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Effect of different pre-treatments of fresh coconut kernels on some of the quality attributes of the coconut milk extracted

Viduranga Y. Waisundara, Conrad O. Perera *, Philip J. Barlow

Department of Chemistry, Food Science and Technology Program, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore Received 11 October 2005; received in revised form 19 February 2006; accepted 23 February 2006

Abstract

Fresh, dehusked and shelled coconut kernels were subjected to blanching and freezing treatments and were tested for chemical and enzymatic deterioration and yield of milk by monitoring the percentage extraction of coconut milk, free fatty acid (FFA) content, peroxide value (PV), lipase (LIP) and peroxidase (POD) activities, once each fortnight for a total period of 8 weeks. The percentage extraction of milk varied between 31.0% and 33.5% in the blanched samples and did not show a significant change at p > 0.05, as compared to the values at week 0. Similar observations were seen in FFA content and the PV. The LIP activity in these samples decreased to almost 0.176% liberated FFA and POD activity to 0.387 Absorbance Units/g fresh-weight. In conclusion, the results indicated the efficiency of the pre-treatment in suppressing the chemical and enzymatic deterioration of coconut kernels, which normally results in the loss of quality of the coconut milk when extracted.

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Keywords: Free fatty acids; Peroxide value; Lipase; Peroxidase

1. Introduction

Many attempts have been made to stabilize coconut milk against microbial and chemical spoilage (APCC, 1994; Gwee, 1988; Seow & Gwee, 1997; Timmins & Kramer, 1977). Coconut milk has an oil content of 30–35%, the actual content vary due to intrinsic factors of the coconuts themselves (Jeganathan, 1970). However, it is recognized that the product is one that is highly susceptible to chemical and biochemical spoilage such as lipid oxidation.

Commercially, shelf life extension of coconut milk has been achieved primarily through canning, aseptic packaging and spray drying (Seow & Gwee, 1997). Heat processing is an effective means of extending the shelf life of coconut milk. Short-term preservation can be easily achieved by pasteurizing the milk at 72 °C for 20 min,

E-mail address: pereraco@hotmail.com (C.O. Perera).

but long-term storage can only be achieved by using a more stringent heating regime that ensures commercial sterility of the product (Seow & Gwee, 1997). For instance, while pasteurized coconut milk has a shelf life of not more than 5 days at $4 \,^{\circ}$ C (Gwee, 1988), canned coconut milk can last for at least 24 months under ambient storage conditions (Anon, 1985). In retorting, the milk is normally processed to an F_0 of about five (APCC, 1994; Timmins & Kramer, 1977). Such drastic heat treatment is required because raw coconut milk is a low-acid liquid food, with a pH of around 6.2. However, coconut milk coagulates readily upon heating to $80\,^{\circ}$ C (Hagenmaier, 1983; Steinkraus, David, Ramos, & Banzon, 1968). This may result in unacceptable curdled products, particularly in more concentrated forms of coconut milk (Seow & Gwee, 1997).

With respect to the different methods employed to extend the shelf life of fresh coconut milk, very little work has been carried out on investigating how pre-treatments could be applied to the coconut-kernel itself prior to milk extraction, in order to obtain commercially acceptable fresh coconut milk. Although, sophisticated methods such

^{*} Corresponding author. Present address: P.O. Box 462, Palmerston North 5301, New Zealand. Tel.: +65 65167961; fax: +65 67757895 (Singapore).

as spray drying may meet the aims and objectives of achieving the required period of shelf life of the extracted milk, such implementations are costly and require technological competency during the application (Punchihewa, 1999). There seems to be a demand for 'fresh' coconut milk in places where availability of coconuts is limited or absent. The effect of different pre-treatments of the coconut kernels on some of the quality attributes of the coconut milk extracted was the main subject of this study.

The pre-treatments studied in this paper involved the application of different blanching treatments followed by freezing of fresh coconut kernels before the extraction of coconut milk. The percentage extraction of coconut milk, free fatty acids (FFA) content, peroxide value (PV), lipase (LIP) and peroxidase (POD) activities were chosen as indicators of, chemical and enzymatic deterioration of the extracted coconut milk.

Special attention was paid to the degree of inactivation of the two enzymes referred to above. The two most common enzymes, which required their activities to be arrested in coconut kernels, are lipoxygenase (LOX) and lipase (LIP). Lipases (glycerol ester hydrolases E.C. 3.1.1.3) in coconut milk hydrolyze the ester bonds of tri-acyl glycerols (TAG). Lipase activity increases with the maturity of any fruit or vegetable thus, increasing the FFA content in the material (Huang, 1984). The predominant FFAs in coconut milk consist of the saturated fatty acids lauric, palmitic and stearic and the unsaturated oleic acid. A higher amount of these FFAs would indicate a higher activity of the lipase enzyme, thus indicating a higher rate of spoilage of the coconut milk due to chemical degradation.

The primary aim of blanching is the inactivation of enzymes that cause undesirable changes during the processing and subsequent storage of food products. Peroxidase (POD) and lipoxygenase (LOX) could be considered as indices of adequacy of blanching (Pilnik & Voragen, 1991). However, in this study, POD was chosen over LOX due to the ease of assaying its activity and also for the fact that LOX would have a lower threshold temperature of inactivation than POD.

2. Materials and methods

2.1. Raw materials

The coconuts were provided by Sin Gin Him Pvt. Ltd., Clementi, Singapore. The chosen variety of coconuts was harvested from Johor Bahru, Malaysia, prior to June, 2004. Care was taken in choosing the coconuts, and only those, which were at the same stage of maturity, as assessed by the colour of the husks, were used.

The coconuts were de-husked and de-shelled at the company and brought to the laboratory in sterilized bags. Each de-shelled coconut was cut into 16 triangular shape pieces of approximately uniform dimensions, by initially cutting a whole coconut into two halves and thereafter, continuing the procedure on each of the halves.

2.2. Sample preparation

Approximately equal, triangular pieces of coconut kernels from 25 large, healthy coconuts were pooled together. Three random samples of approximately 400 g each were selected for coconut milk extraction, and different analyses of the milk as described subsequently. This was considered as the week 0 sample without any treatment.

2.3. Pre-treatments

The pre-treatments consisted of (1) blanching, (2) sanitization, (3) vacuum packing and (4) freezing. The randomized pieces of coconut kernels prepared earlier were divided approximately into three lots of 5 kg each, and the first lot was blanched at 85 °C for 25 min, the second lot at 90 °C for 18 min, and the third lot at 95 °C for 12 min to inhibit the LIP, LOX and POD (Pilnik & Voragen, 1991). The respective blanching times were chosen as the time for the interior of the pieces of coconut to reach the required temperatures as determined previously, by using a thermocouple.

Each of the three lots of blanched samples were sanitized to minimize the microbial load of the samples using Aqua-Plus 5 (Biocide Asia – Pacific Pvt. Ltd., Singapore), a chlorine dioxide based food grade commercial sanitizer. Sanitizer solution containing 100 ppm of ClO₂ was prepared by adding 2 ml of Aqua-Plus 5, to a glass container along with 0.2 g of citric acid to release the ClO₂. The container was swirled for 15 min. Distilled water was added to the resulting mixture and transferred to a volumetric flask to make up to 1000 ml. The blanched coconut pieces were rinsed well and completely immersed in the solution. As the sanitizing process with chlorine dioxide was considered to be spontaneous, the coconuts were kept immersed for a period of only 15 s before taking them out and leaving to air-dry on a previously sterilized stainless steel table.

Each of the three lots of blanched, sanitized samples was packed into 12 bags, each of approximately 400 g (altogether 36 bags of 400 g), vacuum sealed, and stored under frozen conditions at $-18\,^{\circ}\text{C}$.

Three sample bags were randomly drawn from each of the three blanched samples every fortnight, thawed as described below. Milk was extracted and analyzed for different quality parameters as described below.

2.4. Control samples

Another about 10 kg of randomized coconut kernel pieces prepared earlier were sanitized with 100 ppm ClO₂ solution and divided into two lots of approximately 5 kg each. One lot served as a control for no-blanching and no-freezing, which was packed into 12 bags of approximately 400 g each, vacuum sealed and were left at room temperature (23 °C). The other lot served as the control

for no-blanching but with-freezing, which was also packed into 12 bags of approximately 400 g each, vacuum sealed and were frozen at $-18\,^{\circ}\text{C}$.

Three sample bags were randomly drawn from each of the control lots every fortnight for extraction of milk and analysis of the quality parameters as the test samples.

2.5. Packaging

For the purpose of vacuum packing, a Stephanal Alvac vacuum packing machine (Stephanal Alvac, MAP Sensomatic, Almelo, Holland) was used with the default settings at 95.0% vacuum with a sealing pressure of 400 kPa, a sealing time of 2 min and a sealing bar release time of 2 min. The packaging material consisted of high-density polythene bags with a thickness of 0.1 mm available in the dimensions of 21×30 cm.

2.6. Determination of thawing conditions of coconuts for the extraction of coconut milk

The 400 g bags of frozen coconut kernels were thawed using a Certomat® water bath (Certomat® WR, B. Braun, Holland). The chosen thawing temperatures were 30, 35, 40 and 45 °C (Cancel, 1979). The coconut pieces were kept in their bags at these respective temperatures for a uniform period of 20 min. The coconut kernels were grated, placed in a muslin cloth bag, and a pressure of 1379 kN/m² exerted for 15 min to extract the coconut milk.

2.7. Extraction of coconut milk

The pressure and holding time of grated coconuts for the percentage extraction of coconut milk, were maximized prior to the commencement of the experiment as follows. The coconuts were grated in a blender (Braun, Singapore) in samples of approximately 50 g at a blending time of 10 min at maximum speed. Small amounts of exudates observed in the bags after thawing were incorporated into the blender. Uniform amounts of 400 g of grated coconuts were placed in a muslin cloth bag and subjected to extraction pressures of 689, 1034, 1379, 1724 kN/m², respectively, with holding times of 5, 10 and 15 min for each of the values of pressure (Table 1), using a laboratory rack and cloth press. The values of the pressure and the respective holding time for which the maximum percentage milk extraction observed, were chosen as the standard conditions to be used during subsequent extractions of coconut milk throughout the study. The percentage extraction was calculated as volume of milk (ml) extracted per 100 g of grated coconuts.

2.8. Free fatty acid content

The FFA content was determined using the AOAC method 922.02 (2002). The percentage FFA was calculated as lauric acid (molecular weight = 200.32) per 100 ml of coconut milk, considering lauric acid to be the predominant fatty acid present in the coconut milk sample.

Table 1
Percentage extraction of coconut milk for variations of pressure applied at different holding times on fresh coconut kernels

Holding time (min)	Percentage extraction of coconut milk (v/w %) ^A	
5	$26.35 \pm 0.83^{\mathrm{a}}$	
10	27.22 ± 0.91^{a}	
15	$27.45 \pm 1.09^{\mathrm{a}}$	
5	$28.65 \pm 0.89^{\mathrm{b}}$	
10	$28.80 \pm 1.15^{\mathrm{b}}$	
15	28.90 ± 1.15^{b}	
5	$29.34 \pm 0.67^{ m b}$	
10	$29.75 \pm 1.25^{\mathrm{b}}$	
15	$29.80 \pm 0.87^{\mathrm{b}}$	
5	$30.27 \pm 1.67^{\mathrm{b}}$	
10	$30.50 \pm 1.58^{\mathrm{b}}$	
15	$30.86 \pm 0.96^{\mathrm{b}}$	
	5 10 15 5 10 15 5 10 15 5 10	

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups Within groups	20.39629 1.0092	3 8	6.798763889 0.12615	53.8942837	1.19277E - 05	4.066180557
Total	21.40549	11				

 $^{^{\}mathrm{a,b}}$ Values denoted by the same superscript within a column are statistically not significant at p < 0.05.

^A Figures represent means of triplicate determinations (mean \pm SD).

2.9. Peroxide value

The PV was determined as recommended by the method of the Association of Official Analytical Chemists (AOAC, 1998) for which it was necessary to extract the oil content from the coconut milk. Hence, a modification of the method for extraction of oil from food material suggested by Egan, Kirk, and Sawyer (1981) was carried out. A sample of 20 g of coconut milk was added to 20 ml of 2.05 N standardized HCl till the solids were completely dissolved in the acid. The resulting solution was heated in a water bath at 70 °C for 30 min, and thereafter immediately transferred to a water bath at 100 °C for a further 30 min. After cooling the solution, 25 ml of diethyl ether was added, followed by 25 ml of petroleum ether. The mixture was centrifuged at 3000 rpm using an Eppendorf 5084 centrifuge (Eppendorf, London, UK), for 5 min at 25 °C. The diethyl ether layer was separated from the centrifuged sample using a separatory funnel and added to a Soxhlet apparatus at 50 °C, to evaporate the diethyl ether. The remaining solution was left to dry in an oven at 100 °C for another 1 h to remove further traces of diethyl ether.

The PV was calculated as mili-equivalents (meq) of peroxide O_2 per kg of oil by the following formula:

Peroxide Value =
$$\frac{N \times V}{W}$$
,

where V is the Titer volume of $Na_2S_2O_3$ (ml), W the weight of coconut oil (kg) and N the normality of the $Na_2S_2O_3$ solution.

2.10. Lipase activity

An extract of coconut milk lipase in acetone powder was prepared according to Hassanien and Mukherjee (1986), modified as follows. A sample of 25 g of coconut milk was added to cold acetone at 4 °C and homogenized. This crude extract was filtered through a cheese-cloth to remove precipitating material from the solution (Abigor et al., 2002). The residue was left to dry at room temperature (23 °C) to yield the acetone powder and left in the chiller at 4 °C till the assay was conducted.

Lipase activity was assayed by the titrimetric method of Khor, Tan, and Chua (1986) modified as follows. The assay mixture contained 5 g of coconut oil extracted according to the method suggested under Section 2.7, 2.5 ml of hexane to solubilize the oil and 1 g of the crude enzyme extract. The mixture was incubated at 30 °C for a period of 1 h with continuous stirring, using a magnetic stirrer. At the end of the incubation, 25 ml of acetone–ethanol (1:1 v/v) were added to stop the reaction and to extract the FFA's liberated. The FFA's in the mixture were then estimated by direct titration with a standardized solution of 0.0503 N NaOH using phenolphthalein as indicator. Lipase activity was expressed as the percentage of FFA's liberated after 1 h of incubation (Khor et al., 1986).

2.11. Peroxidase activity (POD)

The method employed by Tisiskens et al. (1997) was used to extract soluble POD. A sample of 30 g of coconut milk was homogenized in 90 ml of 50 mM Tris-Mes buffer, pH 8.8, containing 1% polyclar AT in a blender (Braun, Singapore) for 5 min at 4 °C. The resulting solution was centrifuged at 13,000g for 10 min in an Eppendorf 5084 centrifuge (Eppendorf, London, UK), and the supernatant was removed and assayed for soluble POD. The sediment fraction was retained for extraction of bound POD, where it was re-extracted using the steps stated above with 0.4 M CaCl₂ as the extraction buffer. The POD assay was conducted by applying a few modifications to the method suggested by Tisjskens et al. (1997). Extracts of 50 µl were assayed in a cuvette containing 2.7 ml of 50 mM Tris-Mes buffer, at pH 5.5, with 50 µl of O-dianisidine as a hydrogen donor and 100 µL of 1% H₂O₂ as the oxidant. Activity was assayed at 470 nm for 5 min at 25 °C using the UV-vis spectrophotometer – 1240 (Shimadzu, Japan). One unit of activity was defined as the change in absorption of 1.000 per min.

2.12. Chemicals

All chemicals used in the research were of analytical grade and purchased from either Sigma Chemicals (Singapore) or BDH Chemicals (BDH Ltd., Poole, UK) unless otherwise specified.

2.13. Statistical analyses

The results of the analyses were reported as mean \pm SD. The least significance difference (LSD) was calculated using the statistical software, SPSS version 12.0 for Windows. This was performed by comparing the values via one-way ANOVA at a 95% confidence level (p < 0.05).

3. Results and discussion

3.1. Best conditions of extraction of coconut milk from fresh kernels

The percent extraction of milk tended to increase with the increase in pressure and holding time (Table 1). However, none of the values above an applied pressure of 689 kN/m^2 were statistically significant at p < 0.05. At the highest pressure of 1724 kN/m^2 , the muslin cloth bag used to contain the grated coconuts tended to break under pressure and leak its contents. Therefore, a pressure of 1379 kN/m^2 for holding time of 15 min was chosen as the best conditions for the extraction of coconut milk during subsequent trials.

3.2. Best thawing conditions of frozen coconuts for the extraction of coconut milk

An increase in the percentage extraction of coconut milk was observed with the increase in the thawing temperature

Table 2
Percentage extraction of coconut milk versus the temperatures used to thaw frozen coconuts

Thawing temperature of water (°C)	Percentage extraction of coconut milk (v/w %) ^A		
30	$25.67 \pm 0.15^{\mathrm{a}}$		
35	$26.89 \pm 0.44^{\mathrm{b}}$		
40	$27.34 \pm 0.25^{\mathrm{b}}$		
45	$28.95 \pm 0.34^{\rm c}$		

 $[\]overline{a,b,c}$ Values denoted by the same superscript within a column are statistically not significant at p < 0.05.

(Table 2). The results were similar to those reported by Cancel, Rivera-Ortiz, and de Hernandez (1976a, 1976b). The temperature of 45 °C was chosen as the most appropriate thawing condition with reference to the highest percentage extraction obtained under the conditions of the study.

3.3. Percentage extraction of coconut milk

A significant decrease in the percentage extraction of milk was observed throughout the period of analysis, in the coconut samples, which were not subjected to any blanching treatment (Fig. 1). The percentage extraction of milk in unblanched/frozen samples, decreased from $31.21 \pm 0.06\%$ at week 1 to $27.90 \pm 0.20\%$ at week 8 whereas the control samples (unblanched/non-frozen) decreased from $30.45 \pm 0.04\%$ to $25.11 \pm 0.90\%$. However, unblanched/frozen samples, showed a lower rate of decrease than the control samples. The blanched samples did not show any significant differences in values, although they showed a slight tendency to increase with time of frozen storage. This clearly shows that blanching has a beneficial effect on the extractability of milk from frozen coconut kernels.

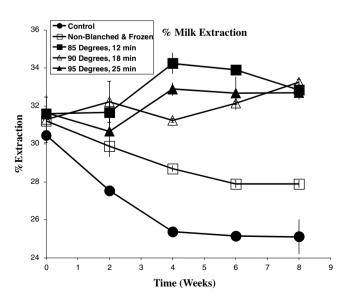


Fig. 1. Effect of different blanching treatments and frozen storage on the extractability of coconut milk.

3.4. Free fatty acid (FFA) content

The FFA content of fresh coconut kernel was $0.55 \pm 0.1\%$ as lauric acid. Similar values were reported by Cancel (1979). The FFA content of the control samples, increased steadily with increase in storage time. This may be due to the microbial lipase that may have been produced due to growth of microorganisms during storage at room temperature of the control samples. However, the FFA content of the unblanched/frozen sample remained constant throughout the storage period (Fig. 2). This could be associated with the arrest of LIP by freezing conditions (Fig. 3). The increase in the FFA content in the control samples, which were not subjected to freezing, further supports this conclusion. The FFA content in the blanched and frozen samples slightly decreased and maintained constant throughout the storage period. This may be due to

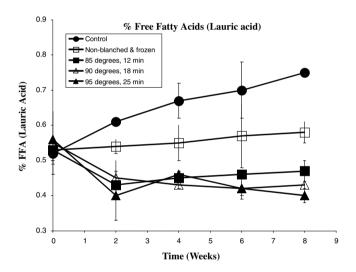


Fig. 2. Effect of different blanching treatments and frozen storage on the FFA content of coconut kernels.

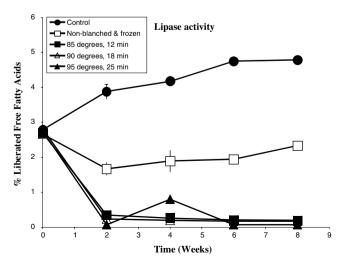


Fig. 3. Effect of different blanching treatments and frozen storage on the lipase activity of coconut kernels.

A Figures represent means of triplicate determinations (mean \pm SD).

possible partial washing out effect of the initial FFA in the fresh coconut kernels during the water blanching treatment.

3.5. Peroxide value

The value for which the PV indicates the onset of rancidity is 35.5 meg of peroxide O2 per kg of oil (Anon, 1985). All blanched samples had PVs below 27.5 meg of peroxide O₂ per kg of oil, indicating that the coconut samples were not classified as rancid. In fact there was no apparent rancid odor in any of the blanched frozen samples. The controls reached the level of rancidity at week 4 (Fig. 4). There was a strong rancid odor in the control samples from week 4 onwards. The samples subjected only to freezing showed an increase in PV although at a comparatively lesser rate than that of the control samples. The minor differences in PV values observed in the blanched/frozen samples during the storage period were statistically not significant. This shows that the blanching treatments given to the coconut kernels were adequate to inactivate peroxidase enzymes (Fig. 5) and hence, lipoxygenase as well. The results were similar to those obtained in the research by McLellan and Robinson (1981) on peroxidase and lipoxygenase enzymes of Cabbage and Brussel Sprouts, where the PV decreased along with the peroxidase and lipoxygenase enzyme activities.

3.6. Lipase activity

Lipase activities of the samples are shown in Fig. 3. The overall lipase activity of the fresh coconut kernels at week 0 was between $2.77 \pm 0.10\%$ liberated FFA. These values were close to those obtained by Enujiugha, Thani, Sanni, and Abigor (2003), who reported lipase activity of

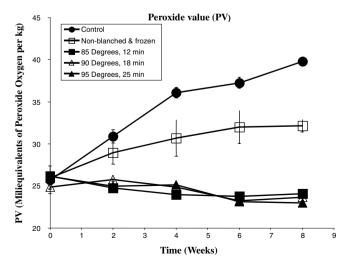


Fig. 4. Effect of different blanching treatments and frozen storage on the PV of coconut kernels.

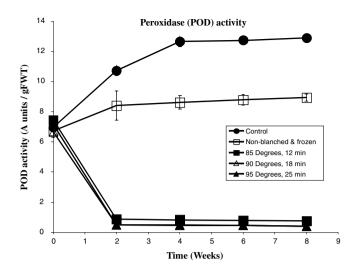


Fig. 5. Effect of different blanching treatments and frozen storage on the peroxidase activity of coconut kernels.

 $2.5 \pm 0.1\%$ liberated FFA in fresh coconut milk. The enzyme activity in the control samples steadily increased in value with increase in time of storage. This may be due to the microbial lipase that may have been produced due to growth of microorganisms during storage at room temperature of the control samples. However, the unblanched/frozen samples maintained a steady lipase activity over the period of the study. Lipase is known to be active under temperature conditions below the freezing point of water, although it may not be as active as under comparatively higher temperatures. The lipase activities in the blanched frozen samples were insignificant, indicating that all the blanching treatments were adequate to inactivate all lipase activity in the coconut kernels. The minor differences in the lipase values seen between blanching temperatures of 85 and 95 °C were statistically not significant at p < 0.05.

3.7. Peroxidase activity

The control samples showed a continuous increase in the POD activity until week 4, followed by a lesser rate of increase until the values remained almost constant (Fig. 5). This may be due to product inhibition after week 4. The unblanched samples subjected to freezing did not show any statistically significant change in the activity at p < 0.05, and thus the values remained constant throughout the 8 weeks of analysis. As in the case of lipase activity, significantly decreased values were observed in the blanched samples where the POD activity was almost completely arrested. The overall POD activity of the blanched samples reduced to almost zero after blanching, indicating the blanching treatments were adequate to inactivate peroxidase enzymes. As with the earlier parameters studied, the difference between the POD activity values of the blanched samples was not statistically significant.

4. Conclusions

The pre-treatments investigated in this research were unique as they were applied to the coconuts before the extraction of coconut milk. Blanching and freezing together had a beneficial affect over maintaining a uniform percentage extraction of coconut milk with respect to time, as compared with the unblanched frozen samples or the controls, over the time period of the study. The FFA and PV values were clearly suppressed by blanching and freezing treatments applied. There was corresponding suppression of the activities of LIP and POD by blanching. No rancid off-flavors were detected in any of the blanched, frozen samples studied. Even though the PV, and FFA were not significantly altered in the frozen unblanched samples, there was an increasing trend in these values with time of storage. However, no rancid off-flavors were detected in these sample, but their milk extractability was significantly reduced with time of storage. These results suggest that the blanching treatments were successful in the elimination of deteriorative enzymatic reactions, enabling the preservation of the coconut kernels over a long period of time under frozen conditions. Therefore, fresh coconut kernels may be blanched and stored frozen for extended periods of time to extract fresh coconut milk without significantly lowering the extractability or the quality of coconut milk.

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