Separation of the Oil and Protein Fractions in Coconut (*Cocos nucifera* Linn.) by Fermentation

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Patented and published methods of separating oil and protein from coconut meat by fermentation were studied and combined to devise a better process. Coconuts available commercially vary in maturity and length of storage before processing, and varied in their response to fermentation processing. Approximately 60% of the milks produced from individual coconuts showed a breaking of the emulsion when fermented under controlled conditions. Forty percent failed to break, indicating that some factor(s) responsible for the coconut milk emulsion stability remained uncontrolled during fermentation. The optimum dilution range for rapid fermentation of coconut milk and separation of the oil and protein was found to be 1:1 to 1:2 (w/v) coconut meat/water. *Lactobacillus plantarum* effected more rapid separation of oil than *Lactobacillus delbrueckii*. The fermentation progressed best under microaerophilic conditions at 40°–50° C. The fermentation was successful in breaking the emulsion at a relatively broad range of pH and titrable acidity.

Oilseeds such as peanut, soybean, and sesame are relatively low in moisture and, when pressed, yield oil directly. Coconut with a fresh moisture content of 50% (Banzon, 1969), when pressed, yields a coconut milk. Coconut milk is a naturally opaque emulsion containing oil, water, sugar, protein, and salts. This is not to be confused with coconut water which is the clear liquid in the center of a fresh coconut. Coconut is often dried to produce copra, which can be pressed to yield coconut oil directly. Thus, coconut oil can be removed from coconut by wet or dry processing methods. Unfortunately, copra produced by traditional drying methods is often moldy and contaminated by insects and rodents. Thus, it does not offer a very wholesome raw material for production of either oil or protein. Furthermore, if the copra is pressed to remove the oil, the high temperatures used result in a copra cake in which the proteins have been largely denatured and insolubilized. Sreenivasan and Rajasekharan (1967) reviewed methods of processing coconut to oil and protein.

Thieme (1968) discussed various methods of processing coconut oil. In the traditional method, discussed further by Andaya *et al.* (1961), the fresh coconut is grated and pressed to yield a coconut milk. The fat-rich fraction separates as a cream, and the cream is then rendered by boiling until the moisture is removed and the oil separates. The residue in this case is a very pleasant flavored, toasted flake retaining relatively large amounts of oil on the surface. The protein is denatured.

Literature on the emulsifying agents in coconut milk is sparse. Clemente and Villacorte (1933) concluded that sugars, dissolved in the aqueous phase, act as emulsifying agents with the proteins colloidally dispersed at the water-oil interface. Birosel and Gonzales (1961) and Birosel *et al.* (1962, 1963), however, reported that the emulsifier system must be the phosphorus and nitrogen-bearing substances that are present in the nut kernel. The emulsifier has the characteristic of a phospholipid.

Adriano and Manahan (1931) showed that the composition of coconut without embryo differs from that with embryo, especially with regards to moisture content, fat, and protein, although the pH and titrable acidity are almost identical. Roxas (1914) reported that lipase is present in germinating coconut as an enzyme which can be activated by dilute acids. When the nuts germinate, the percentage of sucrose and invert sugar in both the meat and the milk increases rapidly (Gonzales, 1914; Vista, 1915). Gonzales (1914) in his studies on the changes occurring in ripening coconuts cited the work of Walker (1906), which showed that coconuts fresh from the trees but fairly ripe (all green husks) and "dead-brown" husks vary in composition, especially with regard to moisture and oil content. The composition of coconut does not change very much even when stored for three months (just beginning to sprout). Those stored for six months which had not sprouted had a high moisture content of 59.30% and 27.27% of oil. These are considered abnormal coconuts for the simple reason that they do not sprout at all. Gonzales further reported that sucrose tended to increase from none in the greenest to 0.1982% in the most mature coconuts. Total solids of the coconut meat (endosperm) increased from about 5% in the greenest to 46.38% solids in the oldest nuts. At the same time, oil content increased from 0.595 to 24.97%. Percent of nitrogen in the dry matter of the coconut meat tended to fall from 2.26% in the green to 1.14% in the most mature nuts. He emphasized that the absolute quantities of sucrose, solids, and nitrogen varied widely from tree to tree.

Fermentation as a method of facilitating oil extraction has been reported by several workers (Alexander, 1921a,b; Beckman, 1930; Horovitz-Vlasova and Novotelnov, 1935; Soliven and de Leon, 1938). These scientists observed that if the emulsion is allowed to ferment, the oil and protein fractions separate. However, they did not specify whether the enzymes or acid produced or the combination of these along with, perhaps, other factors elaborated by the microorganisms are responsible for breaking the emulsion.

Alexander (1921a) used a pure culture of bacteria capable of digesting albumin. Fermentation of the coconut milk took place in 5 to 10 hr if maintained at a desirable temperature (unspecified). The process is not used commercially at the present time.

Alexander (1921b) heated the emulsion in an autoclave and, as soon as the pressure reached 20 psi, the emulsion broke. The mixture was taken out of the autoclave and then centrifuged to separate the oil from the water. This pro-

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cedure denatures the protein, thereby destroying its functional properties.

Another process to extract coconut oil was reported by Beckman (1930) and Horovitz-Vlasova and Novotelnov (1935). Finely macerated coconut meat was inoculated with *Lactobacillus delbrueckii* and incubated under anaerobic conditions at 50° C. Calcium or magnesium carbonate was added to neutralize the acid as soon as it formed, thus preventing the slowing down of bacterial growth. After 6 days, the oil was recovered from the residue by filtration. The 6 days required made it inefficient for commercial application. Lava (1937) patented a process similar to the Alexander and Beckman processes.

In the Philippines, Soliven and de Leon (1938) studied the liberation of coconut oil using a *Bacillus* species obtained from the scum of fermenting coconut milk. The best fermentations occurred between 30° to 40° C for 15 to 20 hr using a 1 to 3 (v/v) dilution of coconut milk. The mixture molded at a higher dilution, and at a lower dilution there was no distinct separation of oil. The method is not used commercially at the present time.

Lava *et al.* (1941) published a semicommercial method of extracting coconut oil. The process consisted of passing the meat through a series of knives until particles the size of sawdust were produced. A water emulsion and an oil cake were obtained after pressing. The emulsion was broken by adjusting the pH of the emulsion in the range of 3 to 5.6 and a layer of oil, proteinaceous and cellulose matter, and water was formed. The oil was recovered by siphoning. The method is not used commercially.

Robledaño and Luzuriaga (1948) obtained the first Filipino patent for a "wet process" of extracting coconut oil. The coconut meat was ground and pressed to yield a solid residue and an emulsion of oil and water. The cream obtained after centrifugation was subjected to controlled enzymatic action, frozen, and thawed. The oil was removed by centrifugation. The process was tried commercially in a factory at Calamba, Laguna, Philippines. At present the factory is not operating. Apparently the process was not commercially economical.

Water-white coconut oil from fresh coconut was reported by Francisco (1961). He failed to give any details of his process, so that it cannot be critically evaluated from the commercial view point.

Since none of the above procedures are in commercial use, even though some have been tried commercially, it can be concluded that all have drawbacks. In most of the procedures the length of time required for separation of oil was too long to be economical and the oil recovery was low.

Wet processing of fresh coconut has potential advantages. The first is that by proper handling the oil is of better quality than that obtained by the usual commercial dry processing. Second, the protein can be obtained undenatured. Coconut protein is a very interesting product in itself. It is of good quality nutritionally. It coagulates with heat much like egg albumin. Thus, obtained undenatured, it could serve as a base for instant puddings where the housewife adds water to a mixture of coconut protein with appropriate flavors, brings the mixture to a boil, and the protein precipitates, giving texture to the pudding.

This study was made to select the best features of the different published and patented methods of wet extraction of coconut oil and to optimize the factors influencing the fermentation in the hope that the process evolved would be economically feasible.

The study included determination of factors which must be controlled in breaking the emulsion, such as numbers and types of microorganisms, optimum pH and temperature, optimum oxygen relationships, optimum dilution, and the use of adjuncts to yield a fermentation in the minimum length of time.

MATERIALS AND METHODS

Coconut milk is the white opaque liquid expressed from finely comminuted meat of sound ripe coconuts. If extracted without the addition of water, it is a thick cream. If extracted with water, a less dense coconut milk is obtained. By gravity separation, however, the coconut cream forms a top layer, and the coconut skim milk forms a lower layer. Both layers are opaque, but the upper is denser and thicker.

Residue is the insoluble solid particles. including fiber remaining after expressing the milk.

Coconuts were randomly selected daily for each experiment. Husked coconut was halved and the meat was removed from the shell by grating. To extract the coconut milk, coconut meat and distilled water were beaten in a Waring Blendor in proportions—coconut meat to water of 1:1, 1:2, 1:2.5 (w/v). The mixture was pressed through a cheesecloth to separate the milk from the residue.

The milk was divided into two portions. One part was allowed to stand at room temperature in a separatory funnel to separate the cream from the skim milk. The other part was inoculated with 5% (v/v) sucrose broth culture of *Lactobacillus* to facilitate separation of the cream. Two species of *Lactobacilli* were used, *Lactobacillus plantarum* and *Lactobacillus delbrueckii.*

The cream was transferred to a fermentation vessel and more inoculum was added to give a total inoculum of 10% (v/v). To determine the relationship between surface area and volume on the rate of fermentation, three types of containers were used as fermentation vessels—Petri dishes, test tubes, and Erlenmeyer flasks. Fermentation vessels, except the Petri dishes, were covered with rubber stoppers. Fermentation was carried out at 30°, 40°, and 50° C.

To determine further the effect of temperature on the rate of fermentation, samples were preheated at 32°, 38°, and 40° C before incubation at 40° C.

Bacterial counts, titrable acidity (as lactic acid) and pH were determined immediately after extraction of coconut milk and every 2 hr thereafter until visible oil separation, if any, occurred.

Yeast extract, tryptone, sucrose, lactose, and a combination of yeast extract, tryptone, and sucrose in 1% concentrations were incorporated as adjuncts to coconut cream to determine if fermentation and emulsion breakdown would be stimulated.

The samples were inoculated with *L. plantarum* and were incubated at the optimum temperature for fermentation. The pH and titrable acidity were taken at intervals until the emulsion started to break, as evidenced by the presence of oil on the surface.

Two methods of mechanical grinding were used—the Waring Blendor in the laboratory and the Rietz Disintegrator with 0.023-in. screen in the pilot plant. The latter method necessitated the use of 1:2 dilutions (coconut to water) w/v basis. With lower dilutions, the slurry would not pass through the screen.

After fermentation the oil and curd layers were separated from the skim milk by siphoning. The curd containing solid proteinaceous and cellular matter separated and formed a layer after fermentation between the skim milk portion and the oil. It still contained a portion of the oil which had to be

separated by centrifugation. The oil was pasteurized at 70° to 75° C for 15 min to kill the microorganisms and stored to determine stability. Free fatty acids expressed as oleic acid, refractive index, and specific gravity were determined after one month storage using the A.O.A.C. (1965) methods.

Moistures and crude fat were determined by the procedures described in A.O.A.C. (1965).

The protein in the residue and in the skim milk before and after fermentation and in the curd were determined using the semi-micro Kjeldahl method (A.O.A.C., 1965).

Bacterial counts on coconut milks were made using serial dilutions plated in duplicate on tryptone, glucose, and yeast extract medium incubated at 37° C for 48 hr.

RESULTS AND DISCUSSION

Based upon wet processes and what appeared to be the most critical factors in the separation of coconut oil and protein by fermentation, as described in the patents and other literature, the following process was devised.

Remove the coconut meat from the shell with a mechanical grater.
Grind the grated coconut meat with distilled water 1:1 (w/v) in a Waring Blendor.
Filter press to separate oil, water, protein and all filterable material from the insoluble residue.
Inoculate with a 10% (v/v) of a culture of *Lactobacillus plantarum* grown on a medium containing tryptone 0.5% yeast extract 0.25% and sucrose 1.5%.
Incubate at 40° C.

The process, when optimized, caused a typical separation of a high quality, water-white oil and precipitation of a protein-rich fraction in from 60 to 75% of the coconut milks tested. Under optimum conditions, the coconut milk emulsion began to break, releasing the oil in about 4 to 8 hr, and separation was complete in 10 hr. The protein precipitated, under the influence of the acid produced, forming a layer at the top of the aqueous phase where it could easily be recovered by centrifugation.

Although the process worked with 60 to 75% of the coconut milks tested, it failed to cause separation with from 25 to 40% of the coconut samples. It was concluded that this variation in batches of coconuts accounts for the fact that no method so far devised for the separation of coconut oil and protein by fermentation has ever succeeded commercially.

INFLUENCE OF COCONUT RAW MATERIAL

The coconuts obtained on the market were not uniform and were constantly changing. They were generally characterized as mature, but varied in age at harvest and in length of storage. It would be impossible to obtain coconuts of the same age and maturity commercially. Some of the coconuts were beginning to form embryos. This again influences the chemical composition and enzymic content of the nut. It was not known how much individual coconuts would vary in their response to fermentation. When 17 individual coconuts were extracted (Table I) in a Waring Blendor with water 1:1 (w/v) and fermented under controlled conditions using the process described, it was found that 10 of the 17 samples (60%) fermented, separating out the water-white oil and protein rich precipitates, while seven samples (40%) failed to separate. None of the uninoculated (control) samples showed breaking of the emulsion.

INFLUENCE OF pH

The pH of freshly extracted coconut milk ranged from 5.9 to 6.7 (Table I). The pH (Tables II and IV) decreased slowly during the first 4 hr of fermentation for the inoculated samples as the organisms started to multiply while titrable acidity increased. Oil separation appeared 4 to 6 hr after incubation at 40° C. At this time all samples showing breaking had a pH of 4.7 to 5.5. Trials with individual coconuts (Table I) also showed that breaking of emulsion may occur outside pH 5.2, the isoelectric point of coconut protein (Strength, 1969). The oil separation was completed when all the curd remained suspended at the upper part of the aqueous phase.

INFLUENCE OF DILUTION

Breaking of emulsion with separation of oil appeared in the 1:1 and 1:2 dilutions (inoculated samples) after 4 hr of

Table I. Changes in Titrable Acidity (as Lactic Acid) and pH of Individual Coconuts during Fermentation[a] at 40° C (Dilution 1:1 w/v)

Coconut No.	0 hour		5 hours				21 hours			
			Control		Inoculated		Control		Inoculated	
	pH	% T.A.	pH	% T.A.	pH	% T.A.	pH	% T.A.	pH	% T.A.
1	5.9	0.15	5.4	0.26	4.9[d]	0.23	4.4	0.45	3.4	0.76
2	6.0	0.19	4.6	0.40	5.0[d]	0.30	4.4	0.62	3.4	0.86
3	6.1	0.17	5.4	0.24	4.5	0.30	4.2	0.55	3.3	0.74
4	6.2	0.14	5.0	0.24	4.8	0.26	4.2	0.64	3.1	0.78
5	6.3	0.15	5.2	0.21	4.8[d]	0.23	4.5	0.57	3.4	0.81
6	6.3	0.10	5.0	0.38	4.9[b]	0.30	4.2	0.57	3.5	0.86
7	6.3	0.17			6.0[c]	0.19	4.3	0.43	3.7	0.65
8	6.3	0.14			5.0[c]	0.45	4.2	0.53	3.6	0.83
9	6.3	0.26			5.1[c]	0.45	4.8	0.53	4.0	0.83
10	6.4	0.19			5.7	0.24	4.7	0.61	3.8	0.78
11	6.4	0.14			5.3[c]	0.33	4.6	0.67	4.1	0.84
12	6.4	0.15			5.9[d]	0.33	4.2	0.48	3.8	0.76
13	6.5	0.14			5.1[c]	0.33	4.4	0.48	3.8	0.84
14	6.5	0.15			4.8[c]	0.28	4.0	0.38	4.0	0.71
15	6.5	0.15			5.6	0.28	4.3	0.52	3.7	0.71
16	6.5	0.15			5.1	0.28	4.2	0.60	3.4	0.86
17	6.7	0.19			5.5	0.26	4.3	0.47	3.9	0.71

[a] Inoculated with *Lactobacillus plantarum*.
[b] Fastest rate of breaking of emulsion.
[c] Emulsion started to break.
[d] Emulsion started to break at 6 hours; T.A. = titrable acidity.

Table II. Effect of Dilution during Grinding on the Rate of Fermentation, pH and Titrable Acidity of Coconut Cream Inoculated with a 24–48 hr Sucrose Broth Culture of *L. plantarum* Samples Incubated at 40° C

Time (Hours)	Dilutions (w/v)[a] 1:1[b] pH	1:1[b] % T.A.	1:1[c] pH	1:1[c] % T.A.	1:1[d] pH	1:1[d] % T.A.	1:2[b] pH	1:2[b] % T.A.	1:2[c] pH	1:2[c] % T.A.	1:2[d] pH	1:2[d] % T.A.	1:2.5[b] pH	1:2.5[b] % T.A.	1:2.5[c] pH	1:2.5[c] % T.A.	1:2.5[d] pH	1:2.5[d] % T.A.
0	6.3	0.12	6.3	0.12	6.3	0.12	6.3	0.21	6.3	0.21	6.3	0.21	6.1	0.24	6.1	0.24	6.1	0.24
2	6.1	0.36	5.8	0.30	5.9	0.30	5.6	...	5.3	0.21	6.1	0.30	5.7	0.21	5.7	0.24	5.2	0.30
4	6.1	0.36	5.3[e]	0.45	5.6	0.42	5.7	0.27	5.1[e]	0.30	4.7[e]	0.30	6.1	0.36	5.6	0.24	5.8	0.30
6	6.0	0.42	5.1	0.54	5.5[e]	0.36	5.7	0.30	4.8	0.30	5.1	0.30	6.0	0.36	5.4	0.54	5.0	0.57
8	5.0	0.45	5.1	0.45	5.1	0.60	5.7	0.30	5.2	0.42	5.1	0.30	...	...	...	...	...	...
23	4.5[e]	0.48	5.1	0.87	5.3	0.66	4.6[f]	0.60	4.7	0.69	5.2	0.69	4.3[f]	0.39	4.6[f]	0.60	5.3	0.57[g]

[a] Grams coconut meat: ml water during grinding.
[b] Uninoculated control.
[c] Cream separated by flotation, then inoculated with organisms.
[d] Cream separated by adding 5% inoculum to whole coconut milk.
[e] Emulsion started to break.
[f] No oil separation.
[g] Oil separation after 26 hours; T.A. = titrable acidity (as lactic acid).

fermentation at 40° C (Table II), while oil separation occurred in the uninoculated samples of the same dilution after 23 hr of fermentation. Breaking of emulsion occurred after 26 hr in inoculated samples (*L. plantarum*) of the 1:2.5 dilution, while no oil was liberated in the uninoculated samples of that dilution.

The optimum dilution range for rapid demulsification and separation of oil was coconut milk containing 1:1 to 1:2 coconut meat to water (w/v). Lower proportions failed to introduce sufficient fluidity for grinding and hindered extraction of the coconut oil, protein, and other extractables. Use of higher proportions of water increased the time required to break the emulsion.

Separation of the cream by flotation normally took several hours. By inoculating the freshly extracted milk with a 5% (v/v) sucrose broth culture of either of the two species of *Lactobacilli*, however, the rate of cream separation was hastened.

Fermentation was accomplished generally without cream separation. The advantage was that the coconut milk could be inoculated and fermented immediately after extraction. The advantage of separating the cream from the skim milk portion, however, was that the bulk of the material to be fermented was reduced. Consequently, the volume of the inoculum was also reduced.

The effect of dilution on the volume of coconut cream and volume of oil and protein recovered after fermentation is shown in Table III. The volume of cream recovered remained relatively constant as dilution increased. However, the volume of oil recovered following fermentation decreased significantly as dilution increased. The higher did not grind as efficiently as the lower dilutions. Apparently the unground solids did not come into contact with the blendor blades as efficiently in the higher dilutions. Thus, more oil was retained in the residue.

Table III. Effect of Dilution during Grinding on Volume of Cream and Oil Recovered from Coconut Milk[a]

Samples	Proportion Coconut Meat[b] to Water 1:1 (w/v)	1:2 (w/v)	1:2.5 (w/v)
Coconut meat, g	1000	1000	1000
H_2O added, ml	1000	2000	2500
Before Fermentation			
Milk, ml	1577	2543	3080
Cream, ml	883	866	888
Residue, g (wet basis)	422	457	420
Residue, oil, % (dry basis)	17.5	23.9	27.9
Residue protein, % (dry basis)	7.8	7.0	6.3
Skim milk, ml	693	1677	2190
Skim milk protein, % (wet basis)	1.6	1.1	0.43
Skim milk protein, g	11.1	18.4	9.4
After Fermentation			
Oil recovered, g	137	77	65

[a] Coconut comminuted in Waring Blendor; Milk inoculated with *L. plantarum*; incubated at 40° C. [b] 3.6% protein; 30% oil.

FERMENTING MICROORGANISMS

The initial counts of microorganisms in coconut milks prepared under reasonably good conditions in the laboratory were surprisingly high, in the vicinity of 1,000,000 organisms per ml. Generally the acid producing microorganisms predominated and the pH of the milk would fall. Sometimes the emulsion would break, freeing the oil. However, under natural conditions without an added inoculum of *Lactobacillus*, the fermentation was prolonged generally beyond 24 hr and breakage of the emulsion and separation of oil often failed to occur.

The changes in pH, titrable acidity, and bacterial counts in uninoculated coconut milk and in coconut milk inoculated with *L. plantarum* or inoculated with *L. delbrueckii* are shown in Table IV. The predominant organisms in the uninoculated samples were gram-positive long and short rods occurring singly. As fermentation progressed what appeared to be a nearly pure culture of gram-positive rods, occurring singly, developed. As the pH slowly decreased and titrable acidity increased, the bacterial count slowly increased.

Inoculating the fermenting coconut milk with *L. plantarum* and incubating at 40° C effected a rapid breaking of the emulsion and liberation of the oil in the majority of batches. *L. plantarum* multiplied faster and was more reliable in this regard than *L. delbrueckii*, which had been suggested and used by several other processes (Beckman, 1930; Horovitz-Vlasova and Novotelnov, 1935; Soliven and de Leon, 1936).

The higher counts of predominating organisms undoubtedly increase the rate of breaking of the emulsion. After 6 hr of incubation at 40° C, samples inoculated with *L. plantarum* showed a total count of 3×10^9 at the start of breaking of the

Table IV. Changes in pH, Titrable Acidity (as Lactic Acid) and Bacterial Counts in Uninoculated and Inoculated[a] Coconut Milks[b]

Time Hours	Uninoculated Control[c]			L. delbrueckii[d]			L. plantarum[e]		
	pH	% T.A.	col./ml	pH	% T.A.	col./ml	pH	% T.A.	col./ml
			10^6			10^6			10^6
0	6.0	0.10	1	6.0	0.10	1	6.0	0.10	1
2	6.0	0.13	3	6.1	0.16	28	5.4	0.16	63
4	6.0	0.13	8	6.2	0.16	30	5.3	0.16	184
6	5.8	0.16	23	6.2	0.16	80	4.7	0.26	2780
8	5.7	0.20	28	5.6	0.20	340	4.4	0.49	3620
10	5.5	0.25	94	5.2	0.25	385	4.3	0.58	4380
12	5.1	0.36	158	4.8	0.30	434	4.2	0.67	5290

[a] 24-hour sucrose broth cultures.
[b] Coconut milks 1:1 (w/v) dilution (coconut meat to water), incubated at 40° C.
[c] Emulsion started to break at 12 hours.
[d] Emulsion started to break at 10 hours.
[e] Emulsion started to break at 6 hours.

emulsion. At the same time, only 8×10^7 cells of *L. delbrueckii* were present and 2×10^7 cells had developed in the uninoculated control. The latter two samples did not show signs of breaking of emulsion at this point.

INFLUENCE OF TEMPERATURE

A temperature of 40° C resulted in a faster breakage of the emulsion with separation of oil than temperatures either higher or lower (Table V).

At 30° C there was separation of oil only after 23 hr of incubation. At 40° C oil separation appeared in from 4 to 8 hr. Delicate coconut aroma was present throughout the fermentation process. At 50° C cooked coconut oil odor was perceptible; separation of oil occurred also at 23 hr of incubation.

Table V. Effect of Temperature on the Rate of Fermentation, pH and Titrable Acidity of Coconut Milk[a]

Time Hours	30[b]		40[c]		50[b]	
	pH	% T.A.	pH	% T.A.	pH	% T.A.
0	5.9	0.13	5.9	0.13	5.9	0.13
2	5.4	0.22	5.4	0.22	5.3	0.19
4	5.1	0.22	5.0	0.29	5.0	0.22
6	4.2	...	4.4	0.35	4.6	0.28
8	4.2	0.31	4.1	0.44	4.2	0.31
10	4.1	0.35	3.9	0.47	4.2	0.38
12	3.8	0.53	3.9	0.63	4.1	0.38

[a] Extracted 1:1 w/v with H_2O and inoculated with *L. plantarum*. Emulsion started to break at 23 hr. [c] Emulsion started to break at 8 hr. T.A. = titrable acidity (as lactic).

INFLUENCE OF OXYGEN TENSION

Samples fermented in Petri dishes developed molds on the surface. Those in the test tubes produced very distinct separation of oil. When Erlenmeyer flasks were used, the fill of containers affected the rate of breaking of the emulsion. Oil separated 4 to 8 hr in containers which were half filled and covered with rubber stoppers. When the flasks were filled completely and covered with rubber stoppers, breaking of emulsion was delayed. Also, the gas produced by the organisms exerted so much pressure on the stopper that the oil overflowed from the container. This suggests that the fermentation process is neither aerobic nor anaerobic, but that the microorganisms involved are microaerophilic. This is characteristic of genus *Lactobacillus*.

EFFECT OF ADJUNCTS

Using individual adjuncts—yeast extract, tryptone, sucrose, lactose, and a combination of tryptone-sucrose—yeast extract did not improve the separation of oil by fermentation. Separation of oil started in 4 to 8 hr after incubation in all samples, except in the uninoculated control in which the emulsion failed to break.

The oil acquired the characteristic odor of the yeast extract or tryptone when they were used. With sucrose and lactose, a delicate coconut odor prevailed during the fermentation process.

INFLUENCE OF GRINDING

To determine the effect of extent of rupturing the cells on the yield of oil and protein, three methods of grinding were used, namely the traditional grater alone, grater plus Waring Blendor, and grater plus Rietz disintegrator.

The results showed that the Rietz disintegrator was the most efficient with regards to rupturing the cell, thus liberating a higher percentage of oil. Although the fermentation process worked quite well with samples ground in the Waring Blendor, in no case was fermentation separation of oil achieved in coconut samples ground in the Rietz. Apparently the more intense mixing achieved in the Rietz produced a coconut milk emulsion in which the factor(s) responsible for stability of the emulsion was intensified.

Attention was focused mainly on the use of grater *vs.* grater plus Waring Blendor. 19.29% oil and 2.84% protein remained in the residue when the grater alone was used. However, when the grated samples were comminuted further in a Waring Blendor, the cells were more disintegrated so that only 11.45% oil and 1.10% protein remained in the residue. The protein in curd after fermentation was 22.15% and 21.45% when grater plus Waring Blendor and grater alone, respectively, were used. The conclusion was reached that grater plus Waring Blendor is the preferred method of rupturing coconut cells, if the fermentation method of oil separation is to be used.

QUALITY OF THE OIL RECOVERED

The oil recovered had a free fatty acid value similar to that found in freshly extracted coconut milk, which was 0.048% expressed as oleic acid. This shows that the fat was definitely unaltered during the liberation of the oil. The moisture content of the oil was 0.20%. Specific gravity was 0.892

at 26° C. Refractive index was 1.4535. The oil had a delicate coconut odor.

After 1 month storage, the free fatty acid of the pasteurized sample rose to 0.37%, and the unpasteurized sample was 0.59%.

DISCUSSION

Results suggest that at least two factors may be related to emulsion stability, and therefore their removal or modification by fermentation may be related to breaking of the coconut milk emulsion with separation of oil. The first is removal of fermentable sugars which may serve as emulsifier. The second is precipitation of soluble protein as acid is produced during fermentation. Removal or modification of either of these factors or both may be responsible for breaking the emulsion. Unfortunately, however, this does not explain why some coconut milk emulsions processed by fermentation under controlled conditions failed to break and release their oil. There must be factors other than fermentable sugars and proteins related to emulsion stability.

In view of the coconut milk emulsions which failed to break using the described process, further studies should be undertaken using other organisms with different enzyme complements in the hope that one may be found capable of breaking the more stable emulsions.

Further studies should include comparison of the fermentability of germinated *vs.* nongerminated coconuts and coconuts of different maturities.

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