purposes. This suggestion is also supported by the high quality of its phyto-molecules consisting in catechins and derivatives, gallic acids and arbutin. These latest are widely involved in the prevention and the therapy of many diseases including tumor and cancer. They also serve in cosmetics as antiaging, lightning and also as repairing agents. Shea press cake also contains oxalates and phytates, but the amounts were at far lower than those of most of current therapeutical teas. Hence, this subproduct and environment pollutant might be considered as a valuable edible bank of bioactive molecules, and be recommended like teas, coffee, cumin etc. in prevention against tumor, cancer and other diseases including metabolism disturbances and degenerative illnesses.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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