Aqueous Enzymatic Extraction of Coconut Oil

Y.B. Che Man[a,*], Suhardiyono[a], A.B. Asbi[a], M.N. Azudin[b], and L.S. Wei[c]

[a]Department of Food Technology, Universiti Pertanian Malaysia, 43400 UPM, Serdang, Selangor, Malaysia, [b]Australian Wheat Board, Melbourne, Victoria 3000, Australia, and [c]Department of Food Science, University of Illinois, Urbana, Illinois 61801

ABSTRACT: Aqueous extraction of coconut oil with various enzymes was investigated. Several enzyme preparations (cellulase, polygalacturonase, protease, and α-amylase) were used at different concentrations, pH, and temperature values to enhance oil extraction. After the oil had been released by the enzyme reaction, it was separated by centrifugation. The results showed that an enzyme mixture at 1% (w/w) each of cellulase, α-amylase, polygalacturonase, and protease at pH 7.0 and an extraction temperature of 60°C represented the most effective extraction conditions with an oil yield of 73.8%. Quality characteristics of the oil were as follows: moisture content, 0.11%; free fatty acid, 0.051%; peroxide value, 0.016 meq oxygen/kg; anisidine value, 0.026; iodine value, 8.3; saponification value, 260; and color, 0.6 (Y + 5R). This technique for recovering oil from fresh coconut meat with enzymes is a significant improvement in both oil yield and quality over the traditional wet process.
JAOCS 73, 683–686 (1996).

KEY WORDS: Aqueous coconut oil extraction, oil yield, quality characteristics.

There are several technologies for removing oil from coconut meat and copra, such as the wet process, dry process, and solvent extraction. Even though more efficient and modern processess for coconut oil extraction are available, processing of fresh coconut meat into oil by the traditional wet process is still practiced at the village level in many coconut-producing countries. The traditional wet process involves grating coconut meat and separating the oil from the extracted milk by cooking. Generally, only 30–40% of the oil is recovered by the traditional wet process, and the quality is poor due to the high moisture content and short shelf life (1). The main problem of the wet process for coconut oil extraction is the inefficient separation of oil from coconut milk (1). In coconut milk, each oil globule is surrounded by a film of interfacially active protein and other cellular materials (2). To extract the oil, the cell walls are normally broken down by means of mechanical action, such as grating and pressing (3). However, plant cell walls can also be hydrolyzed and degraded by the action of various enzymes, thus releasing the oil (4–7). A study of coconut oil extraction by McGlone *et al.* (4), based on the enzymatic action of polygalacturonase, α-amylase, and protease on diluted coconut paste, obtained 80% yield of good-quality coconut oil, even without further purification, compared with the Official Mexican Standards. In this study, the effects of aqueous enzymatic extraction with cellulase, α-amylase, polygalacturonase, and protease at different concentrations, pH values, and temperatures on extraction yield and quality of the coconut oil were investigated.

*To whom correspondence should be addressed.

MATERIALS AND METHODS

Materials. Grated coconut meat was obtained from a local supplier at Taman Sri Serdang (Selangor, Malaysia). Chemicals of analytical grade were obtained from BDH Chemical Ltd. (Poole, England). The enzymes Celluclast (a cellulase preparation), Viscozyme (a polygalacturonase preparation), Alcalase (a protease preparation), and Termamyl type tech. (an α-amylase preparation) were donated by NOVO Industri A/S (Bagsvaerd, Denmark).

Extraction procedure. The extraction of oil was carried out according to Figure 1. Grated coconut meat (150 g) was mixed with 150 mL water. The mixture was kneaded manually for 5 min, squeezed and strained through a layer of cheesecloth, and the milk obtained was placed in a beaker. The coconut milk was heated to 90°C for 30 min and left to cool. The residue was mixed with 600 mL water. The mixture was kneaded for 1 min, aliquots were adjusted to desired pH and placed in a waterbath, which was heated to the set temperature value. The enzyme preparation [1.5 mL or 0.1% (w/w) of final concentration] of cellulase, α-amylase, polygalacturonase, and protease, either singly or in combination, was added to the mixture of 40, 50, or 60°C and pH values 4, 5, 6, 7, and 8. The mixtures were incubated for 30 min. After incubation, the mixture was strained through a layer of cheesecloth. The filtrate was then mixed with the coconut milk previously extracted and allowed to settle for 1 h to facilitate separation of the cream. The upper creamy layer was centrifuged at 12,300 × *g* and 12°C for 20 min. After centrifugation, the water layer was drained off, and the collected cream was allowed to melt. After melting, the cream was centrifuged again, resulting in a frozen oil layer and an aqueous layer. The oil layer was allowed to melt and was then strained through a fine cheesecloth and placed in a sealed bottle for analysis.

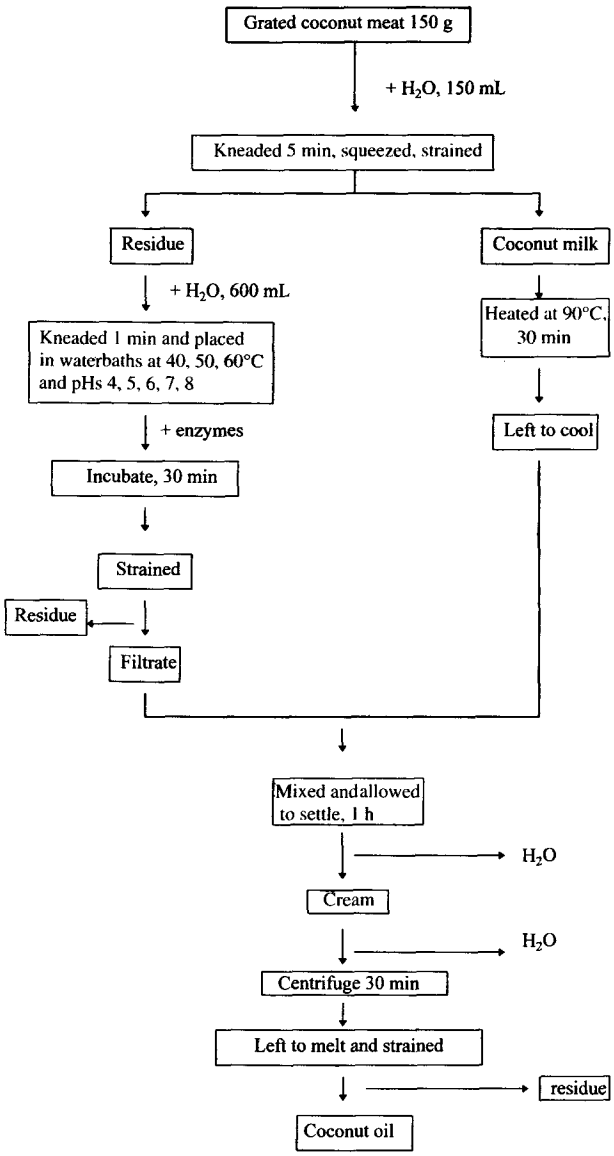

FIG. 1. Block diagram of coconut oil extraction with enzymes.

Extraction yield. The extraction yield was calculated based on the initial oil content of coconut meat, as determined by the Soxhlet method of AOAC (8), and the direct weight measurement of the oil obtained from the aqueous enzymatic extraction after centrifugation.

Analysis of oil quality. Moisture and free fatty acid (FFA) content were measured according to AOAC methods (8). Peroxide, iodine, and saponification values were measured according to British Standard No. 684 (9). The anisidine value was measured according to PORIM Test Methods (10). Color was measured by Lovibond Tintometer (model E) according to British Standard No. 684 (9).

Statistical analysis. The data were analyzed by two-factor analysis of variance techniques. Means that were significantly different at 5% level of probability ($P < 0.05$) were further separated by Duncan's multiple range test (11).

RESULTS AND DISCUSSION

Effect of enzymes on oil yield. The effect of various enzymes at the 0.1% (w/w) level at ambient temperature (*ca.* 25°C) and pH 6.5, both as single and mixed enzymes, on extraction yield of coconut oil is shown in Table 1. By mixing the grated coconut meat with water, squeezing and centrifuging without enzyme (control sample), 19.3% of oil was extracted. Treatment of the mixture of grated coconut meat residue with cellulase presumably caused the cell walls to become more permeable to the flow of oil due to hydrolysis of cell wall material that housed the oil globule. The oil yield rose to 28.1%. A similar result was obtained with α-amylase. Treatment with cellulase or α-amylase increased the oil yield by about 46% compared with the control (without enzyme). When polygalacturonase or protease was used, higher yields of 32.2 or 36.1%, respectively, were obtained. This represents an increase in oil recovery of 67 and 87%, respectively. Combinations of different enzymes at 0.1% (w/w) level resulted in further increases in the extraction yield (Table 1). Enzyme treatment with a mixture of cellulase and α-amylase yielded 36.3% oil. A combination of cellulase, α-amylase, and polygalacturonase yielded 37.1%. When cellulase, α-amylase, polygalacturonase, and protease were used, 41.7% was obtained. This represent an increase of about 89, 93, and 116%, respectively, over the control.

Based on the best oil recovery in Table 1, the effect of three levels of enzyme concentrations, i.e., 0.1, 0.5, and 1% (w/w), was studied. The results are presented in Table 2. Maximum oil recovery of 69.0% was obtained with a 1% (w/w)

TABLE 1
Effect of Various Enzymes on Yield of Oil Extracted at Ambient Temperature (*ca.* 25°C) and pH 6.5

Enzyme	Oil yield (%)[a]
Control (without enzyme)	19.26 ± 0.35
Cellulase	28.12 ± 0.06
α-Amylase	28.17 ± 0.06
Polygalacturonase	32.16 ± 0.14
Protease	36.09 ± 0.51
Cellulase + α-amylase[b]	36.32 ± 0.13
Cellulase + α-amylase + polygalacturonase[b]	37.08 ± 0.14
Cellulase + α-amylase + polygalacturonase + protease[b]	41.67 ± 0.44

[a]Means of two readings ± standard deviation. [b]Concentration of each enzyme, 0.1% (w/w).

TABLE 2
Effect of Enzyme Concentration on Yield of Oil Extracted at Ambient Temperature (*ca.* 25°C) and pH 6.5

Enzyme (%)[a]	Oil recovery (%)[b]
0.1	41.67 ± 0.44
0.5	49.67 ± 0.57
1	69.03 ± 0.40

[a]Concentration of each enzyme. [b]Means of two readings ± SD.

TABLE 3
Effect of pH and Temperature on Oil Recovery with 1% (w/w) Enzyme Mixture

pH	Oil recovery (%)[a]		
	40°C	50°C	60°C
4	53.26^{aA}	60.54^{abB}	68.20^{bB}
5	64.07^{bB}	67.87^{bBC}	71.14^{bcBC}
6	67.02^{bcBC}	70.87^{bcBC}	61.72^{bcBC}
7	72.95^{cC}	73.38^{bcC}	73.83^{cC}
8	71.53^{bcBC}	72.35^{bcBC}	72.60^{bcBC}

[a]Means of two readings. [a–c]Means in a row followed by different letters are different ($P < 0.05$). [A–C]Means in a column followed by different letters are different ($P < 0.05$).

concentration of each enzyme in the mixture of cellulase, α-amylase, polygalacturonase, and protease.

The effects of pH and temperature of the aqueous medium on oil recovery are presented in Table 3. Optimum oil recovery with mixed enzymes was 73.8 at pH 7.0 and 60°C. However, there was no significant difference in oil recovery at 40, 50, and 60°C, which were 72.9, 73.4, and 73.8%, respectively. At each treatment temperature, however, there were significant increases in oil yield with increasing pH up to pH 7. The yield decreased again at pH 8.

Therefore, oil recovery by aqueous enzymatic extraction with the mixture of cellulase, α-amylase, polygalacturonase, and protease at 1% (w/w) concentrations in this study was higher than that obtained by the traditional method (30–40%) or the method developed by the Royal Tropical Institute of Amsterdam (50%) (12,13). It was also higher than the yield obtained after acetic acid treatment (60.2%) (14). However, McGlone *et al.* (4) obtained an extraction of 80% with a mixture of 0.1–1% protease, α-amylase, and polygalacturonase and an incubation time of 30 min at 40°C. McGlone *et al.* (4) used an optimized dilution factor of 1:4 (coconut meat/water). The yield in the present study was slightly lower due to the difference in the enzyme mixture used and also to the dilution ratio.

Oil quality. The effect of enzyme treatment on the quality of extracted oils is presented in Tables 4, 5, and 6. Moisture contents of the extracted oils at 40, 50, and 60°C were constant at 0.11%. FFA contents ranged from 0.051 to 0.055%, peroxide values from 0.016 to 0.018 meq oxygen/kg, anisidine values from 0.027 to 0.028 (1,000 × abs), iodine values from 8.1 to 8.4, saponification values from 260 to 262, and the color of the extracted oil was unchanged at 0.6 (Y + 5R). There were no significant differences among the treatments in all quality parameters measured. The quality of the oil extracted was excellent, and it required no further refining. The oil meets the quality of the proposed International Standard by Asian and Pacific Coconut Community, as shown in Table 7 (13).

In terms of oil yield and oil quality, the technique for recovering oil from fresh grated coconut meat by aqueous enzymatic extraction technology showed a significant improvement over the traditional wet process that is currently prac-

TABLE 4
Effect of pH on Quality Characteristics of the Extracted Oil at 40°C and 1% (w/w) Enzyme Mixture

	pH[a]				
	4	5	6	7	8
Moisture (%)	0.11	0.11	0.11	0.11	0.11
FFA (%)[b]	0.055	0.053	0.052	0.051	0.051
Peroxide value (meq oxygen/kg)	0.018	0.017	0.017	0.016	0.016
Anisidine value (1,000 × abs)	0.028	0.028	0.027	0.027	0.027
Iodine value	8.2	8.3	8.4	8.3	8.3
Saponification value	260	260	261	262	261
Color	0.6	0.6	0.6	0.6	0.6

[a]Means of two readings. [b]FFA, free fatty acid.

TABLE 5
Effect of pH on Quality Characteristics of the Extracted Oil at 50°C and 1% (w/w) Enzyme Mixture

	pH[a]				
	4	5	6	7	8
Moisture (%)	0.11	0.11	0.11	0.11	0.11
FFA (%)	0.054	0.052	0.052	0.052	0.051
Peroxide value (meq oxygen/kg)	0.017	0.0165	0.0165	0.016	0.016
Anisidine value (1,000 × abs)	0.028	0.028	0.028	0.027	0.027
Iodine value	8.1	8.2	8.4	8.3	8.3
Saponification value	262	262	261	261	261
Color	0.6	0.6	0.6	0.6	0.6

[a]Means of two readings. See Table 4 for abbreviation.

TABLE 6
Effect of pH on Quality Characteristics of the Extracted Oil at 60°C and 1% (w/w) Enzyme Mixture

	pH[a]				
	4	5	6	7	8
Moisture (%)	0.11	0.11	0.11	0.11	0.11
FFA (%)	0.052	0.051	0.051	0.052	0.052
Peroxide value (meq oxygen/kg)	0.017	0.016	0.016	0.016	0.016
Anisidine value (1,000 × abs)	0.027	0.026	0.026	0.026	0.026
Iodine value	8.3	8.3	8.3	8.3	8.3
Saponification value	261	260	260	260	260
Color	0.6	0.6	0.6	0.6	0.6

[a]Means of two readings. See Table 4 for abbreviation.

TABLE 7
Proposed International Standard for Coconut Oil by Asian and Pacific Coconut Community[a]

Quality parameter	Grade 1 (RBDO)[b]	Grade 2 refined oil	Grade 3 (white oil)[c]
Moisture (%)	0.10	0.10	0.25
FFA (% lauric)	0.10	0.10	0.10
Iodine value	7.5–9.5	7.5–9.5	7.5–9.5
Saponification value	255 min	255 min	255 min
Color (Y + 5R)	2	2	4

[a]Source: Reference 13. See Table 4 for abbreviation; Y, yellow; R, red.
[b]Refined, bleached, and deodorized oil.
[c]Obtained from traditional wet process.

ticed in many coconut-producing countries. The technology has advantages because it presents no hazards, and the mild processing ensures a high-quality product. The residual cake, which contains about 7% (dry basis) protein (15), can be recovered as a hygienic co-product that is fit for human consumption, or a recovery scheme is possible for a food-grade protein.

ACKNOWLEDGMENT

The authors thank the Government of Malaysia for providing the funds under the IRPA Scheme (1-07-05-083) to carry out this study.

REFERENCES

1. Thieme, J.G., *Coconut Oil Processing,* RAO, Rome, 1968.
2. Gonzales, O.N., *Coconut Milk,* edited by A.J. Benzon, O.N. Gonzales, S.Y. deLeon, and P.C. Sanches, Philippines Coconut Research and Development Foundation Inc., Quezon City, 1990, pp. 15–48.
3. Hagenmaier, R., *Aqueous Process,* Philippines Coconut Research and Development Foundation Inc., Quezon City, 1980.
4. McGlone, O.C., A.L.M. Canales, and J.V. Carter, Coconut Oil Extraction by a New Enzymatic Process, *J. Food Sci. 51*:695–697 (1986).
5. Cheah, S.C., M.A. Augustin, and L.C.L. Ooi, *Enzymatic Extraction of Palm Oil,* Palm Oil Research Institute of Malaysia, Kuala Lumpur, 1987, pp. 12–14.
6. Olsen, H.S., *Aqueous Enzymatic Extraction of Oil from Seeds,* edited by S. Maneepun, P. Varangoon, and B. Phithakol, Food Science and Technology in Industrial Development, Bangkok, 1988, pp. 30–37.
7. Christensen, F.M., Extraction by Aqueous Enzymatic Process, *INFORM 2*:984–987 (1991).
8. *Official Methods of Analysis of the Association of Official Analytical Chemists,* 15th edn., AOAC, Washington D.C., 1984.
9. *Methods of Analysis of Oils and Fats,* British Standard Institution, London, 1976.
10. *PORIM Test Methods,* Palm Oil Research Institute of Malaysia, Ministry of Primary Industries, Kuala Lumpur, Malaysia, 1988.
11. Little, L.M., and J.P. Hill, *Agricultural Experimentation,* John Wiley and Sons, New York, 1977.
12. Loo, T.G., *Small Scale and Home Processing of Fresh Coconut (Oil Manufacture) and Utilization of By-Products,* Royal Tropical Institute, Amsterdam, 1982.
13. Thampan, P.K., *Handbook on Coconut Palm,* Oxford and IBH Publishing Co., New Delhi-Bombay-Calcutta, 1984.
14. Che Man, Y.B., Suhardiyono, A.B. Asbi, and M.N. Azudin, Acetic Acid Treatment of Coconut Cream in Coconut Oil Extraction, *ASEAN Food J. 7*:38–42 (1992).
15. Rasyid, F., M. Manullang, and P.M.T. Hansen, Isolation and Characterization of Coconut Protein, *Food Hydrocolloids 6*:301–314 (1992).

[Received May 9, 1995; accepted February 12, 1996]