Chapter 3

Stabilization as well as Destabilization of Coconut Milk Emulsion
Chapter 3A

Stabilization of Coconut Milk using Silica Nanoparticles
3A.1. Introduction

Coconut milk is the aqueous extract of the solid coconut endosperm which is essentially free from fibre. There appears to be considerable confusion in literature, industry and amongst consumers regarding the terms ‘coconut water’ and ‘coconut milk’. The term ‘coconut water’ refers exclusively to the natural aqueous liquid endosperm of the coconut. Coconut milk is the white, opaque protein–oil–water emulsion obtained by pressing grated or comminuted solid coconut endosperm, with or without addition of potable water or liquid endosperm i.e. coconut water (Seow and Gwee, 1997). The production and packaging of coconut milk is extensively discussed in section 1B.2.4. In most home preparations, coconut milk is extracted and consumed immediately in the form of curries or desserts. Commercially, shelf-life of coconut milk can be extended primarily through canning, aseptic packaging (like Tetra pak) and spray drying. During storage, the coconut milk emulsion separates into an oil-rich cream phase and an aqueous phase. Several attempts have been made to stabilize coconut milk against microbial (fermentation leading to organoleptic changes), chemical (oxidative rancidity) and physical spoilage (breakdown of oil-in-water emulsion). Demulsification of coconut milk is an unacceptable physical defect in both raw and processed forms of coconut milk and sometimes may be mistaken as “spoilt” by the consumer. The two phases can easily be mixed back together temporarily by stirring or shaking.

The phase separation can be retarded by high speed homogenization (to reduce the oil droplet diameter) and/or incorporation of additives such as emulsifiers (to reduce coalescence) and thickeners (to increase viscosity of
continuous phase). Research has shown that homogenising pressure as well as sterilization temperature influences the quality of canned coconut milk (Chiewchan et al., 2006). Fat content and temperature with and without homogenization have significant effect on apparent viscosity of coconut milk. Increase in fat content leads to increase in apparent viscosity whereas temperature has an inverse effect on apparent viscosity (Simuang et al., 2004). The apparent viscosity of coconut milk decreases with an increase in shear rate during homogenization (Peamprasart and Chiewchan, 2006).

Thermal processing is essential for sterilization of coconut milk, but heating of coconut milk is shown to increase flocculation and effects the stability of coconut milk (Tangsuphoom and Coupland, 2005). Hence stabilization is achieved by incorporation of additives such as small surfactant molecules (glycerol monostearate and tween 60) and/or proteins (casein) and/or thickeners (xanthan gum and guar gum). It had also been reported that addition of stabilizers to non-homogenised coconut milk has no effect on its stability (Tangsuphoom and Coupland, 2008).

Silica nanoparticles (also known as fumed silicon dioxide, Aerosil™, Cab-O-Sil™) has multifunctional properties that allows it to act as a viscosity control agent, emulsion stabilizer, suspension and dispersion agent, desiccant, etc (Villota et al., 1986). Silica nanoparticles can be safely used as an anti-caking agent in an amount not to exceed 2 percent by weight of the food (Code of Federal Regulations: 21CFR172.480, www.accessdata.fda.gov). It is added to fruit powders, table salt, vegetable extracts as well as ground spices and is known to increase their flowability and increase their shelf life. In the present study, silica nanoparticles were tested as a potential coconut milk stabilizer.
3A.2. Materials and methods

3A.2.1. Materials

Fresh and mature coconuts were purchased from local market. Sodium caseinate was procured from Best exports, Bangalore, India. Xanthan gum, Tween 60 and Guar gum were purchased from Sigma Aldrich, St. Louis, USA. Glycerol monostearate (GSM) and silica nanoparticles were procured from Loba Chemie Pvt. Ltd., Mumbai, India. Silica nanoparticles used in this study were hydrophilic fumed silica particles, with a primary particle diameter of 12 nm and a specific surface area of 200 m$^2$/g.

3A.2.2. Expelling of coconut milk

Coconuts were broken followed by separation of coconut meat from the shell and paring. The coconut meat was then thinly sliced using food processor (Preethi Mega Magic MG 175, India). Water was added to the sliced meat in the ratio 1:1 and ground to paste using wet grinder attachment of the above mentioned food processor for 1 min at maximum speed. The slurry was pressed through cheese cloth and manually squeezed to obtain coconut milk (without fibre).

3A.2.3. Emulsion stability

Emulsion stability can be indicated by creaming index, which was measured according to method described by Raghavendra and Raghavarao (2010) with a few modifications. Different additives (sodium casienate, glycerol monostearate, tween 60, xanthan gum, guar gum and silica nanoparticles) at different concentrations (0.01, 0.1 and 1% w/w) were added to freshly expelled coconut milk and homogenised at 10,000 rpm for 1 min using high
performance dispersing instrument (T 25 digital Ultra-Turrax®, IKA-Works, Inc., USA). The mixtures were poured into 25 ml measuring cylinders and allowed to stand for 1 h (as creaming of the coconut milk, which served as control stopped at 50 min). Some samples separated into cream (top) and transparent aqueous (bottom) phases. The total height of the emulsion in the cylinder \( H_E \) and the height of the aqueous layer \( H_A \) were measured. The extent of creaming was given by a creaming index and was determined by the following equation:

\[
C.I. (\%) = 100 \times \left( \frac{H_A}{H_E} \right) \tag{3A.1}
\]

### 3A.2.4. Viscosity

Ostwald viscometer was used to measure the time flow of coconut milk samples. The density of coconut milk samples was measured using pyknometer. The reference sample used was distilled water. The viscosity of samples was determined by the following equation:

\[
\eta_1 = \frac{\eta_2 d_1 t_1}{d_2 t_2} \tag{3A.2}
\]

where \( \eta_1 \) and \( \eta_2 \) are viscosities, \( d_1 \) and \( d_2 \) are densities and \( t_1 \) and \( t_2 \) are flow times of coconut milk samples and water, respectively.

### 3A.2.5. Oil droplet size analysis

The mean oil droplet size \( D_{50} \) in coconut milk samples (with/without silica nanoparticles) were analysed using particle size analyser (‘Bluewave’, Blue Laser Diffraction Particle Size Analyzer, Microtrac Inc., USA). The scattering
pattern was used to calculate the particle size of the droplets using internal software by using 1.46 as refractive index of coconut oil.

3A.3. Results and Discussion

Fresh coconut milk has mild sweet taste. It can be consumed raw as such or used as a milk substitute in tea, coffee, or baking by vegans or people allergic to animal milk. Thus, it is essential to retain its flavour as well as form till the time packaged coconut milk reaches the consumer. Effect of various additives (sodium casienate, glycerol monostearate, tween 60, xanthan gum, guar gum and silica nanoparticles) used to impart physical stability to the coconut milk emulsion are discussed in the following sections.

3A.3.1. Effect of additives on stability of coconut milk

Addition of additives/stabilizers like sodium caseinate, glycerol monostearate (GMS), tween 60, xanthan gum, guar gum and silica nanoparticles were found to affect the emulsion stability of coconut milk. From Figure 3A.1. it can be observed that addition of these stabilisers, except for GMS, had a significant effect on stability of coconut milk and the extent of which increased with an increase in the concentration of the additive. The viscosity of coconut milk varied with type and concentration of the additive (Table 3A.1). Homogenization alone (without addition of additives) had no significant effect on the viscosity of coconut milk.

Addition of sodium caseinate to coconut milk has resulted in better emulsion stability, exhibiting better stability at higher concentrations. Sodium caseinate is a well known protein emulsifier because it is a flexible protein which can unfold to form interfacial film on oil droplets in oil-in-water emulsions.
Dickinson et al. (1997) documented the influence of sodium caseinate on the stability of oil-in-water emulsions. At higher protein levels, individual droplets are protected from coalescence by the thick adsorbed protein layer and thus the emulsion becomes stable. A steady increase in viscosity was observed with increase in concentration of sodium caseinate in coconut milk resulting in higher stability of the emulsion.

Although GMS is a good emulsifier, it was not observed to impart stability to coconut milk (Figure 3A.1). This may be attributed to improper dispersal of GMS in coconut milk as GMS is insoluble in water (which is the continuous phase) and requires high speed and high pressure homogenisation. Also much difference in the viscosity (~2.23 mPa.s) was not observed with change in concentration of GMS in coconut milk (Table 3A.1). Tween 60 (Polyoxyethylene sorbitan monostearate also called Polysorbate 60), another common emulsifier, imparted poor stability to coconut milk at low concentrations of 0.01% and 0.1%, but was effective at 1% concentration.

Emulsifiers are known to coat the dispersed phase spheres and avoid coalescence. This aids short term stabilization by interfacial action (Sahin and Sumnu, 2007). Low stability of coconut milk with 0.01% and 0.1% tween 60 may be also due to reduction in viscosity (~2.00 mPa.s).

Gums are not true emulsifiers i.e. they are not involved in stabilising emulsions at interfacial level. Their function is to increase the viscosity of the aqueous phase such that it slightly exceeds that of oil leading to low tendency of dispersed oil phase to coalesce (Panda, 2010). The gums used in this study are xanthan gum and guar gum. The former is of bacterial origin (Xanthomonas campestris) while latter is of plant origin (endosperm of guar
beans). Both gums are extensively used as thickeners in food and stabilizers in cosmetics. Xanthan gum imparted highest stability to coconut milk (Figure 3A.1) but suffers from a serious drawback of very high viscosity (Table 3A.1), which may not be suitable for thin coconut milk consumers. Similar trend was followed by Guar gum as well.

Silica nanoparticles appears to impart good stability to coconut milk emulation even at low concentrations of 0.1% (Figure 3A.1). The viscosity at 0.1% concentration in coconut milk is less than that of coconut milk without homogenization. Silica nanoparticles have shown to stabilise oil-in-water pickering emulsions (Chevalier and Bolzinger, 2013; Frelichowska et al., 2009, 2010). A pickering emulsion is an emulsion that is stabilized by solid particles which adsorb onto the interface between the two phases. In the case of oil-in-water pickering emulsions, oil adsorption as well as capillary condensation of oil within silica aggregates has shown to contribute to emulsion stability.

3A.3.2. Oil droplet size

Figure 3A.2 shows effect of time on the mean diameter ($D_{50}$) of oil droplets in coconut milk with/without homogenisation and addition of 0.1% silica nanoparticles. It can be observed that coconut milk without homogenisation had least droplet diameter ($6.26 \pm 0.04 \mu m$) followed by homogenised coconut milk droplet diameter ($6.70 \pm 0.01 \mu m$) before allowed to cream under gravity. The $D_{50}$ of non-homogenized and homogenized coconut milk samples gradually increased upto 30 min ($6.51 \pm 0.05$ and $6.74 \pm 0.01$ µm, respectively) but showed sharp increase at 60 min ($9.79 \pm 0.04$ µm and $8.71 \pm 0.04$ µm, respectively) indicating coalescence of fat droplets. On the other
hand, homogenised coconut milk containing 0.1% (w/w) silica nanoparticles did not undergo droplet coalescence up to 60 min shown by constant $D_{50}$ of 7.00 ± 0.01 µm to 7.04 ± 0.01 µm.

3A.4. Conclusion

Silica nanoparticles have shown to stabilise coconut milk emulsion, by preventing coalescence of oil droplets, even at low concentrations of 0.1% (w/w). The high stability (indicated by low creaming index) with added advantage of low viscosity indicates the potential of silica nanoparticles to be incorporated in coconut milk emulsion as a stabiliser.
Table 3A.1: Viscosity of coconut milk, with and without additives

<table>
<thead>
<tr>
<th>Sample</th>
<th>Viscosity (mPa.s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>without additives</td>
</tr>
<tr>
<td></td>
<td>0.01%</td>
</tr>
<tr>
<td>Coconut Milk</td>
<td>2.20 ± 0.05</td>
</tr>
<tr>
<td>Homogenized Coconut Milk</td>
<td>2.11 ± 0.06</td>
</tr>
<tr>
<td>Sodium Caseinate</td>
<td>2.11 ± 0.02</td>
</tr>
<tr>
<td>Glycerol Monostearate</td>
<td>2.20 ± 0.01</td>
</tr>
<tr>
<td>Tween 60</td>
<td>2.00 ± 0.01</td>
</tr>
<tr>
<td>Xanthan Gum</td>
<td>3.84 ± 0.06</td>
</tr>
<tr>
<td>Guar Gum</td>
<td>3.54 ± 0.03</td>
</tr>
<tr>
<td>Silica Nanoparticles</td>
<td>2.14 ± 0.02</td>
</tr>
</tbody>
</table>

*ND – Not determined due to gel-like consistency
Figure 3.1: Effect of homogenization and stabilizers on creaming index of coconut milk
Figure 3A.2: Variation of oil droplet size with respect to time
Chapter 3B

Destabilization of Coconut Milk for Production of Virgin Coconut Oil
3B. 1. Introduction

Conventionally, oil from oilseeds is obtained by mechanical expulsion or solvent extraction. A great deal of research in the upcoming area of aqueous and enzymatic methods for achieving high yields of oil has been carried out for oil seeds like rapeseed, soybean, coconut, avocado, sunflower and peanut (Rosenthal et al., 1996). In the case of coconut, oil obtained from fresh coconut gratings through wet (via coconut milk) or dry processing (via dehydration of coconut gratings) is known as Virgin Coconut Oil (VCO). Different methods of VCO production and their details are given in Table 3B.1. VCO has more beneficial health effects than copra pressed oil, since it retains most of the nutraceutical components. The natural antioxidants present in oil make it very stable and are responsible in increasing its shelf life. The medium chain fatty acids (MCFAs) present in coconut oil are similar to that of human milk with corresponding health benefits. The most predominant MCFA is lauric acid (45-53%). The anti-microbial properties of VCO include antibacterial, anti-fungal and anti-viral properties which can be attributed to the presence of MCFAs and their derivatives formed in the gastrointestinal tract.

During wet processing, coconut milk (the aqueous extract of the solid coconut endosperm) is subjected to various treatments to destabilize the emulsion in order to obtain oil. Coconut milk contains about 54% (w/w) moisture, 35% (w/w) fat and 11% (w/w) non-fat solid (Simuang et al., 2004). The naturally present proteins and phospholipids in coconut milk act as emulsifiers by interacting with the oil droplets and provide stability to the coconut milk emulsion (Peamprasart and Chiewchan, 2006; Tangsupphoom and Coupland, 2008). Extra energy (conventionally in the form of thermal, centrifugal, pH,
chilling and thawing treatments) is required to destabilize this emulsion. The effect of different treatments like thermal, pH, chilling, enzyme treatments and combination of enzyme treatment followed by chilling, thawing for the destabilization of coconut milk emulsion to obtain virgin coconut oil have been reported by Raghavendra and Raghavarao (2010).

In order to obtain oil from coconut milk, destabilization of this emulsion is essential. Methods such as enzymatic treatment, application of ultrasound and variation of pH of coconut milk have been explored to destabilize coconut milk. The CFTRI process for production of VCO (Raghavendra and Raghavarao, 2011; Raghavendra et al., 2009) involves several steps such as enzyme treatment of coconut milk, centrifugation (twice) and chilling and thawing. An improved process with fewer steps and reduced processing time is developed as described in this chapter.

3B. 2. Materials and methods

3B.2.1. Materials

Fresh and mature coconuts (10-12 months old) were purchased from the local market. Enzymes; papain, bromelain (from pineapple stem), ficin and subtilisin were procured from Sigma-Aldrich, St. Louis, USA. Fungal protease was procured from Kaypeeyes Biotech Private Ltd., Mysore, India. All chemicals of analytical grade were procured from Merck Chemicals, Mumbai, India.

3B.2.2. Expelling of coconut milk

Fresh and mature coconuts were subjected to deshelling and paring. The white kernels were subjected to disintegration using rotary wedge cutter
(Krauss maffe, Germany) and coconut milk was expelled using screw press.

3B.2.3. Destabilization of coconut milk emulsion

3B.2.3.1. Enzyme treatment
The coconut milk emulsion was treated with papain, bromelain (from pineapple stem), ficin, subtilisin and fungal protease at different concentrations and incubated at 25 ± 2°C (room temperature), 40 ± 1°C and 55 ± 1°C for 3 h. The creaming index was calculated at time intervals of 1h as described in section 3A.2.3.

3B.2.3.2. pH treatment
The pH of coconut milk emulsion was varied between 3.0 - 10.0 by the addition of 1 N HCl or NaOH while stirring for 2 min using magnetic stirrer. The inherent pH of freshly extracted coconut milk was 6 (control). The samples were allowed to stand for 3 h at ambient temperature (25 ± 2°C) and the creaming index was estimated as described in section 3A.2.3.

3B.2.3.3. Ultrasound treatment
The destabilization studies were carried out using a 25 ml measuring cylinder which was filled with freshly extracted coconut milk and placed on the transducer of ultrasonic humidifier (Model HM-460, Holmer Products Corp., USA). The emulsion was subjected to ultrasonication at a frequency of 1.2 MHz. The creaming index was measured at regular intervals of 5 min as described in section 3A.2.3.

3B.2.4. Methods used for VCO production
The coconut milk emulsion (8.5 kg) was obtained from fresh mature coconuts (50 nos.) by the procedure described in section 3B.2.2. As per the existing
VCO process (Raghavendra and Raghavarao, 2011), coconut milk (4 kg) was treated with fungal protease enzyme at 0.1% (v/v) concentration (0.05 tyrosine units/ml of coconut milk) and incubated for 2 h. Coconut milk was then subjected to centrifugation (Disc centrifuge, Model: TA05-00-105, Westfalia separator, Germany) at 12,000 rpm to separate coconut cream (1.43 kg) and whey (2.11 kg). Coconut cream was chilled at 7 ± 1°C overnight. Coconut cream was thawed to ambient temperature followed by centrifugation at 12,000 rpm to obtain clear VCO (1.18 kg) and solid protein residue (80 g).

In the improved process for VCO production, coconut milk (4 kg) was subjected to bromelain enzyme treatment (3 tyrosine units/ml of coconut milk) and allowed to stand for 3 h. The cream and aqueous phases were visibly distinct at the end of this gravity separation for 3 h. The cream was centrifuged (Disc centrifuge, Model: TA05-00-105, Westfalia separator, Germany) at 12,000 rpm to yield clear virgin coconut oil (1.34 kg).

3B.2.5. Oil quality analysis

The oil quality analysis was carried out using AOAC (2007) methods at Food Safety and Analytical Quality Control Laboratory, CFTRI.

3B. 3. Results and discussion

3B.3.1. Enzymatic destabilization of coconut milk emulsion

The enzymes used to destabilize coconut milk emulsion were papain, bromelain (from pineapple stem), ficin, subtilisin and fungal protease and their details are given in Figure 3B.1. Preliminary results of destabilization of coconut milk emulsion at room temperature (25 ± 2ºC) using enzymes
indicated that optimum activities for bromelain and ficin were 3 units/ml and 2 units/ml, respectively, while for papain, subtilisin and fungal protease there was no significant difference in degree of destabilization when used above 1 unit/ml. Further, the experiments of enzymatic treatment for destabilization of coconut milk emulsion were carried out at enzyme activities of 1 unit/ml, 2 units/ml and 3 units/ml at different temperatures (25 ± 2ºC, 40 ± 1ºC and 55 ± 1ºC) for all the enzymes. As shown in Figures 3B.2., 3B.3. and 3B.4, the creaming index increased with an increase in time as well as temperature of incubation. Results indicate that ficin (C.I. ~60% after 3 h incubation) was the most effective at 25 ± 2ºC (room temperature) whereas bromelain was found to be more effective at higher temperatures (C.I. ~65% after 3 h incubation) when compared to the other proteases. Proteases are known to cleave proteins, which are responsible for emulsion stabilization, resulting in coalescence of oil droplets leading to enhanced creaming (Figure 1C.2). The shorter fragments of protein/peptides move towards aqueous phase facilitating the phase separation (Raghavendra and Raghavarao, 2010).

Microstructure of oil droplets obtained using phase contrast microscopy (Figure 3B.5) indicated an increase in the droplet size with an increase in temperature of incubation which is a result of coalescence of oil droplets. It may be noted that the biggest droplet size was observed at the highest temperature (55 ± 1ºC) in case of bromelain. Although ficin resulted in the highest destabilization of coconut milk at 25 ± 2ºC (room temperature), bromelain was chosen for destabilization coconut milk emulsion in the improved process, considering the cost and availability of the enzyme (Tarté, 2009).
3B.3.2. Improved process for VCO production

An improved process for production of VCO using bromelain, consisting of a single centrifugation step was developed. The improved process consumed very less time (less than 8 hrs) compared to existing process which takes more than 24 h. Pilot scale production resulted in ~14% increase in VCO yield with the improved process compared to the existing process. The increase in oil yield can be attributed to reduced number of processing steps. The existing process suffers from a major drawback of being discontinuous. The discontinuity arises due to the freezing and thawing steps involved in the existing process. In the improved process, freezing and thawing is completely eliminated thus makes it a continuous process. The removal of the freezing and thawing steps also reduces the cost of production of oil as the cold storage is not required. The quality of oil produced from existing CFTRI process and improved process was analyzed. VCO obtained from the improved process was filtered through activated charcoal. The quality analysis of the oil obtained from different processes was presented in Table 3B.1. It can be observed from the table that most of the parameters such as moisture content, specific gravity, refractive index, iodine value, acid value, peroxide value and colour were in accordance with the Asian Pacific Coconut Community (APCC) standards. Polenske value (indicator of amount of volatile fatty acid that can be extracted from fat through saponification) was lower than the standard value while unsaponifiable matter and free fatty acid content were slightly higher than the standard values. Passing oil through activated charcoal did not have much effect on the quality of oil except for slight reduction in colour. Bleaching using activated charcoal is reported for
many oils (Erickson, 1990). The fatty acid analysis of the oils obtained from different processes was presented in Table 3B.2. The fatty acid profile is practically identical for all the oils tested. All fatty acids, except for palmitic acid, are fairly in the range of the standard values.

3B.3.3. Effect of pH on destabilization of coconut milk

The inherent pH of fresh coconut milk is generally 6 ± 0.2. The effect of pH in the range of 3 to 10 on destabilization of coconut milk emulsion is shown in Figure 3B.6. Highest C.I. (%) was observed when the pH was 4, while below and above this the stability of coconut milk was not affected by variation of pH. In order to find the optimum pH for destabilization of coconut milk, the pH was varied around 4 (3 to 5). Results indicated the highest destabilization of coconut milk emulsion to be in the range of pH 4 to 4.4 (Figure 3B.7). This can be attributed to the fact that coconut proteins have their isoelectric point at pH ~ 4 which results in precipitation of proteins present at the oil-water interface, leading to faster demulsification (Marina et al., 2009b; Tangsuphoom and Coupland, 2008). Synergistic effect on destabilization was observed when bromelain (3 units/ml) was added to coconut milk at pH 4.4 (C.I. of ~53%) when compared either to pH 4.4 alone (C.I. of ~43%) or bromelain treatment (3 units/ml) alone (C.I. of ~41%) as shown in Figure3B.8.

3B.3.4. Effect of ultrasonication on destabilization of coconut milk

One of the earliest applications of ultrasound was for preparation of stable emulsions which were used in food, cosmetic and pharmaceutical industries (Mason et al., 1996). But recent studies have shown that lower power/high frequency ultrasound can be utilised for splitting oil-in-water emulsions
(Juliano et al., 2011; Nii et al., 2009). Hence, the effect of high frequency ultrasound was studied on the demulsification rate of coconut milk (Figure 3B.9). C.I. of about 20% was observed at the end of 30 min exposure to ultrasound in case of coconut milk emulsion obtained from fresh coconut without the addition of water. (C.I. was 0% for control i.e. without ultrasound treatment). Similarly, the values of C.I. for the coconut milk emulsion obtained from fresh coconut after grinding with meat-to-water ratios of 1:1, 1:2 and 1:5 were 60%, 72% and 85%, respectively, with control values of 0%, 0% and 18%, respectively. In case of undiluted coconut milk emulsion, 10 min of ultrasound treatment was found to be optimum as further increase in ultrasonication did not increase the C.I. An increase in temperature of the cream phase was observed with an increase in time of ultrasound exposure. The temperature of coconut milk was 26 ± 1°C when expelled. The temperature of cream phase after 10, 20 and 30 min of ultrasound treatment were recorded to be 28 ± 1°C, 32 ± 1°C and 38 ± 1°C, respectively. Ultrasonication is known to generate microstreaming which increases the collision frequency thereby increasing the probability of coalescence, which in turn increases demulsification. Application of high frequency ultrasound have shown enhanced creaming of fat globules in cow milk emulsion (Juliano et al., 2011) and floculation of oil droplets leading to separation of phases of canola oil emulsion (Nii et al., 2009). It can be observed from Figure 3B.10 that the combination of treatments (enzyme + ultrasonication) resulted in higher demulsification (C.I. ~53%) when compared to ultrasonication alone for 10 min (C.I. ~ 43%), enzyme treatment alone using 3 units/ml bromelain (C.I. ~48%) and control (C.I. ~19%) on incubation for 3 h. A synergistic effect of
combined treatment (enzyme + ultrasonication) was observed on destabilization of coconut milk emulsion. High power, low frequency ultrasound has been widely used for inactivation of enzymes. However, high frequency ultrasound (7 MHz) has shown to increase amylase activity (Mason et al., 1996). These results indicate potential use of ultrasound as well as proteases and their combination for effective destabilization of the coconut milk emulsion for the production of VCO.

3B. 4. Conclusion

An improved process for production of VCO using bromelain, consisting of a single centrifugation step was developed for the production of virgin coconut oil. This improved process resulted in higher yield of oil (13.5%) (without the need of a overnight cold storage step) and consumed lower time when compared to the current CFTRI process with practically no difference in the oil quality. Pretreatment of coconut milk with protease, variation of pH of coconut milk and ultrasonication have shown to increase the destabilization of coconut milk emulsion. Potential use of ultrasonication in combination with enzyme (protease) treatment for effective destabilization of the coconut milk emulsion for the production of VCO was demonstrated in the present study.
Table 3B.1: Quality analysis of virgin coconut oil obtained from different processing methods

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Parameter</th>
<th>Virgin coconut oil</th>
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<tbody>
<tr>
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<td></td>
<td>Existing process</td>
</tr>
<tr>
<td>1.</td>
<td>Moisture (%)</td>
<td>0.18</td>
</tr>
<tr>
<td>2.</td>
<td>Specific gravity (@ 30°C)</td>
<td>0.9198</td>
</tr>
<tr>
<td>3.</td>
<td>Refractive index (@ 40°C)</td>
<td>1.4480</td>
</tr>
<tr>
<td>4.</td>
<td>Iodine value</td>
<td>5.09</td>
</tr>
<tr>
<td>5.</td>
<td>Polenske value</td>
<td>8.90</td>
</tr>
<tr>
<td>6.</td>
<td>Acid value</td>
<td>1.50</td>
</tr>
<tr>
<td>7.</td>
<td>Saponification value</td>
<td>255.0</td>
</tr>
<tr>
<td>8.</td>
<td>Unsaponifiable matter (%)</td>
<td>0.70</td>
</tr>
<tr>
<td>9.</td>
<td>Peroxide value</td>
<td>BDL</td>
</tr>
<tr>
<td>10.</td>
<td>Free fatty acid (%)</td>
<td>0.80</td>
</tr>
<tr>
<td>11.</td>
<td>Colour</td>
<td>0.70</td>
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Table 3B.2: Fatty acid analysis of virgin coconut oil obtained from different processing methods

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Fatty acid</th>
<th>Virgin coconut oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Existing process</td>
</tr>
<tr>
<td>1.</td>
<td>Caprilic acid (C₈)</td>
<td>7.3</td>
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<tr>
<td>2.</td>
<td>Capric acid (C₁₀)</td>
<td>6.0</td>
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<tr>
<td>3.</td>
<td>Lauric acid (C₁₂)</td>
<td>51.8</td>
</tr>
<tr>
<td>4.</td>
<td>Myristic acid (C₁₄)</td>
<td>19.6</td>
</tr>
<tr>
<td>5.</td>
<td>Palmitic acid (C₁₆)</td>
<td>7.0</td>
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<tr>
<td>6.</td>
<td>Stearic acid (C₁₈)</td>
<td>2.4</td>
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<tr>
<td>7.</td>
<td>Oleic acid (C₁₈:₁)</td>
<td>4.8</td>
</tr>
<tr>
<td>8.</td>
<td>Linoleic acid (C₁₈:₂)</td>
<td>0.8</td>
</tr>
</tbody>
</table>
Figure 3B.1: Proteases used to destabilize coconut milk emulsion
Figure 3B.2: Effect of proteases on destabilization of coconut milk emulsion at room temperature (25 ± 2°C)
Figure 3B.3: Effect of proteases on destabilization of coconut milk emulsion at 40 ± 1°C
Figure 3B.4: Effect of proteases on destabilization of coconut milk emulsion at 55 ± 1°C
Figure 3B.5: Microscopic structure (magnification 400X) of coconut milk emulsion treated with different proteases (3 units/ml) at different temperatures for 1h
Figure 3B.6: Effect of pH (3-10) on destabilization of coconut milk
Figure 3B.7: Effect of pH (3-5) on destabilization of coconut milk
Figure 3B.8: Effect of pH (4.4), enzyme (bromelain) and combination of pH with enzyme treatment on destabilization of coconut milk
Figure 3B.9: Effect of ultrasonication on destabilization of coconut milk
Figure 3B.10: Effect of ultrasonication, enzyme treatment (Bromelain, 3 units/ml) and combination of ultrasonication with enzyme treatment on destabilization of coconut milk