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Enzymatic Aqueous Processing of Coconuts

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Abstract: Finely ground desiccated coconut meat was incubated in an aqueous medium at 50-55ºC with a commercially available enzyme system. After incubation, the coconut slurry was centrifuged into four parts: a clear oil phase, an emulsion layer, an aqueous phase, and a solid phase consisting of the extracted coconut meal. The enzymatic treatment released about 84% of the oil present in the starting coconut meat, producing a brilliant, light yellow oil that had the characteristic pleasant coconut aroma. Some of the water in the aqueous phase was removed to produce a more concentrated protein solution with intense coconut flavour. The wet solid phase was freeze dried, and then extracted with aqueous alkali, dissolving nearly 88% of the protein from the solid. Centrifugation separated the extract solution from the insoluble residue. The acidification of the extract to the isoelectric region precipitated 93.5% of the dissolved protein. The freeze-dried precipitated coconut protein was a fine, odourless white powder with ~90% (N×6.25) protein content.

Keywords: enzymatic aqueous extraction; polysaccharide; endohemicellulase; immobilized enzyme; freeze drying; reverse osmosis; isoelectric precipitation.

1. Introduction

The conventional processes to extract edible oil from oilseeds or fruit pulps involve mechanical expression and/or solvent extraction. While n-hexane is broadly accepted as the most efficient solvent for oil extraction, its flammability, explosiveness, mild toxicity, and environmental impacts are an ongoing concern for the industry. The extraction process leaves low levels of solvent residues in the extracted oil and the meal, which are safe, yet undesirable. Recently, a great deal of research has focused on the development of alternatives to hexane as the extracting solvent. The least expensive, safest and therefore most desirable solvent is water. Aqueous processing of oil-bearing materials eliminates the potential hazards of explosion and fire, eliminates the negative environmental impacts due to emissions of organic solvents, and does not leave toxic or undesirable solvent residues in the resulting food products. Reduced equipment costs and energy consumption are also potentially possible, since oil and protein may be recovered simultaneously (Cater et al., 1974).

As oil and water are immiscible, the separation of pure oil and water is expected to be relatively simple. Unfortunately, in most systems aqueous extraction with pure water is ineffective, as water cannot efficiently release the oil, which is tied up in the plant cell structure. Any oil released often forms emulsions, which are stabilized by proteins and complex carbohydrates present in the cell.

It was reported that an enzyme system could be used to degrade the insoluble cell wall components and thus release oil in aqueous extraction (Rosenthal et al., 1996). Since the structural composition of the cell wall is

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specific to each oil source, the selection of a suitable enzyme system is critical for efficient oil extraction. Despite the fact that a single type of enzyme may achieve a significant oil recovery in some cases, a combination of several enzymes is often required to degrade the wide range of structural components in the cell matrix.

Enzymatic aqueous extraction typically produces a three-phase system consisting of oil, aqueous, and solid phases. Most often a fourth phase consisting of a water-oil emulsion would also form. Depending on the extraction pH and the cell matrix, a significant fraction of the proteins may dissolve into the aqueous and emulsion phases. Recovery of the proteins from these two phases is possible. In addition, the recovery and purification of the proteins remaining in the solid phase can be also desirable, especially if these have a high nutritive value.

Coconuts are an excellent source of lauric oil. With the frequent fluctuations in the supply and price of coconut oil, the development of an aqueous process that would be less expensive and would produce additional value-added products is highly desirable.

Two processes are currently employed to extract oil from coconuts. The dry process is conventionally used for releasing oil from copra, the dried flesh of coconuts. It involves mechanical expressing at high temperature. The residual oil in the meal may be further recovered by solvent extraction. The process results in a defatted copra meal that is a nutrient-poor animal feed and a crude oil of poor quality, which requires extensive refining.

Alternatively, the coconuts can be harvested earlier, and the oil in the fresh coconut kernels is extracted using a wet process. This involves milling, cold pressing, separation of the resulting cream from the coconut milk, and emulsion breakdown. The extracted oil doesn't require further refining and the co-products are edible. However, the oil yield tends to be lower than that from the dry process.

In order to maintain the quality of the extracted oil, to simplify the separation of the oil phase from the aqueous phase, and to increase the oil yield, enzymes can be used in the wet process. Different enzyme preparations have been tested for the recovery of coconut oil in previously reported work. Sant'Anna et al. (2003) evaluated the coconut oil release using a mixture of protease and a multi-component preparation containing a wide range of carbohydrases. An enzyme system having protease, cellulase, and hemicellulase activities was used to release oil from copra by Tano-Debrah and Ohta (1997). Che Man et al. (1996) used a mixture of protease, cellulase, α-amylase, and polygalacturonase to extract oil from grated coconut meat. An enzyme mixture of galactomannase and a soy polysaccharide-degrading enzyme complex was tested by Christensen and Olsen (1990) for releasing oil from desiccated coconut meat.

In order to effectively release oil enclosed in the cell, enzymes specific to the breakdown of the individual types of polysaccharides in the wall structure of the cell must be identified. Balasubramaniam (1976) indicated that the polysaccharides of fresh mature coconut kernels were galactomannans (61%), mannans (26%), and cellulose (13%). Saittagaroon et al. (1982) reported that the major polysaccharides in copra meal were mannans. Therefore an enzyme preparation having efficient activity to degrade these polysaccharides was desirable. To ensure the quality of the released oil, the selected enzyme system should be essentially free of lipase, which brings about an increased release of fatty acids from oil during processing. The enzyme preparation selected for this study was Gamanase™ 1.0L, a commercially available endohemicellulase that accelerates hydrolysis of β-1,4 glycoside linkages in mannans, galactomannans, and glucomannans (Boyce, 1986).

After oil has been released by enzymatic treatment, the extracted meal can be recovered as a food-grade co-product. No reports on the production of value-added products,

such as coconut proteins, were found in the literature. The aim of this work was the development of a safe and economical process based on aqueous recovery of the proteins as a valuable product, while improving oil quality.

2. Materials and methods

2.1. Materials

In this study desiccated coconut meat was used to investigate the recovery of oil and protein by aqueous extraction. The coconut meat was purchased at a local store. It was a white granular material and contained 2.4% moisture, 7.8% protein, and 65.2% oil.

Enzymes were obtained from Novo Nordisk BioChem North America, Inc. They were a hemicellulase preparation, a pectinase preparation, a cullulase preparation, and an enzyme complex having multiple activities. Combinations of these enzyme preparations, recommended by Novo Nordisk BioChem for the treatments of oil-bearing seeds, were tested in our laboratory for their efficiency on coconut oil extraction. Gamanase™ 1.0L was used in the development work. It is an endo-hemicellulase, available as a viscous liquid with density of 1.05 g/mL. The conditions for its optimum hydrolytic activity are 60-70ºC and pH 3-6.

2.2. Oil extraction

The desiccated coconut meat was wet ground with water at a water-to-solid ratio of 10:1 (v/w) using a Szegö mill (General Comminution Inc., Toronto, Canada). Water was added at 35°C during the grinding, while the coconut meat was added at ambient temperature. After the grinding the temperature of the slurry was slowly increased to 50°C with gentle agitation. The pH of the slurry was adjusted to pH 4.5 by adding 3M H_3PO_4. The enzyme, Gamanase™ 1.0L, was then added at 2% (w/w) of the coconut meat. The temperature of the slurry was maintained between 50-55ºC for 5 hours with gentle agitation. Then the slurry sat for approximately 15 hours at 50ºC without agitation. After the 20h incubation the slurry was centrifuged (9,000×g, 25 minutes) at room temperature. The slurry was separated into four parts: (1) a clear oil layer, (2) an emulsion layer, (3) an aqueous solution, and (4) a wet solid phase (i.e., wet coconut meal). Figure 1 shows the flow diagram for the recovery of the oil by the enzymatic aqueous extraction.

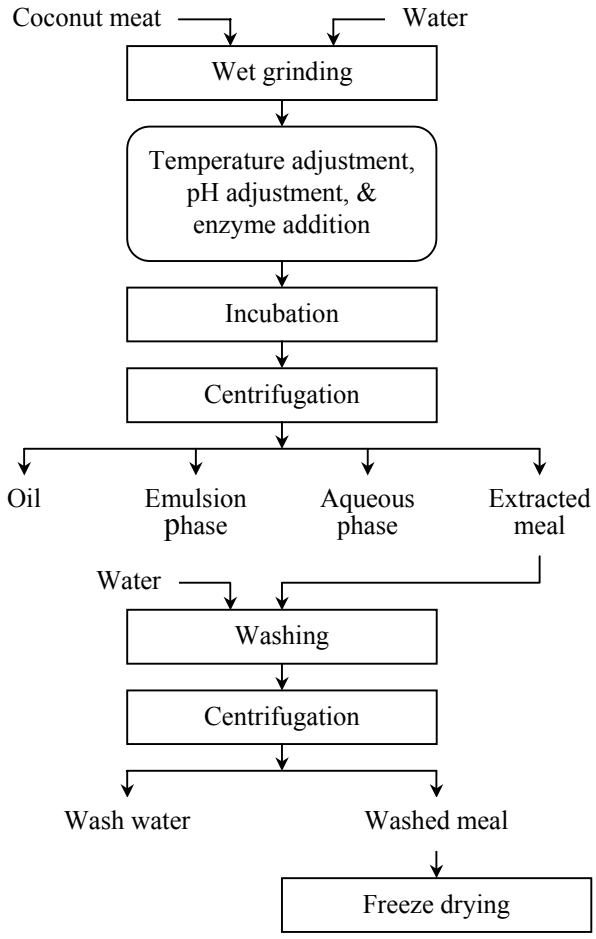

Figure 1. Flow diagram for coconut oil extraction

After sampling, the aqueous solution was concentrated by removing about 2/3 of the water using freeze drying. The protein content in both of the aqueous solution and the concentrated aqueous solution were determined. The wet coconut meal was washed with water, centrifuged, and then freeze dried. The oil and protein content in the freeze-dried solid coconut meal were determined.

2.3. Recovery of coconut protein

The freeze-dried coconut meal was suspended in water at a water-to-solid ratio of 15:1 (v/w). The slurry was then gently stirred for 60 minutes at room temperature after its pH was raised to pH 11.5 by adding 10% NaOH solution. During the alkaline extraction dilute NaOH solution was added to keep the pH constant at 11.5. Centrifugation separated the undissolved residue from the extract, which contained the dissolved protein. The protein content in the extract was determined. The extract was acidified to pH 4.5 by the slow addition of 3M H_3PO_4 to precipitate the protein. The resulting protein suspension was stirred for 30 minutes to complete the precipitation before it was centrifuged. The precipitated protein was collected, freeze dried, and analyzed. The protein content in the clear liquid separated from the precipitate was also determined. The flow diagram for the recovery of the coconut protein is presented in Figure 2.

2.4. Protein analysis

Crude protein content (N×6.25) was determined by the Kjeldahl method, according to AACC Method 46-12 (AACC, 1976). A Büchi 425 digestion unit and a Büchi 320 distillation unit (Büchi Labortechnik AG, Flawil, Switzerland) were used.

2.5. Oil content

A Soxhlet extraction apparatus was used for the oil extraction from the solid with n-hexane. The oil content was determined gravimetrically after evaporation of the n-hexane.

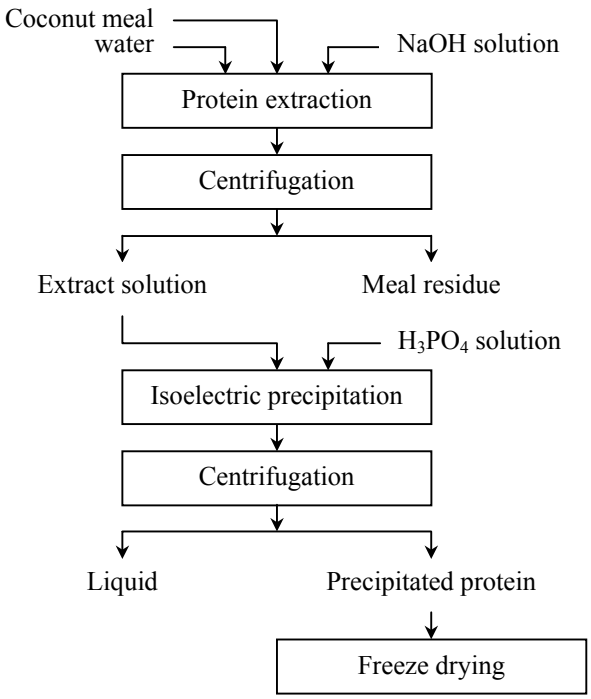

Figure 2. Flow diagram for coconut protein recovery

3. Results and discussion

3.1. Oil extraction with Gamanase™ 1.0L

The treatment with the Gamanase™ 1.0L enzyme preparation released about 84% of the oil originally contained in the coconut meat, leaving the rest in the undissolved solid phase, or meal. The extracted oil was transparent, very light brilliant yellow in colour. It had the characteristic pleasant coconut aroma, and had no off-flavours or unpleasant aftertaste. The free fatty acid content was low and further refining was not required.

The extraction efficiency compared favourably with results from other enzymatic treatments reported earlier. An enzyme system with protease, cellulase, and hemicellulase activities recovered 65.5% of the oil from copra (Tano-Debrah and Ohta, 1997). A mix-

ture of proteases, cellulase, α-amylase, and polygalacturonase used by Che Man et al. (1996) released approximately 73% of the oil from grated coconut meat. The yield obtained in our study was consistent with the results of Christensen and Olsen (1990). They reported an oil yield of 81.8% in the bench-top test when they used an enzyme mixture of galactomannase and SP-249 (a soy polysaccharide-degrading enzyme complex produced by Novo Industri A/S) at 2.6% (w/w) and 0.6% (w/w), respectively, of desiccated coconut meat. A yield of 86% was found in their pilot plant trial with the same concentrations of the enzymes. They also obtained a high yield of 100% when they doubled the amounts of both enzymes. The use of galactomannase not only contributed to the efficient hydrolysis of the cell wall structure, but it also simplified the separation of the resulting water-oil emulsion (Christensen, 1991).

Although in this study the use of the specific hydrolytic enzyme preparation, Gamanase™ 1.0L, facilitated the separation of the oil from the aqueous phase, a thin but distinctive layer of emulsion between these two phases remained. As the focus of this work was on the recoveries of protein and flavour, we did not attempt to completely recover the oil from the emulsion, as there are well-known commercial processes available to accomplish this task. For example, heating followed by centrifugation, or the addition of excess oil into the emulsion, will typically result in clear separation of the emulsion into an oil and an aqueous phase.

During the aqueous enzymatic process, 44% of proteins present in the starting material, the desiccated coconut meat, were dissolved into the aqueous phase. The aqueous phase contained 0.4% protein and the typical pleasant coconut aroma was retained in the solution. In order to produce a more concentrated protein solution, ultrafiltration could be used. However, this operation would deprive the solution of its coconut aroma. The characteristic aroma and flavour of coconut are primarily attributed to the cyclic esters known as γ- and δ-lactones (Allen, 1965; Pai et al., 1979). While the coconut protein would be retained in the retentate stream during ultrafiltration, the lactones would easily pass through the membrane and be left in the permeate stream. Reverse osmosis is the appropriate choice to concentrate this aqueous solution without the loss of its aroma. Since a laboratory reverse osmosis unit was not available during this study, freeze drying was used to concentrate this solution to 1.5% protein. As expected, the concentrated liquid had a more intense, pleasant aroma.

The aqueous solution retains the enzyme, which is then likely denatured. As this is the most expensive component of the process, its recovery and reuse should be investigated. Immobilization of the enzyme system, or the use of commercially manufactured immobilized enzymes should be investigated. A simpler approach, though less effective, would be to reuse some 80% of the extract solution, resulting in a 4-fold reuse of the enzyme in the process. This may also reduce the extractability of the protein into the aqueous phase, resulting in a higher isolate yield.

Table 1. Protein distribution in separate phases after oil extraction

Coconut meat	Aqueous phase	Oil phase	Washed solid phase	Wash water [(a)]	Emulsion phase
100%	44.0%	N/D [(b)]	31.8%	4.2%	20% [(c)]

(a) Water used to wash the solid phase (coconut meal)
(b) Protein was not detected in the oil phase
(c) By difference

When Kwon and Rhee (1996) investigated the nitrogen solubility profile of the hexane-defatted coconut meal, they reported that the minimum solubility was ~5% in water and ~30% in 2% NaCl solution at approximately pH 4.5. Accordingly, we had expected low protein solubility during the aqueous extraction of oil at this point. We found that the nitrogen solubility was remarkably high. While the enzymatic extraction at pH 4.5 released 84% of the oil in the meat, it also dissolved 68% of the nitrogen. Evidently the hydrolytic cleavage of the cell wall structure facilitated by Gamanase™ 1.0L enhanced the dissolution of the proteins as well. The protein distribution among the different phases after the extraction at pH 4.5 is presented in Table 1. The proteins dissolved in the aqueous phase and in the wash water and remaining in the wet solid phase accounted for 80% of the proteins in the starting desiccated coconut meat. Most of the remaining 20% were assumed to be in the emulsion phase.

The enzymatically extracted, freeze-dried coconut meal contained 14.8% protein and 64.5% oil (both on moisture-free basis). It was odourless and white in colour. It contained 31.8% of the original protein, 16.5% of the original oil, and 17% of the original mass in the starting material, desiccated coconut meat.

3.2. Protein extractability and recovery

Kwon and Rhee (1996) reported that some 80% of the nitrogen in hexane-defatted coconut flour dissolved in aqueous NaOH at pH 11. When the coconut meat containing oil was studied (Kwon et al., 1996) the maximum nitrogen extractability was found to be slightly lower (~75%) at pH 12. Accordingly, we extracted the protein from the freeze-dried coconut meal at pH 11.5, and found that 87.9% of the protein in the meal was dissolved. The acidification of the extract solution to pH 4.5 precipitated 93.5% of the dissolved protein. Since the enzymatic treatment was carried out at pH 4.5, the majority, if not all, of the protein fractions soluble at this pH have already been removed from the meal. As a result, a high yield of the precipitated protein was expected. When freeze-dried, the precipitated protein was a fine white powder, odourless, and bland in taste. Its protein content was ~90% (N×6.25, on moisture-free and oil-free basis). This product contained 26% of the protein present in the original desiccated coconut meat.

4. Conclusions

We successfully developed a process that gives three potentially valuable products: (1) coconut oil, (2) an aromatic protein-containing liquid, which may be used as a beverage base, and (3) a protein isolate. A high oil yield was achieved in this aqueous laboratory process for oil extraction from coconut meat, due to the effective enzymatic release of oil from coconut matrix. Further refining of the oil would not be required. The concentrated aqueous extract containing protein and the characteristic coconut aroma has a potential application in nutritive specialty drinks. The process also led to the efficient recovery of the solubilized protein as an isolate of high purity and nutritional quality. All of the processing steps are readily scaleable, and therefore the commercial viability of the process should be investigated.

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