



Effect of different treatments for the destabilization of coconut milk emulsion

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ABSTRACT

Coconut milk is an emulsion which is stabilized by naturally occurring proteins. The main objective of the present work is to explore different methods employing thermal, pH, chilling, enzyme treatments and combination of enzyme treatments followed by chilling and thawing for effective destabilization of the coconut milk emulsion. Stability of emulsion is evaluated by measuring the creaming index and observed for the changes in structure of oil droplets, using phase contrast microscope. Combination of treatments (enzyme treatment at 37 °C followed by chilling and thawing) of coconut milk emulsion has resulted in highest yield of 94.5%. Physico-chemical properties and fatty acid compositions are evaluated for coconut oil obtained by combination of treatments and compared with that of commercial coconut oil. It is found that the oil obtained by combination of treatments is low with respect to free fatty acids and peroxide value and high in lauric acid content.

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1. Introduction

Coconut milk is the natural oil-in-water emulsion extracted from the endosperm of mature coconut (*Cocos nucifera* L.) (Seow and Gwee, 1997) and it plays an important role in many traditional foods of Asian and Pacific regions (Chiewchan et al., 2006). Coconut milk contains about 54% moisture, 35% fat and 11% solid non-fat (Simuang et al., 2004; Tansakul and Chaisawang, 2006). Freshly extracted coconut milk is a stable emulsion, which requires extra energy to destabilize this emulsion (McG-lone et al., 1986). It is naturally stabilized by coconut proteins such as globulins and albumins as well as phospholipids (Tangsu-phoom and Coupland, 2008). Some of the proteins present in the aqueous phase of the coconut milk interact with fat globules and act as emulsifier by surrounding its surface (Peamprasart and Chiewchan, 2006).

Conventionally, coconut oil is produced by expelling dry copra, followed by refining during which oil is exposed to high temperatures. Oil obtained from fresh and mature coconuts without any refining is known as virgin coconut oil (VCO) (Shilhavy and Shilhavy, 2004; Marina et al., 2009a). It is colourless with characteristic coconut flavour and finds several applications in medicinal, cosmetics and cooking purposes. VCO retains the fresh aroma

and taste of coconuts whereas, the copra-based refined coconut oil will have a bland taste due to the refining process. It has more beneficial effects than copra pressed oil, since it retains most of the nutraceutical components (Nevin and Rajamohan, 2004). The natural antioxidants present in oil makes it very stable having long shelf life. The health benefits of coconut oil are mainly from the medium chain fatty acids (MCFAs). These MCFAs are similar to that of human milk and have corresponding nutraceutical benefits. The most predominant MCFA is lauric acid (45–53%). German and Dillard (2004) cited the virtues of lauric acid of having antiviral, antibacterial, and antifungal functions. Traditionally, virgin coconut oil is produced by fermentation method, where coconut milk expelled from freshly harvested coconuts is fermented for 24–36 h during this period, the oil phase gets separated from aqueous phase. Further, oil is slightly heated for a short time to remove the moisture and finally filtered (Madhavan et al., 2005). The main disadvantages of this process are low oil recovery and fermented odour, which masks the characteristic coconut flavour of the oil.

Systematic research has been in progress at CFTRI for the production of value added products from coconut (Raghavarao et al., 2008; Raghavendra et al., 2004, 2006, 2007, 2009; Rastogi and Raghavarao, 2006). The present work is one such attempt in that direction. The main objective of the present work is to explore different methods for effective destabilization of coconut milk emulsion based on thermal, pH, chilling, enzyme treatments and also combination of enzyme treatment followed by chilling and thawing.

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2. Materials and methods

2.1. Materials

Fresh and mature coconuts (10–12 months old) were procured from the local market. Commercial coconut oil sample is procured from the market (Departmental Store, Mysore). Enzyme aspartic protease [EC 3.4.23] (Activity: 2500 tyrosine units/g) of commercial grade was procured from Kaypeeyes Biotech Private Ltd., Mysore, India. All chemicals of analytical grade were procured from Merck Chemicals, Mumbai, India. For GC analysis, hexane (HPLC grade) was procured from Ranbaxy Fine Chemicals Ltd, Mumbai, India.

2.2. Extraction of coconut milk

The mature coconuts were subjected to deshelling, paring and removal of water. The white coconut kernel was disintegrated using rotary wedge cutter (Krauss Maffei, Germany). The grating was subjected to expelling in a screw press to extract coconut milk. The fat content of coconut milk ($39 \pm 1\%$) was determined by Rose–Gottlieb method (AOAC, 1990).

2.3. Destabilization of coconut milk emulsion

2.3.1. Thermal treatment

Freshly extracted coconut milk was heated in a constant temperature stirred water bath at different temperatures (40, 50, 60, 70, 80 and 90 °C) for 20 min to destabilize the coconut milk emulsion. Then each sample was subjected to centrifugation (Model: TC-4100 D, Elctrocraft, India) at 3585g for 10 min to separate coconut cream and aqueous phases. Finally cream was centrifuged at 4880g for 15 min to obtain clear oil.

2.3.2. pH treatment

Freshly extracted coconut milk has a pH 6 (control). The pH of coconut milk emulsion was varied between 3 and 5 using 0.1 N HCl and pH 7 and 10 using 0.1 N NaOH. The samples were allowed to stand for 2 h at ambient temperature (29 ± 2 °C) to destabilize the coconut emulsion. Then they were subjected to centrifugation at 3585g for 10 min to separate coconut cream and aqueous phases. Finally, coconut cream was centrifuged at 4880g for 15 min to obtain oil.

2.3.3. Chilling treatment

The coconut milk emulsion were chilled at different temperatures (5, 10, 15 and 20 °C) for 6 h and thawed to ambient conditions (29 ± 2 °C). Further, thawed coconut milk emulsion was centrifuged at 3585g for 10 min to obtain coconut cream and aqueous phases. Coconut cream was subjected to centrifugation at 4880g for 15 min to obtain oil.

2.3.4. Enzyme treatment

The coconut milk emulsion was treated with aspartic protease (Activity: 2500 tyrosine units/g) of 0.1% concentration and incubated at 25 and 37 °C for 3 h. Then it was centrifuged at 3585g for 10 min to separate coconut cream and aqueous phases. Finally coconut cream was centrifuged at 4880g for 15 min to obtain oil.

2.3.5. Combination of enzyme and chilling treatments

The coconut milk emulsion was treated with enzyme protease of 0.1% concentration and incubated at 25 and 37 °C for 2 h. Enzyme treated emulsion was centrifuged at 3585g for 10 min to obtain coconut cream and aqueous phase. Then cream was chilled at

5 °C for 6 h and then thawed to ambient temperature (29 ± 2 °C). Finally, cream was centrifuged at 4880g for 15 min to obtain oil.

2.4. Emulsion stability measurements

Creaming index, an indicator of emulsion stability, was measured according to method reported by White et al. (2007) with a little modification. Coconut milk emulsion subjected to different treatments (thermal, pH, chilling, enzyme and combination of enzyme and chilling treatments) was allowed to stand for 6 h at ambient temperature (29 ± 2 °C). All samples were separated into the cream (top) and the transparent aqueous (bottom) phases. The total height of the emulsion in the test tube (H_E) and the height of the aqueous layer (H_S) were measured. The extent of creaming was characterized by a creaming index = $100 * (H_S/H_E)$.

2.5. Microstructure of oil droplets

Coconut milk emulsion destabilized by different treatments was observed under phase contrast microscope (Olympus BX-40, USA) equipped with camera. Emulsion samples were placed on a glass slide, covered with cover slip and observed at 45× magnification using a phase contrast microscope.

2.6. Fatty acid composition

Analysis of fatty acid composition was done by gas chromatography (GC) (Model: GC-15A, Shimadzu) as per the AOCS method Ce1-62 (AOCS, 1998). Fatty acids present in oil were first converted to fatty acid methyl esters (FAME) before injecting into GC column to obtain the fatty acid profile. The injector and detector temperatures were 230 and 240 °C, respectively. The column temperature was 220 °C and nitrogen was used as a carrier gas at a flow rate of 1 ml/min.

2.7. Physico-chemical properties

The coconut oil obtained by combination of treatments was evaluated for moisture, specific gravity, refractive index, iodine value, polenske value, acid value, saponification value, unsaponifiable matter, peroxide value according to standard methods (AOAC, 2000). A commercial oil sample was also evaluated for the purpose of comparison. Free fatty acids were analyzed according to AOCS method Ca 5a-40 (AOCS, 1998) and expressed as percentage FFA as lauric acid.

2.8. Statistical analysis

All the physico-chemical analysis and fatty acid composition was carried out in triplicates for oil obtained from combination of treatments and commercial sample. Significant differences between means were determined by *t* test (independent samples for mean) using statistical package for social science (SPSS). Significance of differences was defined at $p < 0.05$.

3. Results and discussion

3.1. Effect of thermal treatment

The effect of thermal treatment on the stability of the coconut milk emulsion (quantified by oil yield) is shown in Fig. 1. The coconut oil yield was found to increase with an increase in temperature. The maximum oil yield of 86% was observed at 90 °C. Destabilization of coconut milk emulsion is due to denaturation of heat labile proteins during heating, which results in the aggrega-

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