Extraction of Coconut Oil with *Lactobacillus plantarum* 1041 IAM

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ABSTRACT: Extraction of coconut oil with a pure culture of *Lactobacillus plantarum* 1041 IAM was investigated. Grated coconut meat and water at 30, 50, and 70°C were mixed in various ratios (1:1, 1:2, and 1:3) and allowed to settle for 2–6 h. The most efficient coconut cream separation was obtained at the 1:1 ratio of grated coconut meat to water at 70°C, followed by 6 h settling time. Fermentation was then conducted on coconut cream emulsion with the sample from 1:1 ratio, 70°C, and 6-h settling time. Oil yield from the fermentation process with 5% inoculum of *L. plantarum* 1041 IAM after 10 h at 40°C was 95.06% Quality characteristics of the extracted oil were as follows: moisture content, 0.04%; peroxide value, 5.8 meq oxygen/kg; anisidine value, 2.10; free fatty acid, 2.45%; iodine value, 4.9; and color, 0.6 (Y + 5R). Extraction of coconut oil from coconut meat with *L. plantarum* 1041 IAM was significantly improved in both oil yield and quality over the traditional wet process.

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KEY WORDS: Coconut cream, coconut meat, fermentation, *Lactobacillus plantarum* 1041 IAM, oil yield, quality characteristics.

About 10% of the total oils and fats entering the world market is coconut oil (1). Several methods are currently practiced for removing oil from either fresh coconut meat or copra (dried coconut kernel). These technologies include the (i) wet process, (ii) the dry process, and (iii) solvent extraction. (i) The wet process can be carried out by grinding coconut meat with water and filtering it to produce coconut milk or coconut cream. This emulsion contains protein and coconut oil, which can be separated either through common kitchen utensils or hydraulic presses. However, coconut oil extraction by these wet process techniques has not been commercially successful (2–4). (ii) The dry process is the present commercial technique for coconut oil extraction. Copra is cleaned, ground, steamed, and pressed through an expeller for coconut oil extraction. This extracted oil is further purified by neutralization, bleaching, and deodorization to remove free fatty acids (FFA), odors, flavors, and pigments. (iii) Solvent extraction is possible with an appropriate solvent, such as benzene or *n*-hexane. Even though oil recovery is high, the process is rarely applied owing to its high risk and high investment cost.

Usually, the recovery of coconut oil by the traditional wet process is low, about 30–40% (5). Moreover, the oil obtained is of poor quality owing to the high moisture content (MC) dark color, and short shelf life (6). The process is also energy- and time-consuming. On the other hand, the traditional method is easy to handle, and the extracted coconut oil has a pleasant aroma and low FFA (7). Several workers have investigated alternative wet extraction methods to recover coconut oil. Che Man *et al.* (8) studied the use of 0.1–0.4% acetic acid (25%) for coconut oil extraction and showed a recovery of 58.3–60.3% of good-quality oil. In another study, Che Man *et al.* (9) obtained a yield of 73.8% of good-quality oil with 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. The extracted oils in these studies required no further purification to meet the quality of the proposed International Standard by the Asian and Pacific Coconut Community (4). An earlier study by McGlone *et al.* (10), in which a mixture of polygalacturonase, α-amylase, and protease were used on diluted coconut paste, obtained an 80% yield of good-quality oil, compared to the Official Mexican Standards (10). Suhardiyono (11) investigated the use of baker's yeast to extract coconut oil. The mixed culture of baker's yeast grew in coconut milk and broke the emulsion into good-quality oil. Moreover, the yield of extracted oil was double that of the traditional wet process. Based on this work, we postulated that higher recovery of coconut oil could be obtained by using pure cultures of microorganisms. Therefore, the objectives of this study were to investigate the effect of a pure culture of *Lactobacillus plantarum* 1041 IAM to break the coconut cream emulsion for separating the oil and to determine the yield and quality characteristics of the extracted oil.

MATERIALS AND METHODS

Materials. Fresh coconut of the Mawar variety was obtained from Universiti Pertanian Malaysia Farm (Selangor, Malaysia). A pure culture of *L. plantarum* strain 1041 IAM was obtained from the University of Tokyo, Japan. Commercial coconut oil was purchased from Kilang Minyak Tanjung

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Karang Sdn. Bhd. (Selangor, Malaysia). All chemical reagents were of analytical grade and obtained from BDH Chemical Ltd. (Poole, England).

Extraction of coconut cream. The extraction of coconut milk was carried out as follows: Grated coconut meat and water at 30°C were mixed in proportions of 1:1, 1:2, and 1:3. The mixture was kneaded manually for 5 min, and the milk was extracted, squeezed, and strained through a layer of cheesecloth. The coconut milk obtained was then left to settle for 2, 4, and 6 h. The samples based on the ratio of 1:1 were used to determine the effect of different settling times (2, 4, and 6 h) and temperatures (30, 50, and 70°C) on the oil extraction yield. Coconut milk was then allowed to settle and separate into two layers: the upper cream emulsion layer, which was thick and dense, and the lower aqueous layer, which contained mainly water and was drained off.

Treatment of coconut cream by chemicals and heat. The freshly extracted coconut cream was treated chemically as described by Bhowmik and Marth (14) with some modifications. The coconut cream (200 mL), containing 1 g of 30% hydrogen peroxide (H_2O_2), was incubated at 40°C for 2 h. Catalase (10.5 mg) was then added to decompose the H_2O_2. Coconut cream was further incubated at 30°C for 3 h. A preliminary experiment was carried out to determine the degree of stability of the product by a plate count. As a result of the peroxide/catalase treatment, the bacterial plate count was reduced to 3870 cells/mL coconut cream compared to 4.01×10^8 cells/mL coconut cream in the original sample.

Fermentation. A pure culture of *L. plantarum* strain 1041 IAM was transferred to the MRS medium for cell activation at 30°C for 48 h (13). Pellets of *L. plantarum* were obtained after centrifugation with phosphate buffer saline (PBS) solution several times. Dilution was carried out in PBS with different concentrations between 10^{-1} and 10^{-6}. These dilutions were later checked with a spectrophotometer at 540 nm to obtain the appropriate optical density (OD). The number of lactic acid bacteria cells was determined by hemacytometer at the selected and specified OD (0.01–0.06). Coconut cream was divided into two parts. Portion of the cream were transferred to fermentation vessels where 1, 3, and 5% inocula of *L. plantarum* 1041 were added; another portion was used as a control. Fermentation was carried out from 2 to 10 h at 40°C (12).

Determination of lactic acid. Coconut cream (10 mL) was augmented with five drops of 0.5% phenolphthalein, followed by titration with 0.1 N NaOH solution. The acid produced by *L. plantarum* 1041 IAM was calculated as lactic acid (%, wt/vol) with the formula: 1 mL 0.1 N NaOH = 0.009 g lactic acid.

Oil recovery. The oil recovery was calculated based on the initial oil content of the coconut meat as determined by the Soxhlet method of AOAC (15) and the direct weight measurement of oil obtained after extraction.

Analyses of oil quality. MC and percentage (%) FFA were measured according to AOAC methods (15). Peroxide (PV) and iodine (IV) values were measured according to British Standard No. 684 (16). The anisidine value (AnV) and fatty acid composition (FAC) were measured according to PORIM Test Methods (17). Color was measured by Lovibond Tintometer (Model E) according to British Standard No. 684 (16).

Statistical analysis. The data were analyzed by analysis of variance techniques. Means that were significantly different at a 5% level of probability ($P < 0.05$) were further separated by Duncan's Multiple Range Test (18).

RESULTS AND DISCUSSION

Effect of coconut meat/water ratio on oil yield. The effect of different amounts of water at 30°C added to coconut meat on extraction yield of oil is shown in Table 1. The results show that the 1:1 ratio gave the highest oil yield, compared to 1:2 and 1:3. As the ratio of water added was increased, the oil content decreased. Higher proportions of water increased the dilution effect and therefore decreased the oil yield in coconut cream from 37.09 to 32.50 and 28.48% in 1:1, 1:2, and 1:3, respectively, after 2 h of settling time. The same trend was found for 4- and 6-h settling times. Banzon *et al.* (1) found that the composition of coconut cream is largely based on the amount of water added for the extraction of oil. This result showed that adding less water contributed to a higher proportion of oil and is in agreement with the finding of Banzon and co-workers.

Effect of water temperature and settling times on oil yield. Table 2 shows that as water temperature was increased from 30 to 70°C, there was a significantly increased ($P < 0.05$) oil yield for the 1:1 ratio of coconut meat/water when followed by 2–6 h settling times. The oil recovery from coconut cream with 70°C water was 46.23%, while those of water at 30 and 50°C were 37.09 and 40.83%, respectively. The most effective time of settling the coconut milk was 6 h, which yielded 51.33% oil when the water was at 30°C. The yield was further increased to 83.88% with water at 70°C. Therefore, a long settling time is required at higher temperature to efficiently separate the cream emulsion so that higher oil yield is obtained.

Oil yield after fermentation. Based on the previous experiments, coconut cream from the 1:1 coconut meat/water ratio (wt/vol) was used for fermentation study. The concentrated coconut cream contained lower proportions of skim milk and therefore reduced the volume of inoculum added. Puertollano *et al.* (12) also reported that the optimum dilution for rapid

TABLE 1
Effect of Coconut Meat/Water Ratio on Oil Yield (%) at 30°C[a]

	Settling time (h)		
Coconut meat/water	2	4	6
1:1	37.09[a]	43.04[a]	51.33[a]
1:2	32.50[b]	38.38[a,b]	41.70[b]
1:3	28.48[c]	36.18[b]	41.61[b]

[a]Mean of three readings. Means in a column followed by different superscript letters (a–c) are different ($P < 0.05$).

TABLE 2
Effect of Settling Times and Water Temperatures Based on 1:1 Ratio of Coconut Meat/Water on Oil Yield (%)[a]

Settling time	Water temperature (°C)		
(h)	30	50	70
2	$37.09^{c,f}$	$40.83^{c,e}$	$46.23^{c,d}$
4	$43.04^{b,f}$	$61.42^{b,e}$	$70.63^{b,d}$
6	$51.33^{a,f}$	$75.76^{a,e}$	$83.88^{a,d}$

[a]Means of three readings. Means in a column followed by different superscript letters (a–c) are different ($P < 0.05$). Means in a row followed by different superscript (d–f) letters are different ($P < 0.05$).

TABLE 3
Effect of Incubation Time and Inoculum Level on Formation of Lactic Acid (%)[a] During Fermentation of Coconut Milk Extracted at 30°C, Followed by Settling (2 h)[b]

Inoculum	Incubation time (h)				
(%)	2	4	6	8	10
0	$0.180^{d,h}$	$0.190^{d,h}$	$0.20^{c,g}$	$0.225^{b,g}$	$0.315^{a,f}$
1	$0.20^{d,g}$	$0.288^{c,g}$	$0.315^{b,f}$	$0.322^{b,f}$	$0.330^{b,g}$
3	$0.225^{b,f}$	$0.304^{b,f}$	$0.336^{a,e}$	$0.342^{c,f}$	$0.352^{c,g}$
5	$0.243^{d,e}$	$0.323^{a,e}$	$0.350^{a,e}$	$0.358^{c,e}$	$0.368^{b,e}$

[a]Means of three readings. Means in a row followed by different superscript letters (a–d) are different ($P < 0.05$). Means in a column followed by different superscript letters (e–h) are different ($P < 0.05$). [b]Fermentation with *Lactobacillus plantarum* was carried out at 40°C. Coconut milk was derived by a water extraction of grated coconut meat (1:1, coconut meat/water).

de-emulsification and separation of oil was coconut milk of 1:1 coconut meal/water ratio (wt/vol). Lower proportions of water, for example 1:0, can hinder the extraction of coconut oil, protein, and other extractable materials owing to insufficient fluidity for grinding. However, a higher proportion of water increased the time required to break the emulsion, such as a ratio of 1:3 for sample and volume of inoculum.

Coconut cream contained a high bacterial count, and the predominant organisms were gram-positive bacteria with long and short rods (12). Therefore, H_2O_2 was added to the cream, followed by heating at 40°C to kill the background mesophilic bacteria, to reduce competition between the background bacteria and *L. plantarum* strain 1041 IAM. Fermentation in an Erlenmeyer flask provided a microaerophilic condition for growth of the lactobacilli.

The formation of lactic acid during fermentation is presented in Tables 3 and 4. Inoculation of coconut cream with *L. plantarum* strain 1041 IAM resulted in rapid breaking of the emulsion. We believe that *L. plantarum* used glucose, the only sugar present in coconut cream, at 1.23% (14), for growth and consequently caused the production of lactic acid. Lactic acid increased as the amount of *L. plantarum* inoculated increased to 5%, indicating that a higher rate of fermentation occurred in samples with a ratio of 1:1 coconut meat/water at 30°C and 2-h settling time. The lactic acid produced ranged from 0.207 to 0.368% in inoculated coconut cream, compared to lower lactic acid levels, 0.18 to 0.315%, without inoculation. However, the lactic acid produced in the treatment of 1:1 coconut meat/water with a water temperature of 70°C, followed by 6 h settling time, ranged from 0.0351 to 0.0522%, which was lower than the former treatment. Precipitation of soluble protein in the interfacial film occurred as a result of lactic acid formation during fermentation. Lactic acid destabilized the protein and caused water to be released (11).

Oil recovery. The effect of various concentrations of *L. plantarum* strain 1041 IAM during fermentation at 40°C and 10 h incubation time on oil yield is presented in Table 5. Shorter incubation times were less effective. The amount of oil obtained ranged from 37.09 (control) to 59.91% (after fermentation with water at 30°C and 2h settling time) and 83.88 (control) to 95.06% (after fermentation with water at 70°C and 6h settling time). The highest oil yield was 95.06% at 5% inoculum with a sample of 1:1 ratio, 70°C and 6 h settling time. This study showed that oil recovery was improved after fermentation, compared to the traditional method, which was about 30–40%. However, at 70°C and 6 h settling time, oil recovery was tremendously improved.

Quality characteristics of extracted coconut oil. The FAC of the extracted coconut oil is presented in Table 6. The results are in agreement with the Codex Alimentarius Commission International Standard (CACIS) value (19). As a comparison, the FAC of commercial coconut oil was in close agreement with the extracted oil. However, extracted coconut oil showed higher total saturated fat, or 92.43%, compared with 88.45% in commercial coconut oil. The high degree of saturation caused a higher resistance to rancidity (20).

The color of the oil was unchanged at 0.3 (Y + 5R), as shown in Table 7, for low-temperature and 2-h settling time treatment and 0.6 (Y + 5R) for high-temperature and 6-h settling time treatment, as shown in Table 8. PV in the extracted oil ranged from 2.55 to 5.15 meq oxygen/kg, compared to 8.95 meq oxygen/kg in commercial oil. A 5% inoculum contributed to the lowest PV, 2.55 meq oxygen/kg, compared to 3.50 and 4.05 meq oxygen/kg in 3 and 1% inoculum in low-temperature short settling time. For the high-temperature, 6-h settling treatment, a 5% inoculum gave a PV of 5.8, compared to 7.2 and 7.6 meq oxygen/kg in 3 and 1% inoculum, which

TABLE 4
Effect of Incubation Time and Inoculum Level on Formation of Lactic Acid (%)[a] During Fermentation of Coconut Milk Extracted at 70°C, Followed by Settling (6 h)[b]

Inoculum	Incubation time (h)				
(%)	2	4	6	8	10
0	$0.0342^{e,i}$	$0.0378^{d,i}$	$0.0405^{c,h}$	$0.045^{b,i}$	$0.0477^{a,i}$
1	$0.0351^{e,h}$	$0.0387^{d,h}$	$0.0423^{c,i}$	$0.0477^{b,h}$	$0.0486^{a,h}$
3	$0.0369^{e,g}$	$0.0405^{d,g}$	$0.0432^{c,g}$	$0.0495^{b,g}$	$0.0504^{a,b}$
5	$0.0459^{e,f}$	$0.0477^{d,f}$	$0.0495^{c,f}$	$0.0513^{b,f}$	$0.0522^{a,f}$

[a]Mean of three readings. Means in a row followed by different superscript letters (a–e) are different ($P < 0.05$). Means in a column followed by different superscript letters (f–i) are different ($P < 0.05$).
[b]Fermentation with *Lactobacillus plantarum* was carried out at 40°C. Coconut milk was derived by a water extraction of grated coconut meat (1:1, coconut meat/water).

TABLE 5
Effect of Inoculum Level, Settling Time, and Extraction Temperature on Oil Yield (%)[a] Following Incubation[b]

Inoculum (%)	1:1 ratio, 30°C, 2 h settling	1:1 ratio, 70°C, 6 h settling
0	37.09[c]	83.88[c]
1	54.49[b]	90.46[b]
3	57.32[a,b]	93.64[a,b]
5	59.91[a]	95.06[a]

[a]Mean of three readings. Means in a row followed by different superscript letters (a–c) are different ($P < 0.05$).
[b]Fermentation with *Lactobacillus plantarum* IAM 1041 was carried out at 40°C on coconut milk derived by water extraction of grated coconut meat (1:1, coconut meat/water).

TABLE 6
Fatty Acid Composition (%) of Standard, Extracted, and Commercial Coconut Oil

Fatty acid	Standard oil[a]	Extracted oil	Commercial oil
Caproic (C_6)	<1.2	n.d.	n.d.
Caprylic (C_8)	3.4–15	6.32	5.78
Capric ($C_{10:0}$)	3.2–15	5.59	5.39
Lauric ($C_{12:0}$)	41–46	49.69	49.49
Myristic ($C_{14:0}$)	13–23	19.94	18.33
Palmitic ($C_{16:0}$)	4.2–12	8.83	10.38
Stearic ($C_{18:0}$)	1–4.7	2.06	2.08
Oleic ($C_{18:1}$)	3.4–12	6.36	9.01
Linoleic ($C_{18:2}$)	0.9–3.7	1.206	2.545

[a]Reference 19; n.d., not detected.

TABLE 7
Effect of Inoculum Level of *Lactobacillus plantarum* 1041 IAM on Quality Characteristics of Oil Extracted with 30°C Water, Followed by Settling (2 h)[a,b,c]

Inoculum (%)	Color	PV	AnV	FFA	MC	IV
0	0.3[a]	5.15[b]	0.49[b]	0.075[b]	0.023[b]	5.89[b]
1	0.3[a]	4.05[c]	0.163[c]	0.05[c]	0.018[c]	3.80[c]
3	0.3[a]	3.50[c]	0.140[d]	0.04[d]	0.016[d]	3.70[d]
5	0.3[a]	2.55[e]	0.120[e]	0.035[e]	0.013[e]	3.10[e]
C[d]	0.3[a]	8.95[a]	0.73[a]	1.55[a]	0.034[a]	8.71[a]

[a]Reported results are means of three readings. Means in a column followed by different superscript letters (a–e) are different ($P < 0.05$).
[b]Coconut meat was extracted with water (1:1, coconut meat/water) at 30°C as described in the Materials and Methods section, then allowed to settle for 2 h. Samples were then inoculated with *L. plantarum* and incubated for 10 h at 40°C.
[c]Color, determined as Y + 5R; PV, peroxide value (meq oxygen/kg); AnV, anisidine value (1,000 × abs); FFA, free fatty acid content (%); MC, moisture content (%); IV, iodine value (g I/100 g sample). [d]C: Commercial oil.

TABLE 8
Effect of Inoculum Level of *Lactobacillus plantarum* 1041 IAM on Quality Characteristics of Oil Extracted with 70°C Water, Followed by Settling (6 h)[a,b,c]

Inoculum (%)	Color	PV	AnV	FFA	MC	IV
0	0.6[a]	9.6[a]	4.59[a]	3.48[a]	0.057[a]	7.61[b]
1	0.6[a]	7.6[c]	2.56[b]	3.04[b]	0.049[b]	7.61[b]
3	0.6[a]	7.2[d]	2.21[c]	2.76[c]	0.044[c]	6.73[c]
5	0.6[a]	5.8[e]	2.10[d]	2.45[c]	0.039[d]	4.95[d]
C[d]	0.3[a]	8.95[a]	0.73[a]	1.55[a]	0.034[a]	8.71[a]

[a]Reported results are means of three readings. Means in a column followed by different superscript letters (a–e) are different ($P < 0.05$).
[b]Coconut meat was extracted with water (1:1, coconut meat/water) at 70°C as described in the Materials and Methods section, then allowed to settle for 6 h. Samples were then inoculated with *L. plantarum* and incubated for 10 h at 40°C.
[c,d]For abbreviations see Table 7.

was higher than the former treatment. However, the maximum PV according to CACIS is 10 meq oxygen/kg. Although the oil quality of the latter treatment was not so good as the former, it was still within the range for standard good oil. Peroxides are the primary products formed by oxidation of the oil. They are unstable and break down to many types of secondary products. The deterioration of oils cannot be measured accurately by using this method alone (21) because a decrease in PV does not necessarily indicate the oil is in good condition. Theoretically, coconut oil should show a low rate of oxidation because it contains low levels of unsaturated fatty acids.

The comparison of AnV between extracted coconut oil and commercial oil is presented in Tables 7 and 8. There was a significant difference ($P < 0.05$) in AnV as the percentage inoculum was increased. Commercial oil had higher AnV, 0.73 compared to extracted coconut oil, which ranged from 0.12 to 0.163 in low-temperature short settling time and from 2.10 to 4.59 in high-temperature long settling time. According to Russell (22), as a rule of thumb for good-quality oils, the AnV should be less than 10. Inoculum at 5% showed the lowest AnV, 0.12 and 2.10, for low temperature, 2-h settling time and high-temperature, 6-h settling time, respectively.

Tables 7 and 8 also show the results obtained for FFA and MC. The higher the MC in the oil, the higher the percentage of FFA. MC in extracted coconut oil after fermentation ranged from 0.013 to 0.018% in low-temperature short settling time treatment, which was lower than the 0.034% in commercial oil. Inoculum at 5% showed the lowest MC at 0.013%, compared to 3 and 5% inoculum, whereas uninoculated coconut cream produced oil with 0.023% MC, which is higher than the inoculated coconut cream. MC in high-temperature longer settling time treatment ranged from 0.039 to 0.057%. Inoculum at 5% showed the lowest MC, 0.039%, compared to 0.044 and 0.049% in 3 and 1% inoculum, respectively.

FFA content ranged from 0.035 to 0.05% in low-temperature short settling time treatment, 0.07% in uninoculated coconut cream, and 1.55% in commercial oil, indicating that the extracted oil has a better quality. However, high-temperature longer settling time treatment showed a higher percentage of FFA, 2.45, 2.76 and 3.04% at 5, 3, and 1% inoculum, respectively, compared to low-temperature short time treatment. Maximum percentage FFA, according to CACIS, is 4, which means that an oil with a higher value is considered rancid. Production of FFA through hydrolysis is related to the secondary products of oxidation (23).

The IV of extracted coconut oil and commercial oil is shown in Tables 7 and 8. The IV indicates the quality of oil based on the unsaturation of fatty acids. The results obtained ranged from 3.1 to 3.8 in low-temperature short settling time treatment and 4.95 to 7.61 in high-temperature long settling time treatment. The IV of commercial oil was 8.71, which is within the standard range of CACIS of 6–11 (19). A lower IV can be reflected by lower amounts of unsaturated fatty acids from the FAC profile in Table 6. Therefore, the stability of the recovered oil was better than the uninoculated coconut oil and commercial oil in terms of its resistance to oxidation.

The technique for coconut oil extraction by fermentation with a pure culture of *Lactobacillus plantarum* 1041 IAM showed a higher oil extraction yield over the previous techniques of acetic acid and aqueous enzymatic extraction as reported by the senior author and co-workers (8,9). All these techniques had shown a significant improvement over the traditional wet process (30–40%) that is currently practiced in many coconut-producing countries.

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