Enzyme-Assisted Aqueous Extraction of Fat from Kernels of the Shea Tree, *Butyrospermum parkii*

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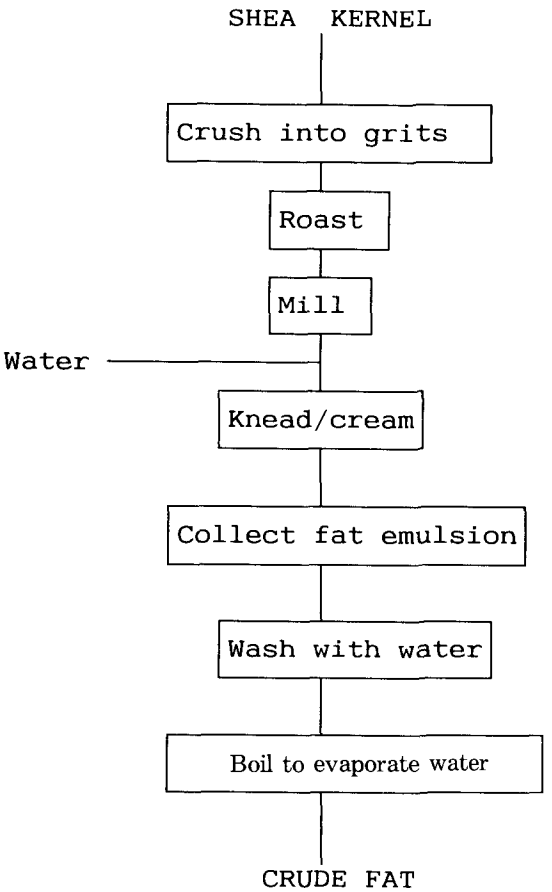
Chemical characteristics of kernels of the shea tree (*Butyrospermum parkii*) from Ghana were determined in order to design and evaluate studies on a traditional enzyme-assisted fat extraction of the kernels. The effectiveness of a number of cellular degrading enzymes in assisting the shea fat extraction were also tested by treating meals of the kernels with one or more of these enzymes before extraction and comparing the yield with control extractions. Proximate composition of the kernel on dry-matter basis was: total lipids, 59.04%; crude fat, 54.85; protein, 7.81%; total carbohydrates, 34.77%; ash, 2.57%. Starch content was 7.59%; hemicellulose, 10.84%; cellulose, 5.95%; and pectic substances, 2.93%. Total fiber content was 20.35%. The fat extracted by the Soxhlet method was pale-yellow in color and solid at room temperatures. Its physicochemical characteristics were: melting range, 34–36°C; iodine value, 58.53; saponification value, 180.37; and unsaponifiable matter content, 7.48%. The predominant fatty acids were: palmitic (3.55%), stearic (44.44%), oleic (42.41%), linoleic (5.88%) and linolenic (1.66%) acids. The enzyme-assisted extraction tests showed increases in extraction yield when the shea kernel meals were treated with the enzyme(s) before extraction. An increase of about 20% was realized when a protease and an enzyme with both cellulase and hemicellulase activities were used together. These observations confirmed the fact that the shea kernel is a rich source of fat. They also indicate the possibility of improving shea kernel extraction processes by pretreating the kernel meal with cell structure-degrading enzymes.

KEY WORDS: Enzyme-assisted, fat extraction, fatty acid composition, microbial enzymes, shea fat, shea kernels, shea tree.

The shea tree, also known as bambuk butter tree (1), is a member of the Sapotaceae family. It is an equatorial plant, growing wild in West and Central Africa. The tree grows to about 12 m high and 80–120 cm in girth when fully matured. The kernels are borne in the tree's spherical to ellipsoidal berry fruits, which are 5–7 cm long. The kernels are enclosed in thin shells, together forming the shea nuts. The matured kernel contains 40–55% fat (1,2). The fat is extracted, and used for edible, technical and medicinal purposes (1–3). Shea fat was considered a highly prized medicinal substance in certain parts of Africa as far back as 1728 (4).

The extraction of shea fat is done by either one of two basic methods, broadly described as traditional or modern. The modern methods involve use of mechanical presses and organic solvents (1). The traditional methods, which predominate the extraction technologies in many of the shea kernel-producing areas, involve water extraction (Scheme 1) (2). Each of these methods apparently has some problems. The modern methods are, reportedly, difficult to perform because of a high latex content of the kernel, although the methods are quite high-yielding (with extraction rates of about 80%) (1). The latex agglutinates and clogs the equipment, or it prevents the efficient penetration of the solvent used for the extraction. It is also indicated that products from these modern processes are sometimes difficult to refine (1).

The traditional methods, on the other hand, are grossly inefficient, yielding not more than 35% of the oil contents of the kernels, and their product quality is often low (2). It is obvious that a major contributing factor to the low yield in the traditional methods is the inability of the treatments involved to adequately rupture the cellular structure of the kernels, a necessity for efficient vegetable oil extraction (5). These problems have invariably contributed to the underdevelopment of the shea fat industry. And presently, even though nonoil resources are being explored for oil production to supplement future global oil supplies (6), tons of shea kernels (a naturally-occurring, useful fat resource) are underutilized. It is important to improve the shea fat extraction process to make the shea industry more economically viable.



SCHEME 1

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The use of enzymes to enhance oil seed processing has been reported by many researchers. For instance, Fullbrook (7) reported on soybean and rapeseed; Buenrostro and Lopez-Munguia (8) on avocado; McGlone *et al.* (9) on coconut; Sosulski *et al.* (10) on canola; Cheah *et al.* (11) on palm oil; Sosulski and Sosulski (12) on canola; and Smith *et al.* (13) on soybean. These studies reveal that treatment of the oil-bearing materials involved with some enzymes after mechanical milling further degrades the cellular structure of the material. This occurs through partial hydrolysis of cell walls and membranes, and of some of the macromolecules linking to the fat globules, to release more oil to be extracted by either mechanical pressing or solvent extraction.

The studies on enzyme-assisted extraction methods quoted, like most other previous studies on oil extraction, sought to improve upon the modern extraction methods. However, irrespective of how small the scale of production is, or how crude the technologies involved are, these traditional methods contribute to the global supply of fats and oils. These methods are favored by the socio-economic and agricultural systems of the countries where they predominate in oil extraction industries (many developing countries). The methods are major channels of losses of oily materials and thus require improvement. This work was done to gather data to assist in the design and evaluation of studies on enzyme-assisted fat extraction of shea kernels, based on a traditional water extraction method. It is hoped that data obtained would also add to the otherwise scanty literature available on shea kernels and shea fat.

MATERIALS AND METHODS

Shea kernels of the *poisonni* variety (2) were purchased from markets in Ghana. The pooled quantity was sorted, packed in polythene containers and air-lifted to Japan. They were stored in a cold room (about 5°C) until analyzed (within six weeks).

The kernels were comminuted in a high-speed mixer (Hitachi VA 895 mixer; Hitachi Co., Tokyo, Japan), followed by grinding to a fine meal in a porcelain mortar. Samples for crude fat determinations were mixed with known quantities of diatomaceous earth and further ground to obtain a finer, loose meal. Fat samples analyzed were obtained by solvent extraction (Soxhlet) (14).

Kernels were analyzed for moisture, crude fat, crude protein, total fiber, ash and carbohydrates. The fat was analyzed for melting point, free fatty acid content, iodine value, saponification value, peroxide value, unsaponifiable matter content and fatty acids composition.

Analyses. Moisture, crude fat, crude protein, ash, iodine value and melting point determinations were done by standard methods (14). Total lipids were estimated with the chloroform–methanol method of Folch *et al.* (15). Total fiber was estimated by the method of Lee *et al.* (16) with heat-stable α-amylase (EC 3.2.1.1 Type XII-A), α-glucosidase (EC 3.2.1.20) and a protease (P-5380) from Sigma Chemical Co. (St. Louis, MO). Free fatty acid content was determined with the method described by Pearson (17). Carbohydrates were estimated by methods described by Southgate (18) and Kobayashi *et al.* (19), with some modifications. Southgate's method (18) for alcohol-soluble sugar determination was modified by refluxing the sample two times with methanol at 65°C for 2 h. In the Kobayashi *et al.* method (19) for hemicellulose and cellulose determination, the ammonium oxalate was substituted with ammonium oxalate–oxalic acid solution (20), and the 24% KOH with 16% KOH. Precipitates of fractions were recovered by centrifuging at 16,000 × *g* for 30 min in weighed centrifuge tubes. Precipitates were washed with 95% ethanol, water and acetone. Residual weights were determined, and the residues were resuspended in water, digested with 72% sulfuric acid, diluted and analyzed for total sugars (21). Total soluble sugar analyses were all done by the phenol-sulfuric acid method (22). High-performance liquid chromatography (HPLC) of the sugars in the shea kernel carbohydrates was carried out in a Hitachi L-6000 chromatograph equipped with a Hitachi L-3300 RI monitor. The column was a gelpack GL-C620 (Hitachi Chemical Co. Ltd., Tokyo, Japan), and the eluant was deionized deaerated water. Samples of the fat-free meal were hydrolyzed with sulfuric acid as described earlier and neutralized with sodium hydroxide. The ethanol-extracted and lead acetate-clarified sugar solution was used for the analyses.

Fatty acid composition. The shea kernel fat was hydrolyzed and methylated by the sodium methoxide–boron trifluoride method (14). Prepared esters were extracted with heptane and analyzed with a gas chromatograph equipped with an flame-ionization detector and an integrator (Hitachi Co.). A glass column (2.0 m × 3 mm i.d.) with Unisole 3000 on uniport C 80/100 packing (GL Sciences Inc., Tokyo, Japan) was used. The carrier gas was nitrogen at a flow rate of 30 mL/min. Injection temperature was 220°C, column temperature was programmed at 7.5°C/min from 100 to 230°C. The fatty acids were identified with reference to the retention times of esters of standard fatty acids that were under similar conditions.

Enzyme-assisted extraction. A number of crude enzymes (amylases, proteases, hemicellulases, cellulases and pectinases) were used. Table 1 shows the producer's information on some of the enzymes. Weighed quantities of meals were mixed with water in meal-to-water ratios of 1:4. Meals were autoclaved at 100°C for 5 min, with the aim of inactivating any enzyme present, particularly lipases. Enzymes were added to the cooled meals singly and in combination with others, each enzyme in concentrations calculated to obtain activities of 500 units/gram of enzyme preparation based on the manufacturer's reported activities. A uniform meal weight of 20 g was used in each treatment. The mixtures were thoroughly stirred and were incubated in a water-bath-shaker at 50°C and 100 revolutions per min for about 4 h. All the enzymes were obtained from the Shin Nihon Chemicals Co. (Anjoh, Japan). After incubation the meals were autoclaved again at 100°C for 3 min. They were then quantitatively transferred into centrifuge bottles with hot water, and centrifuged at 12,300 × *g* for 20 min. The supernatants were extracted with petroleum ether in separatory funnels, and the ether phase was washed several times with warm water until the washings were clean. The ether phases were collected in weighed dishes, evaporated on a water-bath and dried in an air-oven at 100°C for 2 h. Weights of oil extracted were determined after cooling the dishes and were expressed as a percentage of the value obtained by the Soxhlet method. Extraction with each enzyme was done in triplicates.

TABLE 1

Producer's Information on the Crude Enzymes[a]

Enzyme	Commn. name	Source	Specified contaminants	Specific activity (units/gram)
α-Amylase	Sumizyme-L	*A. oryzae*	—	12,000
Acid protease	Sumizyme-AP	*A. niger*	—	50,000
Cellulase/hemicellulase	Sumizyme-C	*T. reesei*	—	1,500
Pectinase	Sumizyme-AP2	*A. niger*	Cellulase, hemicellulase	2,000
Glucanase	Sumizyme-TG	*T. reesei*	—	100

[a]*A. = Aspergillus; T. = Trichoderma.*

RESULTS AND DISCUSSION

The shea kernels, generally ellipsoidally shaped, measured from 1.6 to 3.1 cm and from 1.3 to 2.0 cm across the length and breadth, respectively. The mean weight per kernel was 3.22 g. Moisture content (4.92%) was appreciably low for good storage. Molds, which are the predominant spoilage organisms, would not grow at this moisture content. Some of the kernels, however, showed mold contamination when split. The shea fruits are usually gathered from the shea tree fields, and are boiled and depulped by light pounding to obtain the kernels. These kernels are then washed and sun-dried to the appropriate moisture contents. The kernels could thus be infested with mold either before gathering or during the later drying stage. The meal appeared fatty and was sticky during milling; this made sample preparation quite difficult.

The chemical composition of the kernel are shown in Table 2. The crude fat value, about 55%, is really high, which together with the large kernel mass, makes the kernel a rich source of fat. The kernel also has a high carbohydrate content. The high starch content and low levels of pectic substances are peculiar for these carbohydrates. Hemicellulose was higher than the cellulose composition. The HPLC analyses for sugar types showed maltose, rhamnose, xylose, arabinose and glucose as the predominant simple sugars. There were high concentrations of dextrin in all the digests, indicating incomplete digestion. Quantitation of the sugars was therefore not done.

Table 3 shows the physicochemical characteristics of the solvent-extracted shea fat. When in a liquid state, the fat was clear and pale yellow in color. It solidified quickly at room temperatures. The melting range was similar to that of cocoa butter (32–35°C) (3) and was near mouth temperature. The free fatty acid value was quite high. Aye and Adomako (23), however, reported the following values: nonmoldy kernels, 2.3–4.5%; moldy kernels, 15.2–21.6%; germinated kernels, 6.41%; and unblanched kernels, 33.9–43.2%. The designations of the kernels were given based on their state before treatment. Some of the fruits gathered might have germinated prior to kernel extraction. Some of the kernels might also be extracted without prior boiling of fruits (unblanched kernels) (2). The implications from the values quoted are that the free fatty acid values are highly variable, depending on the state or pretreatment of the kernels. The apparently high value observed might have resulted from activities of lipases naturally present in the kernels or produced by infesting molds (24). On the other hand, the comparatively low value observed in the present study suggests that the kernels analyzed were of good quality.

TABLE 2

Chemical Characteristics of Shea Kernels

Characteristics	Composition (% by wt)
Total lipids	59.04
Crude fat	54.85
Total nitrogen	1.37
Crude protein	7.81
Ash	2.57
Carbohydrates (by difference)	34.77
Alcohol-soluble sugars	5.45
Starch	7.59
Pectic substances	2.93
Hemicellulase	10.84
Cellulose	5.95
Total fiber	20.35

TABLE 3

Physicochemical Characteristics of Soxhlet-Extracted Shea Fat

Factor	Value
Free fatty acids (%)	1.28
Iodine value	58.53
Saponification value	180.37
Peroxide value (meq/kg)	9.65
Unsaponifiable matter (%)	7.48
Melting range	34–36°C

Another distinct feature of shea kernel fat is the high unsaponifiable matter content. Codd *et al.* (3) have also reported values of 2–11%. The high unsaponifiable matter content seems to be a limitation to the use of the fat in the soap and detergent industries. The fatty acid constituents, however, suggest that the fat is suitable for these purposes. On the other hand, some of the numerous medicinal properties (particularly dermatological and pharmacological) of the fat, which are already known in many African countries, and are currently becoming recognized in European medical communities, are due to some constituents of the unsaponifiable matter (25,26).

The fatty acid compositions are presented in Table 4. The predominant fatty acids are stearic, oleic, linoleic, palmitic and linolenic acids. Quite a similar pattern has been reported in the literature (27). The characteristic pattern of fatty acids seems to explain the fat's suitability to the formulation of hydrogenated products and cocoa butter substitutes, and also underlies its uses in some cosmetic and pharmaceutical preparations (1–3,25,28).

TABLE 4

Fatty Acids Composition of Soxhlet-Extracted Shea Fats

Fatty acids	Composition (% by wt)
Caprylic (C_8)	0.23
Capric (C_{10})	0.15
Lauric (C_{12})	0.33
Myristic (C_{14})	0.23
Palmitic (C_{16})	3.55
Stearic (C_{18})	44.44
Oleic ($C_{18:1}$)	42.41
Linoleic ($C_{18:2}$)	5.88
Linolenic ($C_{18:3}$)	1.66

In the fat-extraction trials it was observed that addition of each of the enzymes tested significantly increased the extraction yield. In single-enzyme trials, an α-amylase, an enzyme with both cellulase and hemicellulase activities, and a protease gave the best yield. Pectinases produced the lowest increases. Some of the data from large number of trials are presented in Table 5. Data presented are means of three separate extractions. Yield increased further when the enzymes were combined. It was at its best when an acid protease was combined with the cellulase/hemicellulase and a glucanase. The higher effectiveness of α-amylase, cellulase/hemicellulase and protease and of the various combinations seems to reflect the general composition of the kernel (Table 2). The various researchers (7–13) on enzyme-assisted oil extraction observed increased effectiveness when proteases and carbohydrases were used together. Fullbrook (7) observed improved extractability of soybean and rapeseed when the seeds were pretreated with β-glucanase, hemicellulase and proteolytic enzymes. Buenrostro and Lopez Munguia (8) found a combination of cellulase, papain and α-amylase necessary for more efficient extraction of avocado. McGlone *et al.* (9) observed maximum yield of coconut oil when a polygalacturonase, α-amylase and a protease were used in combination. Sosulski *et al.* (10) also observed more efficient extraction of canola with a mixture of carbohydrases. Because the enzymes were compared at the same activity levels, the observations suggest, within the limits of the properties of the enzymes and the extraction conditions used, that an optimum combination of enzymes for the extraction of shea fats is a protease, a cellulase and a hemicellulase together. A more detailed study on the extraction is in progress. However, this preliminary work has shown the possibility of using hydrolytic enzymes to enhance the extraction of shea fat.

The effects of the enzyme treatment on the quality characteristics of the fat will be determined in subsequent work; it is anticipated, however, that there will not be any apparent adverse effects. Improvements in product quality were observed in some of the enzyme-assisted extraction studies cited (7,9). With the aqueous extraction techniques, the water-soluble, low-molecular weight fatty acids, fatty alcohols, phosphatides and many other undesirable nontriglyceride odorous constituents would be washed out of the fat, resulting in a more desirable crude shea fat. Future work will seek to develop an optimum extraction process, based on a traditional water extraction method, and to investigate the possibility of substituting the enzymes with microorganisms that produce those enzymes, under various conditions in the process, for cost effectiveness.

TABLE 5

Effect of Enzyme Combinations on Extraction Yield of Shea Fat

Enzymes[a]	Yield (%)[b]
0	53.7
1	62.0
2	59.4
3	63.9
4	55.5
5	59.2
1,2	68.5
1,3	70.6
1,4	69.1
1,5	65.8
2,3	71.3
2,4	64.7
2,5	66.4
3,4	66.1
3,5	65.9
1,2,3	72.8
1,2,4	72.1
1,3,4	71.6
2,3,4	73.2
2,3,5	74.1

[a]0, No enzyme (control); 1, α-amylase; 2, protease; 3, an enzyme with both cellulase and hemicellulase activities; 4, pectinase; 5, glucanase.
[b]Yield estimations were based on the fat content as determined by the Soxhlet method.

REFERENCES

1. Salunkhe, D.K., and B.B. Desai, *Post-Harvest Biotechnology of Oil Seeds*, CRC Press, Inc., Boca Raton, 1986, pp. 204–205.
2. Ata, J.K.B.A., The Study of the Shea Kernel in Relation to the Traditional Process of Shea Fat, Ph.D. Thesis, University of Ghana, Legon-Accra, 1978.
3. Codd, L.W., K. Dijkhoff, J.H. Fearson, C.J. van Oss, H.G. Roeberson and E.G. Stanford (eds.), *Materials and Technology*, Vol. VIII, Longman J.H. De Bussy, Amsterdam, 1975, pp. 60–61.
4. Ruyssen, 1957. Cited by J.K.B.A. Ata, in The Study of Shea Kernal in Relation to the Traditional Process of Shea Fat, Ph.D. Thesis, University of Ghana, Legon-Accra, 1978.
5. Graille, J., M. Pina and D. Montet, *Oleagineux 43*:188 (1988).
6. Ratledge, C., and C.A. Boulton, in *Comprehensive Biotechnology*, Vol. 3, Pergamon Press, New York, 1985, pp. 983–1003.
7. Fullbrook, P.D., *J. Am. Oil Chem. Soc. 60*:428 (1983).
8. Buenrostro, M., and C.A. Lopez Munguia, *Biotechnology Lett. 8*:505 (1986).
9. McGlone, O.C., C.A. Lopez Munguia and J.C. Carter, *J. Food Sci. 51*:695 (1986).
10. Sosulski, E., F.W. Sosulski and E. Coxworth, *J. Am. Oil Chem. Soc. 65*:357 (1988).
11. Cheah, S.C., M.A. Augustin and L.C-L. Ooi, *PORIM Bulletin 20*, Palm Oil Research Institute of Malaysia, Kuala Lumpur, 1989.
12. Sosulski, K., and F.W. Sosulski, *J. Am. Oil Chem. Soc. 70*:825 (1993).
13. Smith, D.D., Y.C. Agrawal, B.C. Sarkar and B.P.N. Singh, *Ibid. 70*:885 (1993).
14. *Official Methods of Analysis of the Association of Official Analytical Chemists*, Association of Official Analytical Chemists, Washington, D.C., 1984, Methods 27.005, 27.0066, 27.007, 27.009, 28.014 and 28.024.
15. Folch, J., M. Lees and G.S.H. Stanley, *J. Biol. Chem. 226*:497 (1957).

16. Lee, S.C., L. Prosky and J.W. De Vries, *J. Assoc. Off. Anal. Chem. 75*:395 (1992).
17. Pearson, D., *The Chemical Analyses of Foods*, 7th edn., Churchill Livingstone, 1976.
18. Southgate, D.A.T., *J. Sci. Fd. Agric. 20*:327 (1969).
19. Kobayashi, M., K. Funane, H. Ueyama, M. Tanaka and Y. Kato, *Biosci. Biotech. Biochem. 57*:998 (1993).
20. Sabir, M.A., F.W. Sosulski and N.W. Hamon, *J. Agr. Food Chem. 23*:16 (1975).
21. Southgate, D.A.T., *Ibid. 20*:331 (1969).
22. Dubois, M., K.A. Gills, J.K. Hamilton, P.A. Rebers and F. Smith, *Analy. Chem. 28*:350 (1956).
23. Aye, F.O., and D. Adomako, *Processing of Shea Fruits*, Rep. Cocoa Research Institute of Ghana, 1986/87.
24. Bailey, A.E., *Industrial Oils and Fats Products*. Interscience Publication Incorporated, New York, 1951.
25. Pesquet, J-J., *Aproma/Bimonthly Review 27-29*:8 (1992).
26. Lozano, Y.F., C. Dhuique Mayer, C. Bannon and E.M. Gaydou, *J. Am. Oil Chem. Soc. 70*:561 (1993).
27. Godin, V.J., and P.C. Spensley, *Crop and Product Digest, No. 1*, Tropical Products Institute, London, 1971.
28. Swern, D. (ed.), *Bailey's Industrial Oil and Fat Products*, Vol. 2, 4th edn., A. Wiley-Interscience Publication, John Wiley & Sons, New York, 1982, pp. 372-376.

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