Application of Enzyme-Assisted Aqueous Fat Extraction to Cocoa Fat

Kwaku Tano-Debrah* and Yoshiyuki Ohta

Laboratory of Microbial Biochemistry, Faculty of Applied Biological Sciences, Hiroshima University, Higashi-Hiroshima-shi 724, Japan

ABSTRACT: An enzyme-assisted aqueous fat extraction method was used to extract fat from dry cocoa beans. The beans were dehulled and finely ground. Samples were mixed with water in predetermined ratios. A protease and an enzyme with high activities of both cellulase and hemicellulase were added at 1% of the sample weight, mixed, and incubated for about 6 h at 37°C. The treated samples were then extracted by a water flotation technique. The preextraction treatment with enzymes effectively increased fat yield by about 26%. The observation suggests that the enzyme-assisted aqueous fat extraction method could improve the efficiency of rural fats and oils technologies currently in use in many developing countries.
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KEY WORDS: Aqueous extraction, cocoa fat, enzyme-assisted, rural process.

In two previous papers (1,2), we reported how the incorporation of a preextraction enzyme treatment of shea kernel meal in an aqueous shea fat extraction process increased the yield of fat. The technique has now been applied to the extraction of cocoa fat from dried cocoa beans, to explore its applicability in rural cocoa fat extraction processes.

Many rural oil extraction processes (2–9) are based on two general principles: (i) disintegration of the oil-bearing material by either mechanical or mechanical and heat applications, and (ii) water extraction of the fat and oil involving the formation of an emulsion with water, separation of the emulsion, and boiling to remove the water from the emulsion. These two principles are used in the rural extraction of palm oil, palm kernel oil, coconut oil, shea fat, groundnut oil, cocoa fat, and many other oil-bearing materials (3–10). These similarities suggest a potential applicability of the enzyme-assisted process to the extraction of many other vegetable fats and oils.

Aqueous extraction of cocoa butter in Ghana is not a major rural technology, as is the extraction of palm oil, palm kernel, and the other oils mentioned above. The national importance of raw cocoa beans, the commercial value, and probably lack of efficient extraction processes may be among the major impediments. However, current world technological developments seem to suggest that, in the future, local use of raw cocoa beans should be promoted, and perhaps, there would be need to develop a rural cocoa butter extraction industry. This study was therefore undertaken to search for an effective way to improve rural cocoa butter extraction.

*To whom correspondence should be addressed at the Laboratory of Microbial Biochemistry, Faculty of Applied Biological Sciences, Hiroshima University, Kagamiyama 1-4-4, Higashi-Hiroshima-shi 724, Japan.

MATERIALS AND METHODS

Dry cocoa beans were obtained from a cocoa farmer in Ghana. It was a commercial grade. The chemical characteristics were determined as follows.

Chemical characteristics. With the exception of crude fiber, all characteristics were determined as previously described (1). The crude fiber content was determined by the AOAC Fritted Glass Crucible method (11).

Enzyme treatment. The enzymes used were crude preparations, obtained from Shin Nihon Chemicals Co. (Anjoh City, Japan). The types and producers' information on the enzymes are provided in Table 1. Weighed samples of the shelled bean meal were mixed with water in ratios of about 1 to 3 (wt/vol). The mixtures were boiled for 5 min and then cooled. The enzymes were added to the cooled samples singly and in combination, at 1% rate of sample's weight, and thoroughly mixed. The samples were then incubated in a water bath-shaker at 37°C and 80 rpm for 6 h. In a separate experiment, the effect of meal/water ratio on extraction yield was studied. Control experiments were done for evaluation purposes as described before (1,2).

Fat extraction. The enzyme-treated meal samples were extracted with either hexane or water. The extractions were done as previously described (2). In samples extracted with hexane, the quantities of meal were uniformly fixed at 20 g. For water-extracted samples, weights ranged from 50 to 100 g and were held uniform within specific experiments. The yield of fat was expressed as the percentage of the Soxhlet-extracted values.

All experiments were repeated at least once, and mean values of data are reported.

RESULTS AND DISCUSSION

Table 2 shows the chemical characteristics of the cocoa beans. Analysis was done separately for shelled and unshelled beans.

TABLE 1
Producers' Information of the Crude Enzymes

Enzyme	Commercial name	Source[a]	Specified contaminant	Activity (U/g)
Protease	Sumizyme LP	*Aspergillus oryzae*	—	50,000
Cellulase/hemicellulase	Sumizyme C	*Trichoderma reesei*	—	1,500
Pectinase	Sumizyme AP2	*A. niger*	Cellulase, hemicellulase	2,000

[a]All enzymes were supplied by Shin Nihon Chemicals Co. (Anjoh City, Japan).

TABLE 2
Chemical Characteristics of Cocoa Beans

Characteristics	Composition (g/100 g)	
	Shelled	Unshelled
Moisture	3.57	2.97
Crude fat	53.72	46.23
Total nitrogen	2.15	2.26
Crude protein	12.25	12.87
Ash (total)	2.83	nd[a]
Fiber (crude)	2.01	14.96
Carbohydrates:		
Alcohol-soluble sugars	1.12	nd
Starch	5.9	nd
Cellulose	8.9	nd

[a]nd = Not determined.

The proportion of shells was estimated to be about 13.5%. The estimation was done by carefully separating the shells of previously weighed, randomly selected beans and weighing. Five batches of 100 beans each were analyzed for a mean value.

There was a high content of both fat and protein. Starch and cellulose were also quite high in the shelled bean. The proximate composition suggested a combination of enzymes for the preextraction treatment. Based on experience from the shea kernel process (1) and some of the reported findings on enzyme-assisted fat extraction processes (12–15), a protease, an enzyme with both cellulase and hemicellulase activities, and a pectinase were tried in different combinations. The results of these trial extractions are presented in Table 3. Combination of the protease and cellulase/hemicellulase enzymes, each at 1% rate of samples weight, gave the best yield. The two enzymes were thus combined in subsequent experiments. Table 4 shows the yield values when the enzyme-treated meal was extracted with water. The relative increase in yield was about 26%, compared to control 1. Compared to the extraction of shea fat (2), the effective increase in yield after enzyme treatment was significantly less under the same treatment conditions (data of the shea fat process not shown here). One possible reason is the differences in commodity characteristics. The dry cocoa beans were more crispy and could be milled into a finer meal than the shea kernels. The fineness of the cocoa bean meal caused high yields in the control treatments. Treatment control 1 simulates the rural process; however, the yields reported here seemed quite a bit higher than usual. The improved techniques employed under laboratory conditions (2) may also have contributed to this. In the real situation, the increase of about 26% (relative to control 1) could mean over 50% if the estimated mean yield of only up to 40% for traditional processes (5,6) is considered. The lower yield may have resulted from the trapping of oil in a fine-textured paste formed by the end of boiling. Attempt to dry the paste sometimes resulted in darkening the oil. This problem seemed to have originated from the high protein content and fine particle size of the meal extracted, causing the emulsion to contain a high load of colloidal materials.

Table 5 shows the effect of meal/water ratio on extraction yield of fat. The pattern was as observed in the extraction of shea fat. Thus, fat yield generally decreased with increasing dilution of the meal.

The results indicate that it is possible to apply the enzyme-assisted aqueous extraction technique to the extraction of cocoa fat. It suggests that the enzyme treatment could significantly increase the extraction efficiency of a rural cocoa fat extraction process. The potential applicability of the enzyme-

TABLE 3
Effect of Enzyme Type and Combination on Extraction Yield of Cocoa Fat[a]

Enzyme type	Extraction yield (%)
Control 1	58.7 ± 1.5
Control 2	63.5 ± 1.3
Sumizyme LP (SuLP)	69.2 ± 2.6
Sumizyme C (SuC)	68.3 ± 1.1
SuLP and SuC	72.7 ± 2.8
SuLP, SuC, and Sumizyme AP2	71.0 ± 3.5

[a]Control 1, meal samples were extracted without the hydrolytic treatment; Control 2, hydrolysis of meal samples were done but without the enzyme; the treated samples were extracted with hexane. Each enzyme was added at a 1% rate of sample weight. See Table 1 for company source.

TABLE 4
Effect of Enzyme Treatment on Extraction Yield of Cocoa Fat by the Aqueous Extraction Method[a]

Treatment	Extraction yield (%)
Control 1	49.2 ± 2.7
Control 2	54.7 ± 1.7
Enzyme treated (0.5%)	59.3 ± 1.2
Enzyme treated (1%)	63.1 ± 2.7

[a]Meal samples were treated with a mixture of Sumizyme LP and Sumizyme C, each enzyme at 1% of sample weight. The controls were as explained in Table 3, company source as in Table 1.

TABLE 5
Effect of Meal/Water Ratio on Extraction Yield of Cocoa Fat[a]

Meal/water	Extraction yield (%)
1:1	76.1 ± 1.8
1:3	71.5 ± 0.7
1:5	69.9 ± 0.5
1:7	65.1 ± 1.3
1:10	66.5 ± 1.1

[a]Meal samples were treated with Sumizyme LP and Sumizyme C, each added at 1% rate of sample weight. Extraction was done with hexane. See Table 1 for company source.

assisted aqueous fat extraction method in many other rural oil and fat extraction technologies is also expressed.

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