Enzyme-Assisted Aqueous Extraction of Shea Fat: A Rural Approach

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ABSTRACT: The use of enzyme-assisted partial hydrolysis as a preextraction treatment in a rural shea fat extraction process to improve upon the extraction rates of the process was explored following an observed possibility in a preliminary investigation. Finely ground shea kernel meal samples were mixed with water in predetermined ratios and heated to inactivate any enzymes present. A crude protease and an enzyme with both hemicellulase and cellulase activities were added and mixed, also in predetermined concentrations. The suspensions were incubated in a waterbath shaker at temperatures ranging from 30 to 45°C for specified periods of time. The treated meal samples were then extracted using an adapted traditional aqueous extraction process. At optimum meal-to-water ratio of 1:2, enzyme concentration of 1%, the natural pH of the meal (about 5.3), and incubation time of 4 h, the enzyme treatment increased the extraction rate from about 40% in the typical traditional system (control) to about 75%, of the total fat content (estimated by the Soxhlet method). The enzyme-treated meal samples were very easy to extract as there was no need to cream or whip out the fat, as is laboriously done in the traditional process. The extracted fat samples had apparently less unsaponifiable matter content and slightly less free fatty acid content and peroxide value, compared to samples from the typical traditional process and, in some cases, the Soxhlet extracted samples. The observations confirmed the results of the preliminary investigations and suggest that the enzyme-assisted preextraction treatment could significantly improve upon the aqueous shea fat extraction process.

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KEY WORDS: Aqueous extraction, enzyme-assisted, rural process, shea fat.

Rural (traditional) vegetable oil extraction processes, irrespective of how crude the techniques, significantly contribute to global supplies of fats and oils. These processes employ simple size-reduction and household equipment and aqueous extraction techniques to produce highly edible and technical crude fats and oils. They are the predominant extraction processes in many developing countries. In some countries, they are estimated to account for over 60% of the total oil-bearing materials processed (1). These processes have persisted for several years in spite of the proliferation of conventional methods (using presses and organic solvents). Some of the reasons for their persistence had been indicated earlier (2).

These rural processes are, however, not efficient. It is estimated that they extract only about 20 to 40% of the total oil content of oil seeds (3–5), compared to about 80% of the conventional processes (3,6). Considering their widespread applications, these processes thus constitute major channels of losses of fats and oils in the world's food production system. This is an issue worth considering as efforts are intensified to increase fats and oils production to meet the quickly growing global demands.

It is indicated that a major contributing factor for the low extraction efficiencies of the traditional processes is the inadequacies of their preextraction treatments in disrupting the cellular structures of the oil-bearing materials (7). The types of size-reduction equipment (attrition mills) used to achieve this are limited by their design and status in operation. To achieve better results, vigorous heat treatments, such as roasting and boiling, are used to augment the grinding operations. However, these treatments are invariably detrimental to the quality of products (8,9). The strategies for improving upon the traditional processes thus include developing techniques that would better assist the mechanical treatments, efficiently rupturing the cellular structure of the materials, and avoiding extensive heat treatments.

A number of reports have indicated the possibility of using enzyme-catalyzed hydrolysis to effect this (7). Pretreatment of meals of some oil-bearing materials with enzymes prior to oil extraction in both high-pressure pressing and solvent (including water) extraction systems resulted in higher oil yield, higher than usual extraction rates, and improved product characteristics (7,10–13). The purpose of this work was to explore the use of enzyme-catalyzed hydrolysis as a preextraction treatment in an aqueous shea fat extraction process to improve upon the extraction rates of the process. The premise was that, since enzymes act perfectly well in aqueous systems, it could be possible to adapt the enzyme-assisted technique, as various reporters have described, to improve upon the traditional processes. This possibility was also observed in a preliminary investigation of this work (2).

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Shea fat is one of many fats that are mostly extracted with the implied traditional processes. The fat, obtained from kernels of the shea tree, *Butyrospermum paradoxum* subsp. *parkii*, is an important edible and technical fat. It is used in the formulation of cocoa butter substitutes for chocolate and confectionery products; in the production of shortenings, margarine and candles; and as a base for cosmetics and pharmaceuticals (3,4,14). In the West African region where shea fat is predominantly produced, it is an important cooking fat. The kernel contains 50 to 55% of its weight as fat (2–4,14).

MATERIALS AND METHODS

Materials. Shea kernels purchased from markets in Ghana were used. Not much was known about their history except that the fresh shea nuts were boiled prior to the separation of kernels (3). The samples had been sorted, washed and well-dried in an air-oven at 50°C, and then air-lifted to Japan in polythene containers. They were kept in a cold-room until used.

Enzymes. Crude protease (Sumizyme-AP) from *Aspergillus niger* and a crude enzyme (Sumizyme-C) with both cellulase and hemicellulase activities from *Trichoderma reesei*, donated by Shin Nihon Chemicals Co. (Anjoh, Japan) were used. Their properties are shown in Table 1.

Sample preparation. Samples of kernels were crushed with a hammer on a cardboard and then kept in an air-oven for about 1 h at 100°C to dry and condition them for milling. This was a modification of the traditional process in which the kernels are crushed and roasted at higher temperatures [ranging up to about 180°C (15)]. The oven-conditioned mass was cooled and milled into a fine particle-sized meal, which was sticky in consistency, using a high-speed mixer, the Sanyo Food Factory (model SKM 1580 EK; Sanyo Electric Co. Ltd., Osaka, Japan). The meal samples become fluid-like during prolonged milling. To achieve the desired fineness with the equipment, the samples were kept in a cold-room to cool-dry after two successive milling runs, and then remilled.

Chemical analyses of sample. These were done as reported earlier (2).

Fat extraction. Initial experiments were done to compare the extraction yields from enzyme-treated samples and untreated samples, as described below. The effects of some treatment conditions were then investigated.

In the initial experiment, a set of accurately weighed 50-g meal samples in conical flasks was mixed with water, in a ratio of about 1 to 4 wt/vol, gently boiled for 5 min, and then cooled to about 30°C. The crude enzymes were added, each at the rate of 1% of the meal's weight and thoroughly mixed. The mouths of the flasks were covered with parafilm. The flasks were then placed in a waterbath shaker and incubated at 45°C for 4 h, shaking at 80 rpm. Another set of samples prepared similarly but without the enzymes was also incubated alongside for the same period of time as controls. After the treatment, the digests were transferred into wide-mouthed receptacles and extracted as described later. A third set of samples was also extracted without the hydrolysis step. They were, however, boiled for 5 min and cooled just before extraction. The enzyme-treated samples and some of the untreated samples were extracted using a water flotation technique. In a typical extraction, hot water, about the amount of the treated or untreated sample, was added to the mixture and vigorously stirred. Cold water was then added to cool the mixture to about 30 to 40°C. The mixture was left to stand undisturbed for 1 to 2 h to settle (a unit operation termed as aging). The emulsion which formed the top layer was collected into another container. Fresh warm water was added and stirred to wash and again allowed to settle. Finally the emulsion was collected into a beaker and boiled gently until a clear oil was obtained. The oil was decanted into another beaker and placed in an air-oven at 100°C for about 1 h to dry and clarify. It was then decanted into a weighed aluminum dish, cooled and weighed to estimate the yield. The rest of the untreated samples were extracted using the creaming method as described by Aye and Adomako (16). The general enzyme-assisted preextraction treatment and water extraction process are summarized in Figure 1. Yield was estimated as follows:

$$\% \text{ yield} = (\text{weight of oil extracted/total weight of oil in meal extracted, estimated by Soxhlet method}) \times 100 \quad [1]$$

Effects of treatment conditions. The effects of enzyme concentration, treatment time, temperature, pH, meal dilution, and shaking on fat yield were determined. Single-factor experiments were done using the following factor levels: enzyme concentration—0, 0.25, 0.5, 1, 3, 5%; time—1, 3, 6, 12, 20 h; temperature—30, 37, 45°C; meal-to-water ratio—1:2, 1:4, 1:7, 1:10. Variations in pH were achieved by replacing the water used to mix the meal with 0.05 M citric acid/sodium citrate buffer solutions of different pHs. The pHs of the solutions were 3.05, 3.11, 4.62, 4.85, 5.71 and 5.92. [The solutions were prepared at random to have values in the pH range for the buffer (17) and were added after boiling the meal samples with limited amounts of water and cooling.] Multifactor

TABLE 1
Characteristics of Crude Enzyme Preparations Used

Enzyme	Source	Activity (u/g)	Optimum pH	Optimum temp. (°C)	Stability
Sumizyme AP[a]	*Aspergillus niger*	50,000–200,000	3	50	Stable in aqueous solution below 50°C
Sumizyme C[a]	*Trichoderma reesei*	1,500	4.5	50	Stable in aqueous solution below 50°C

[a]Company source: Shin Nihon Chemicals Co. (Anjoh, Japan).

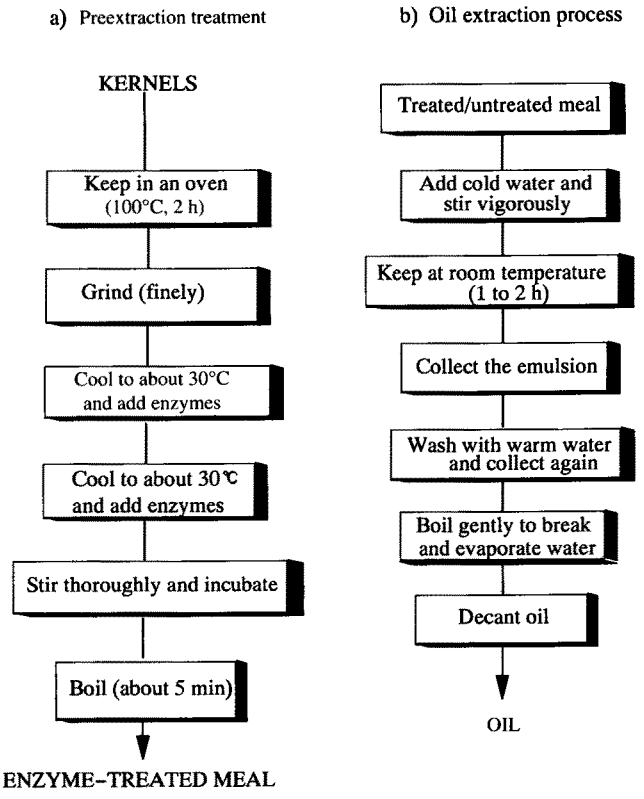

FIG. 1. Summary of the enzyme treatment and the fat extraction processes.

experiments were also done to determine the interactive effects of enzyme concentration and treatment time; enzyme concentration, treatment time and meal dilution; and continuous shaking and treatment time. The factor levels were within the ranges used for the single-factor experiments. The following fixed conditions were used in the unspecified cases: enzyme concentration, 1%; temperature, 30°C; time, 4 h; pH, unmodified pH of meal–water mixture (about 5.3); meal/water, 1:2; continuous shaking at 80 rpm.

Comparison of extraction techniques. Yield on extracting the enzyme-treated meal by the water flotation technique, centrifugation or hexane extraction were compared. In the centrifugation method, the digested meal was mixed with an equal volume of water and centrifuged at 10,000 × *g* for 10 min. The oil layer was collected into a beaker and gently boiled to evaporate the water and then clarified as described above. By the hexane method, the digest was diluted and centrifuged as described above. Hexane was used to wash the residue and then used to extract the supernatant in a separating funnel. The hexane was evaporated in a water bath, and the oil was dried in an oven at 100°C to constant weight.

Analysis of fat samples. The fat samples extracted under optimal enzyme treatment conditions and in the controls were analyzed for free fatty acid (FFA) contents and anisidine values (AVs), peroxide values (PVs), iodine and saponification values and unsaponifiable matter (UM) contents using standard methods (18).

RESULTS AND DISCUSSION

The method for sample preparation and the selection of enzymes were based on some preliminary experimental observations. The initial parameters selected were, however, also guided by the findings of some enzyme-assisted extractions reported (10–13). The samples had to be cool-dried for further grinding because the equipment used was not capable of grinding efficiently in the earlier attempts, during which generated heat melted the fat and made the meal fluid-like. The cold drying thus hardened the meal to make regrinding possible. In a preliminary investigation, eleven crude enzymes, made up of amylases, proteases, cellulase/hemicellulases and pectinases, were tested for their effectiveness in assisting shea fat extraction. Although the combination of a protease, a cellulase, a glucanase and a pectinase resulted in the highest yield, the two enzymes selected for the study together gave a mean yield which was not significantly different from the highest, under similar extraction conditions (2).

Data on the effect of enzyme treatment are presented in Table 2. Values presented are means of three to six determinations. The enzyme treatment significantly increased the extraction yield. The more than 70% yield with the enzyme compared to the nearly 40% yield in the traditional process implies a relative increase in yield of the traditional rate by about 75%. It is reported that the current extraction rate of shea fat by the Cocoa Processing Company (Takoradi, Ghana) is about one-quarter of the weight of the kernel (19). This amounts to about 50% extraction rate, assuming a mean fat content of about 50%. This rate is significantly lower than the rate observed with the enzymes in this experiment. The data also indicate that the creaming technique used in the rural process to extract the fat is a major source of loss in the process. In this traditional process, this operation is also critical with respect to the processing time. It has been estimated that this unit operation takes about 5 to 6 h in processing about 10 to 13 kg of shea kernel meal (3,20). The water flotation technique used seemed simple and suitable for the enzyme-assisted process. It was adapted from a modified Ghanaian traditional palm kernel process (15). It was observed that the extent of dilution of the meal in the enzyme-treated samples could not make the creaming effective, and hence the adaption of the flotation method. The creaming technique had a lower extraction rate

TABLE 2
Effect of Enzyme Treatment and Preextraction Hydrolysis on Extraction Yield

Treatment[a]	Yield (%)
Enzyme-aided hydrolysis	72 ± 3
Hydrolysis without enzyme	48 ± 4
No hydrolysis-1	46 ± 3
No hydrolysis-2	39 ± 3

[a]The treatment described as hydrolysis without enzyme was similar to the preextraction treatment in the text, except that no enzyme was added. In the no hydrolysis-1 case, meal samples were extracted without enzyme or incubation. The no hydrolysis-2 represents the typical traditional process; these latter samples were extracted by the method of Aye and Adomako (16).

as compared to the flotation technique (Table 2). On the other hand, with the flotation technique, the length of time for heating to evaporate the water depended on the amount of water collected with the emulsion. It was thus important to allow effective separation of the emulsion before collection, by keeping the suspension for a longer time: in this experiment, 1–2 h.

Microstructural studies in search for evidence on the enzymes' actions on the cells of the shea kernel meal are yet to be done. However, after the aging, the aqueous phase in the cases treated with enzymes showed deeper coloration (brownish) and more clarity than the controls. The intensity of the color corresponded well with the treatment time at enzyme concentrations from 0.5%, other factors being constant, suggesting the increased release of soluble substances into the aqueous phase. McGlone *et al.* (13) observed changes in the reducing sugar and soluble protein contents during enzyme treatment on the extraction of coconut oil. Such changes are, however, yet to be evaluated during the search for evidence on enzyme actions. The fat emulsions were also more fluid-like (softer) for the enzyme-treated samples, suggesting that most of the fat had been freed. These observations may suggest, as expected, biodegradation during the enzyme treatments.

Increasing the enzyme concentration and treatment time, both generally, increased fat yield. Rapid increases in yield occurred as the enzyme concentration increased from 0 to 1% (Fig. 2). Beyond this level, the rate of increase fell sharply. However, the substrate concentration (meal) was fixed at all enzyme concentrations, and therefore its limitation in the cases of higher enzyme concentration may limit the interpretation of this observation. In the case of treatment time, increasing the time from 1 to 6 h increased the yield by about 6% (from about 62 to 68%), whereas further increase to 20 h saw additional increase of only 1% (from 68 to 69%). From these observations and the observations in the multifactor experiments, it was evident that 1% enzyme concentration and 4-h treatment time were adequate to obtain optimum yield, and so they were used in other experiments as control treatments.

The fat yield was also affected by the meal dilution. The yield decreased as the meal became more diluted (watery), up to the 1:7 level, and then began to rise again. The optimum yield occurred in the least diluted samples (meal/water of 1:2) (Fig. 3). However, at this low water dilution, the treatment had to be done at a temperature slightly above the melting range of the fat, so as to keep the mixture in suspension during treatment. Within the range studied, the effect of temperature on yield was not significant (data not shown); therefore, 37°C was usually used. The reason for the trend is not known; however, McGlone *et al.* (13) and Sosulski *et al.* (12) observed comparable trends in the meal dilution effect, on the extraction of coconut oil and canola oil, respectively, with enzymes.

The enzymes used were reported to have their optimum activities in the acid range (Table 1); however, the yield of fat was higher when the meal–water mixture became less acidic. The fat yield, for instance, increased from 59 to 69% and to about 74% when the meal samples were mixed with buffer solutions (see Materials and Methods section) of pH 3.05, 4.62 and 5.92, respectively. In earlier extraction trials at the unmodified pH of the dissolved meal (about 5.3), yield was persistently high, closer to the higher values of the less acidic pH treatments. In subsequent experiments, therefore, pH adjustments were not done. This also suggests that in such aqueous extraction process pH adjustment may not be necessary.

Carrying out the enzyme treatment under continuous shaking did not cause significant increases in fat yield (data not

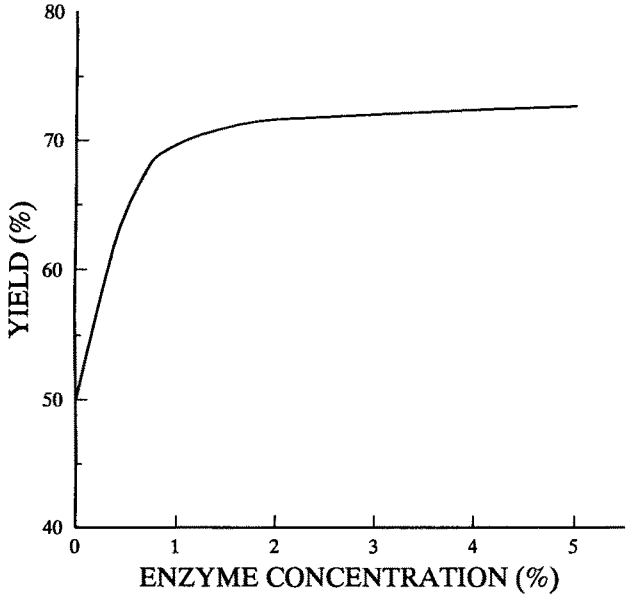

FIG. 2. Effect of enzyme concentration on fat yield.

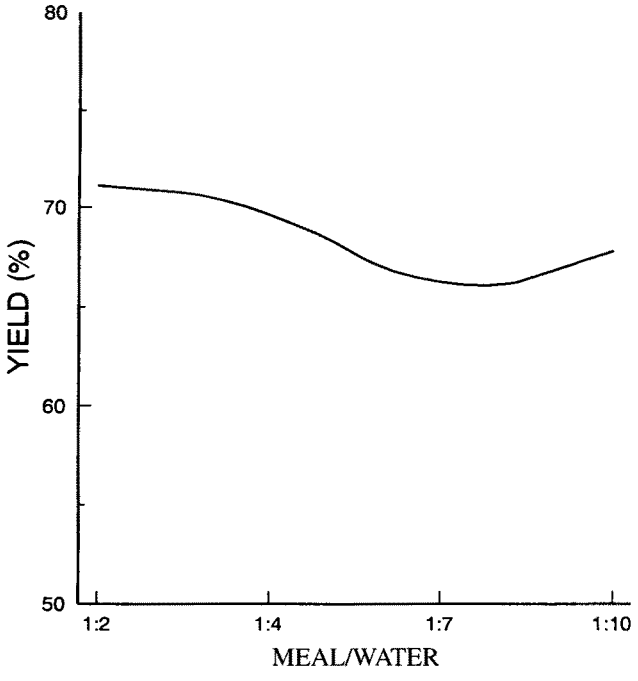

FIG. 3. Effect of meal dilution on fat yield.

presented). However, it was observed that, without shaking, the meal should be adequately diluted to keep it in suspension even when the fat solidifies on standing. Meal/water of about 1:4 was found adequate to ensure this.

Another major observation made was the difference in yield values when the enzyme-treated meals were extracted using different techniques (Table 3). Generally, yield values were high. As expected, the hexane extraction method gave a higher yield compared to the enzyme-assisted traditional method. However, the differences in the yield values were not so big as they are usually seen when the traditional processes are compared with the modern (conventional) processes. The improvement in yield on centrifugation, on the other hand, reveals the limitation in the flotation technique.

It could be observed that yield values apparently changed from experiment to experiment. One major contribution to this was variations in milling efficiencies. Yield tended to be higher in more efficiently milled meals. To limit the effects of these differences on the interpretation of the data, control samples (no enzyme treatment) were included in all determinations. All the meal samples in specific experiments were taken from the same bulk of sample.

The data on the fat analyses are presented in Table 4. Apparently the enzyme treatment did not have detrimental effects on the characteristics of the fat. Rather, the FFA content, AVs, PVs and UM content were apparently lower in the enzyme-assisted extracted samples, compared to samples from the typical traditional process and the Soxhlet-extracted samples. These may be beneficial effects. Considering all the data, it could be inferred, however, that the low FFA value was probably not due to the enzyme treatment but rather the washing effect of the water during the treatment and extraction stages. The FFAs, which are mostly water-soluble, might be extracted into the water. On the other hand, the reduced UM content seems to be due to the effects of the enzymes. How this occurs would be investigated in another experiment. The chemical constants of the crude fat observed here confirm the data in the preliminary investigations (2).

The results generally indicate that the enzyme-assisted extraction method is capable of increasing the fat extraction rate in rural shea kernel processing. Given the fact that the enzyme-assisted process still maintains the features of the traditional process which make the latter predominant in the shea nut-producing countries (2), its adaptation might not be difficult and its exploitation could significantly improve upon the rural shea nut industry. Perhaps, the cost of crude enzymes may limit the adaptation; however, there may be a way out. It may be possible to explore simpler and cheaper ways of producing the enzymes in the targeted areas, where, fortunately, the tropical conditions favor such an exercise throughout the year. It may also be possible to explore the direct use of the organisms which produce the enzymes, taking care of their lipase activities. In Ghana, there is a traditional inoculum called *Kudeme* used to biodegrade grated cassava to make it finer (21). This has been demonstrated to have microbial and enzymatic actions. The inoculum, which is made from cassava, is ground and mixed with the grated cassava and incubated for some time to exert its effects. There is also the *Pito* fiber belt (22), containing immobilized yeasts used in the fermentation processes of *Pito* (23), a traditional alcoholic beverage from sorghum. Developing similar techniques for oil processing would thus not be a completely new idea. This would hopefully be considered in the present studies.

TABLE 3
Effect of Extraction Technique on Fat Yield from Enzyme-Treated Shea Kernel Meal

Technique	Yield
Water flotation	76.29 ± 2.16
Centrifugation	79.81 ± 1.84
Hexane extraction	87.39 ± 2.01

TABLE 4
Chemical Characteristics of Laboratory-Extracted Shea Fat Samples

	Fat sample[a]			
Characteristics	1	2	3	4
FFA (%)	2.89	2.75	3.45	3.29
AV	5.75	5.48	6.86	6.54
PV (meq/kg)	11.18	15.55	17.68	10.60
IV	56.68	56.82	56.53	57.77
SV	180.33	180.52	179.63	180.34
UM (%)	5.15	6.70	7.66	7.35

[a]Sample labels correspond to the following treatments or samples: 1, enzyme-assisted extracted sample; 2, hydrolysis without enzyme; 3, no hydrolysis-1 (see Table 2); 4, Soxhlet-extracted sample. FFA, free fatty acid; AV, acid value; PV, peroxide value; IV, iodine value; SV, saponification value; UM, unsaponifiable matter content.

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