PROPERTIES AND STRUCTURE OF COCONUT MILK EMULSIONS

A Dissertation in
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by
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ABSTRACT

Coconut milk is the natural oil-in-water emulsion extracted from the endosperm of mature coconut (Cocos nucifera L.), and is widely used as a food ingredient in tropical countries to provide creaminess and a unique aroma. The emulsion is naturally stabilized by coconut proteins (i.e., globulins and albumins), but it has relatively poor stability due to the insufficient quantity and quality of the proteins present. In order to improve the stability and quality of coconut milk products, various processes and food additives are normally used by the producers. However, the emulsion science underlying the mechanisms by which additives and processing affect the physical stability and properties of the emulsions are still unclear.

The first objective of this study was to investigate the effects of homogenization and heat treatment (1 h at 50°C to 90°C) on the stability of coconut milk. Fresh coconut milk had large ($d_{43}$~12 μm) but non-flocculated droplets. Homogenization reduced the size of coconut milk droplets ($d_{43}$~6 μm), but increased the degree of flocculation, presumably via a bridging mechanism. Extensive flocculation and slight coalescence was observed in either non-homogenized or homogenized coconut milk after treatment at temperatures above the denaturation temperature of coconut proteins. Flocculation was responsible for increased viscosity and retarded creaming.
The second objective was to determine the influence of pH (3 to 7), and ionic strength (up to 200 mM NaCl) which are known to affect the stability of protein-stabilized emulsions. Coconut milk largely flocculated when the pH was adjusted to close to the isoelectric point (pI~4) of coconut proteins as the electrostatic repulsion between emulsion droplets is reduced due to the loss in surface charge. The addition of NaCl induced flocculation only when the surface charge of the emulsion droplets was insufficient to prevent aggregation due to the screening effect. Again, flocculation resulted in a more viscous product with greater resistance to creaming.

The third objective was to understand the changes in bulk quality and surface properties of coconut milk emulsions due to the addition of model surface-active proteins and surfactants [up to 1 wt% sodium caseinate, whey protein isolate (WPI), sodium dodecyl sulfate (SDS), or polyoxyethylene sorbitan monolaurate (Tween 20)]. When added after the homogenization step, small-molecule surfactants broke up the flocs while protein stabilizers did not. The addition of any surface-active stabilizer before homogenization increased the efficacy of homogenization step and produced stable sub-micron sized emulsion droplets. The improved stability in all cases resulted from the displacement of interfacial coconut proteins by the added stabilizers.

The final objective of this work was to determine the effect of various thermal treatments on the stability of the stable emulsions prepared with different surface-active stabilizers. Coconut milk emulsions homogenized with proteins were stable to the freeze-thaw cycles while those prepared with small-molecule surfactants were not. The caseinate
and SDS emulsions were able to withstand the heat treatments whereas WPI and Tween 20 samples extensively coalesced following autoclave treatment at 120°C.
TABLE OF CONTENTS

LIST OF FIGURES ........................................................................................................ v

LIST OF TABLES ........................................................................................................... xii

ACKNOWLEDGEMENTS ............................................................................................. xiii

Chapter 1  Statement of the Problem ....................................................................... 1

Chapter 2  Literature Review .................................................................................. 7

  2.1  Basic emulsion science ................................................................................... 7
      2.1.1  Definition .............................................................................................. 7
      2.1.2  Emulsion ingredients ........................................................................... 7
      2.1.3  Emulsion formation ............................................................................. 10
      2.1.4  Emulsion characterization .................................................................... 12
      2.1.5  Emulsion stability ................................................................................. 22

  2.2  Coconut milk .................................................................................................. 30
      2.2.1  Coconuts ............................................................................................... 30
      2.2.2  General characteristics of coconut milk ............................................... 31
      2.2.3  Coconut milk extraction ....................................................................... 32
      2.2.4  Composition and properties .................................................................. 32
      2.2.4  Coconut milk emulsion ......................................................................... 41

References.................................................................................................................. 44

Chapter 3  Effect of Homogenization Process and Heat Treatment on the
           Stability of Coconut Milk Emulsions ......................................................... 58

  3.1  Introduction ..................................................................................................... 59
  3.2  Materials and methods ................................................................................... 60
  3.3  Results and discussion .................................................................................... 64
  3.4  Conclusions ..................................................................................................... 77

References.................................................................................................................. 77

Chapter 4  Effect of pH and Ionic Strength on the Physicochemical Properties of
           Coconut Milk Emulsions ............................................................................. 80

  4.1  Introduction ..................................................................................................... 81
  4.2  Materials and methods ................................................................................... 83
  4.3  Results and discussion .................................................................................... 88
      4.3.1  Effect of pH .......................................................................................... 89
      4.3.2  Effect of NaCl ....................................................................................... 99

References.................................................................................................................. 99
LIST OF FIGURES

**Figure 2.1**: Schematic presentation of the structure of the electric double layer........ 18

**Figure 2.2**: Schematic presentations of physical changes in oil-in-water emulsions. ................................................................................................................. 24

**Figure 2.3**: Model of 11S coconut globulin. ............................................................. 40

**Figure 3.1**: Representative particle size distribution of non-homogenized (a) and homogenized (b) coconut milks dispersed in distilled water (solid line) or SDS solution (dashed line). ..................................................................................65

**Figure 3.2**: Micrographs taken of non-homogenized (a, c, e) and homogenized (at 40/4 MPa) (b, d, f) coconut milks either unheated (a, b) or heated to heated to 50°C (c, d) or 90°C (e, f)...............................................................................................67

**Figure 3.3**: Mean particle size of coconut milks homogenized at (●, ○) 20/2, (■, □) 40/4, and (▲, Δ) 60/6 MPa..............................................................................68

**Figure 3.4**: Mean particle size of (●, ○) non-homogenized and (■, □) homogenized (at 40/4 MPa) coconut milks heated at different temperatures. .....70

**Figure 3.5**: Flow behavior index (a), and consistency coefficient (b) of (●) non-homogenized and (○) homogenized (at 40/4 MPa) coconut milks heated at different temperatures. .................................................................72

**Figure 3.6**: Creaming index after 24 h storage of (●) non-homogenized and (○) homogenized (at 40/4 MPa) coconut milks heated at different temperatures. .....74

**Figure 3.7**: Free oil solvent-extracted from (●) non-homogenized and (○) homogenized (at 40/4 MPa) coconut milks heated at different temperatures. .....76

**Figure 4.1**: Mean particle size of coconut milk emulsions adjusted to different pH values. .............................................................................................................90

**Figure 4.2**: Optical micrographs taken of coconut milk emulsions adjusted to pH 3 (a), 3.5 (b), 4 (c), and 6 (d). .................................................................91

**Figure 4.3**: ζ-potential of coconut milk emulsions adjusted to different pH values...92

**Figure 4.4**: LSCM images overlaid with corresponding images from DIC transmitted light of coconut milk emulsions adjusted to pH 4 (a), and 6 (b). ......95
Figure 4.5: Steady shear viscosity of coconut milk emulsions adjusted to pH (●) 3, (♦) 3.5, (■) 4, and (▲) 6. ................................................................. 96

Figure 4.6: Creaming index after 24 h storage of coconut milk emulsions adjusted to different pH values. ................................................................. 98

Figure 4.7: Mean particle size of coconut milk emulsions adjusted to pH (●) 3, (■) 4, and (▲) 6 as affected by the addition of NaCl .................................................... 100

Figure 4.8: Optical micrographs of coconut milk emulsions adjusted to pH 3 (a to c), 4 (d to f), and 6 (g to i) as affected by the addition of 50 (a, d, g) 100 (b, e, h), and 200 (c, f, i) mM NaCl. ................................................................. 101

Figure 4.9: ζ-potential of coconut milk emulsions adjusted to pH (●) 3, (■) 4, and (▲) 6 as affected by the addition of NaCl ............................................................ 102

Figure 4.10: Steady shear viscosity of coconut milk emulsions adjusted to pH 3 (a), 4 (b), and 6 (c) as affected by the addition of (●) 0, (♦) 50, (■) 100, and (▲) 200 mM NaCl ................................................................. 104

Figure 5.1: Representative particle size distribution of (●, ○) non-homogenized and (■, □) homogenized coconut milks. ...................................................... 120

Figure 5.2: Representative images from optical microscopy (a, b), LSCM overlaid with corresponding images from DIC transmitted light (c, d), and cryo-SEM (e, f) taken of non-homogenized (a, c, e), and homogenized (b, d, f) coconut milks. ................................................................. 121

Figure 5.3: Creaming index after 24 h storage of (●) non-homogenized and (■) homogenized coconut milks. ................................................................. 122

Figure 5.4: Mean particle size of coconut milk emulsions with (●, ○) sodium caseinate, (■, □) WPI, (▲, Δ) SDS, or (♦, ◊) Tween 20 added without (a), after (b), or prior to (c) homogenization.................................................... 124

Figure 5.5: Representative micrographs taken of coconut milk emulsions with 1 wt% sodium caseinate (a to c), WPI (d to f), SDS (g to i), or Tween 20 (j to l) added without (a, d, g, j), after (b, e, h, k), or prior to homogenization (c, f, i, l). ............................................................................................................................ 126

Figure 5.6: Creaming index after 24 h storage of coconut milk emulsions stabilized with sodium caseinate (a), WPI (b), SDS (c) or Tween 20 (d) added (●) without, (▲) after, or (■) prior to homogenization. .................................................... 127

Figure 5.7: Representative particle size distribution of coconut milk emulsions with sodium caseinate (a), WPI (b), SDS (c) or Tween 20 (d) added at
concentrations of (●) 0, (∇) 0.1, (■) 0.25, (◊) 0.5, or (▲) 1 wt% prior to homogenization.................................................. 131

Figure 5.8: Creaming index of coconut milk emulsions with sodium caseinate (a), WPI (b), SDS (c) or Tween 20 (d) added at concentrations of (●) 0, (∇) 0.1, (■) 0.25, (◊) 0.5, or (▲) 1 wt% prior to homogenization.................................................. 134

Figure 6.1: Surface protein load of coconut milk emulsions stabilized with SDS (a), Tween 20 (b), WPI (c), or sodium caseinate (d) added (■) after, or (●) prior to homogenization.................................................. 150

Figure 6.2: SDS-PAGE gel and the corresponding densitometric profiles of total (lane A) and interfacial proteins (lane B) in homogenized coconut milk.................. 153

Figure 6.3: ζ-potential of coconut milk emulsions stabilized with SDS (a), Tween 20 (b), WPI (c), or sodium caseinate (d) added (■) after, or (●) prior to homogenization.................................................. 155

Figure 6.4: SDS-PAGE patterns of coconut milk emulsions with WPI (a, c), and sodium caseinate (b, d) at 0.1, 0.25, 0.5, and 1 wt% added after (a, b) or prior to (c, d) homogenization.................................................. 162

Figure 6.5: SDS-PAGE patterns and the corresponding densitometric profiles of WPI (a), and sodium caseinate (b).................................................. 164

Figure 6.6: Peak area of selected protein fractions in coconut milk emulsion stabilized with WPI (a), and sodium caseinate (b) added (■) after, or (●) prior to homogenization.................................................. 165

Figure 7.1: Visual appearance after thermal treatments of homogenized coconut milk (a), coconut milk emulsions homogenized with 1 wt% sodium caseinate (b), WPI (c), SDS (d), and Tween 20 (e)............................................................................ 182

Figure 7.2: Typical thermograms of coconut oil.................................................. 190

Figure 7.3: Successive cooling curves and a heating curve of homogenized coconut milk repeatedly cycled from 30°C to -15°C (a), and -40°C (b) at 1.5°C min⁻¹............................................................................ 191

Figure 7.4: Differential scanning microcalorimetric thermograms of coconut milk homogenized with 1 wt% no additive (a), sodium caseinate (b), and WPI (c) after heated different temperatures for 1 h. ............................................................................ 194

Figure 7.5: Successive cooling curves and a heating curve of coconut milk emulsions homogenized with 1 wt% sodium caseinate repeatedly cycled from 30°C to -15°C (a), and -40°C (b) at 1.5°C min⁻¹. ............................................................................ 197
Figure 7.6: Successive cooling curves and a heating curve of coconut milk emulsions homogenized with 1 wt% WPI repeatedly cycled from 30°C to -15°C (a), and -40°C (b) at 1.5°C min⁻¹.................................................................198

Figure 7.7: Successive cooling curves and a heating curve of coconut milk emulsions homogenized with 1 wt% SDS repeatedly cycled from 30 min⁻¹°C to -15°C (a), and -40°C (b) at 1.5°C min⁻¹. .................................................................201

Figure 7.8: Successive cooling curves and a heating curve of coconut milk emulsions homogenized with 1 wt% Tween 20 repeatedly cycled from 30°C to -15°C (a), and -40°C (b) at 1.5°C min⁻¹. .................................................................202
LIST OF TABLES

Table 1.1: Schematic outline of the dissertation. ........................................................6

Table 2.1: World coconut production between 1997 and 2001. .................................31

Table 2.2: Proximate composition of fresh coconut meat...........................................33

Table 2.3: Physical properties and chemical composition of coconut milk ..............34

Table 2.4: Composition standard of coconut milk products. .....................................35

Table 2.5: Fatty acid composition of coconut oil....................................................36

Table 2.6: Amino acid composition of coconut proteins. .........................................38

Table 5.1: Mean effective and primary particle sizes of coconut milk emulsions with 1 wt% surface-active stabilizers added without, after, or prior to homogenization.....................................................................................................137

Table 5.2: Creaming index after 24 h storage of coconut milk emulsions with 1 wt% surface-active stabilizers added without, after, or prior to homogenization.....................................................................................................138

Table 6.1: Mean effective and primary (in parentheses) particle sizes of coconut milk emulsions with surface-active stabilizers added after or prior to homogenization.....................................................................................................157

Table 6.2: Molar ratio of surface-active stabilizer and coconut proteins in coconut milk emulsions stabilized with different surface-active stabilizers......................158

Table 7.1: Temperature protocols. ..............................................................................177

Table 7.2: Mean effective and primary particle sizes after thermal treatments of coconut milk emulsions homogenized with 1 wt% stabilizer. .............................181

Table 7.3: Summary of stability after thermal treatments of coconut milk emulsions homogenized with 1 wt% stabilizer. .......................................................188
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Chapter 1

Statement of the Problem

Coconut milk is a natural oil-in-water emulsion in which coconut oil droplets are dispersed throughout an aqueous continuous phase. It is produced in either household or industrial scale by extracting the endosperm of mature coconut (Cocos nucifera L.) with water. The white, opaque emulsion has been used widely as an important food ingredient, especially in Asia and Pacific regions due to its unique sensory characteristics. Coconut milk is naturally stabilized by coconut proteins (i.e., globulins and albumins), and phospholipids. However, like all emulsions, coconut milk is thermodynamically unstable and readily separates into cream and serum layers, known as coconut cream and coconut skim milk, respectively. The physical instability of coconut milk is suspected to be the consequence of the inadequate quantity and quality of coconut proteins. Fresh coconut milk is also microbiologically unstable as it is a good source of nutrients, especially fat, has a neutral pH and typically carries a large microbial load.

Various attempts have been made to preserve the coconut milk in order to prolong the shelf life for commercial purposes, mostly by using temperature treatments (e.g., heat-treated, dried, and frozen coconut milk). The problem of eliminating microorganisms in coconut milk by thermal processing is aggravated by the instability of the coconut proteins to heat. In order to obtain coconut milk product with good quality
and stability, various kinds and amounts of emulsifiers and/or stabilizers have been normally used. It is common practical knowledge that the choice of process and additives affects the stability of the emulsion and the quality and sensory characteristics of the processed coconut milk products. However, the physical basis for the changing functional properties and the underlying emulsion science are still unclear.

My dissertation, therefore, concentrates on determining the properties and structure of coconut milk emulsions prepared with various additives and processes. I have taken a model system approach, and used simple combinations of fundamental process operations (homogenization, temperature, pH, and salt) and surface active stabilizers to understand the mechanisms of change with the expectation that this understanding will be helpful in developing more complex and industrially practical processes.

**Goal, Objectives, and Dissertation Layout**

The overall goal of this dissertation is to characterize the effects of processing and ingredient interactions on the microstructure, properties, and colloidal stability of the coconut milk emulsion. In order to accomplish this goal the specific objectives of my research were to:

1) Investigate the effect of heating and homogenization on the stability of coconut milk emulsions.

2) Determine the influence of the addition of acid/base and salt on the stability and structure of coconut milk emulsions
3) Determine the influence of the addition of widely-used surface-active stabilizers on the bulk quality, structure, and surface properties of coconut milk emulsions.

4) Examine the changes in stability of coconut milk emulsions homogenized with different surface-active stabilizers due to various cooling and heating treatments.

The stability of coconut milk emulsion, like most protein-stabilized emulsions, is expected to be affected by denaturation of the proteins either by heat, or the loss of its electrical charge. Therefore, I started my dissertation by determining effects of those factors on the stability and properties of coconut milk emulsions. Chapter 3 is concerned with the changes in stability and bulk quality of coconut milk after homogenization and heating (i.e., Objective 1). Such processes were selected since they are the fundamental operations in coconut milk manufacture.

[It should be noted that the coconut milk used only in Chapter 3 was prepared from whole fresh coconut, and the fat content of the samples was 15% to 17%. The method of coconut milk preparation was modified and used in later chapters. The in-house grated coconut meat was replaced with the ready-to-use frozen grated meat, of which the composition is less varied and more controllable. The extracted milk was diluted in buffer to a final fat content of 10% in order to normalize the fat content of the emulsions and make the sample more experimentally convenient.]
In Chapter 4, the physicochemical properties of coconut milk emulsions with different pH values and ionic strengths were investigated (i.e., Objective 2). The pH of many coconut milk based products is about 6.2 and any decrease in pH normally is a consequence of microbial contamination or of the addition of acidulants. The level of acidity is known to affect the quality of coconut milk and often used by manufacturers as a measure of product quality.

In Chapter 5 and 6, various types and amounts of model surface-active stabilizers were added to coconut milk in order to improve the stability and quality of the emulsion (i.e., Objective 3). Four commonly used proteins and small-molecule surfactants, namely sodium caseinate (i.e., model disordered protein), whey protein isolate, (WPI, i.e., model globular protein), sodium dodecyl sulfate (SDS, i.e., model anionic surfactant), and polyoxyethylene sorbitan monolaurate (Tween 20, i.e., model uncharged surfactant), were selected. The bulk quality of samples was determined in Chapter 5, and the surface properties of samples were examined in Chapter 6 to provide evidence of the surface chemistry underlying changes in bulk properties.

The susceptibility of coconut milk emulsions prepared with surface-active stabilizers to various thermal treatments normally involved in the processing of coconut milk products, e.g., freezing, chilling, heating, and autoclaving, was investigated in Chapter 7 (i.e., Objective 4). The last chapter (Chapter 8) is the conclusions of my dissertation.
Chapters 3 to 7 of this dissertation are presented in form of manuscripts co-authored with my major advisor each with a separate introduction, materials and methods, results and discussion, conclusions, and references. The structure of the dissertation is outlined in Table 1.1.
### Table 1.1: Schematic outline of the dissertation.

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Paper</th>
<th>Objective covered</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Statement of the problem</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Literature review</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Conclusions</td>
<td></td>
</tr>
</tbody>
</table>
Chapter 2

Literature Review

2.1 Basic emulsion science

2.1.1 Definition

An emulsion is a mixture of two immiscible liquids - one is the dispersed or internal phase as small spherical droplets, and the other is the continuous, external phase. In food systems, the two liquid phases are usually oil and water. Food emulsions can be categorized as oil-in-water or water-in-oil depending upon which phase is continuous (Dalgleish, 1996; McClements, 2004b). Some examples of oil-in-water emulsions include mayonnaise, milk, cream, soups, salad dressings, and sauces. This work is solely concerned with oil-in-water emulsions which are hereafter simply referred to as emulsions.

2.1.2 Emulsion ingredients

In addition to oil and water, a typical food emulsion may contain emulsifiers, thickening agents, buffering systems, preservatives, sweeteners, salt, antioxidants, chelating agents, colorants, and flavors (Dickinson, 1992). The physiochemical and organoleptic properties of an emulsion depends on the type and amount of these
ingredients and their physical distribution, i.e., in the dispersed phase, the continuous phase, or at the interface.

2.1.2.1 Fats and oils

For oil-in-water emulsions, the dispersed phase consists of food oils (i.e., largely mixed triglycerides). Fats and oils influence the physicochemical, organoleptic, and nutritional properties of food emulsions in a variety of ways. The turbidity, cloudiness, or opaque appearance of emulsion is largely the result of light scattering by the dispersed oil droplets (McClements, 2004b). The lipid content and droplet concentration of emulsions contribute to the rheological properties as well as the perceived creaminess of the emulsions.

2.1.2.2 Water and aqueous solutions

The aqueous phase of an oil-in-water emulsion contains water and a variety of water-soluble functional ingredients. The unique molecular and structural properties of water largely determine the solubility, conformation, and interactions of such components, which in turns influence the bulk physicochemical and sensory properties of emulsions (McClements, 2004b).
2.1.2.3 Emulsifiers

Emulsifiers are important ingredients in emulsion stabilization. Oil and water are almost completely insoluble in each other. Emulsifiers are amphiphilic, surface-active substances that are capable of retarding the phase separation in emulsions by adsorbing to the oil-water interface and hence lowering the interfacial tension. The commonly used emulsifiers for food emulsions are small-molecule surfactants, amphiphilic biopolymers, and solid particles.

Small-molecule surfactants

Surfactant molecules consist of a hydrophilic head group attached to a hydrophobic tail group. In addition to their principal role in stabilizing the emulsions, small-molecule surfactants may affect emulsion properties by forming micelles, interacting with biopolymers, or altering the fat crystallization in oil droplets (Krog, 1997; Krog & Sparso, 2004). Small-molecule surfactants can be classified by the characteristics of their head groups into nonionic (e.g., monoglycerides, polyoxyethylene sorbitan fatty acid monoesters, sorbitan fatty acid monoesters, and sucrose fatty acid esters), anionic (e.g., alkyl sulfate salts alkyl benzene sulfonate, fatty acid salts, and stearyl lactylate salts), cationic (e.g., alkyl trimethylammonium salts), and zwitterionic (e.g., lecithin and other phospholipids). Each type of surfactant has functional properties that are determined by its molecular structure and the environment (McClements, 2004b).
Amphiphilic biopolymers

Biopolymers that have significant amount of both polar and nonpolar residues tend to exhibit surface activity. Proteins (e.g., dairy proteins, gelatin, egg albumin, and plant proteins) and polysaccharides (e.g., gum arabic and modified starches) are the two most important types of amphiphilic biopolymers used in food emulsions. Under appropriate environmental conditions, biopolymers can hydrophobically adsorb to oil-water interfaces and reduces the contact area between the oil and water molecules at the oil-water interface, thus lowering the interfacial tension. The thick interfacial layer formed by biopolymers also provides steric stability to the emulsion. Biopolymers often rearrange their structures after they have adsorbed to an interface to maximize the number of contacts between oil and their nonpolar groups. Such rearrangements depend on the molecular structure and interactions of the biopolymers, which in turn influence the characteristics of the interfacial membrane (Das & Kinsella, 1990; Dickinson, 1992).

2.1.3 Emulsion formation

The process of converting bulk oil and bulk water into an emulsion or of reducing the size of the droplets in an existing emulsion is known as homogenization and is normally achieved by applying intense mechanical agitation to a liquid, in the presence of emulsifier, using a homogenizer (Breen, Wason, Kim, Nicolov & Shetty, 1996). Homogenization increases the interfacial area and hence the total interfacial free energy of the emulsion. The formation of emulsions by homogenization is a highly dynamic process that involves the violent disruption of droplets and the rapid movement of
surface-active molecules from the bulk liquids to the interfacial region of the newly divided surface.

During the homogenization, the size of the droplets formed depends on two opposing physical processes: droplet disruption and droplet re-coalescence (McClements, 2004b). The disruption of a large droplets into smaller ones depends on the balance between the force that hold the droplets together, i.e., interfacial force; and that tries to pull them apart, i.e., disruptive force generated by the homogenizer (Walstra, 2003). The disruptive force acting on the droplets depends on the nature of flow conditions during homogenization and also the type of homogenizer used. In the presence of emulsifiers, the decrease in interfacial tension also facilitates droplet disruption during homogenization (McClements, 2004b). During homogenization, the emulsifiers present in the solution rapidly adsorb onto the freshly generated droplet surface and thus prevent them against recoalescing. Smaller emulsion droplets can be achieved when the time taken by the emulsifiers to adsorb at the interface is much faster than the time between droplet-droplet collisions (McClements, 2004b).

The mechanical agitation required for homogenization can be applied using a number of different types of devices including high speed mixers, colloid mills, high-pressure valve homogenizers, and ultrasonic homogenizers. High-pressure valve homogenizers are the most common methods of producing fine emulsions in food industry. The homogenizer disrupts the droplets by forcing the coarse emulsion through a narrow gap of the valve where it experiences a combination of intense disruptive forces.
Thus, the large droplets are broken down to smaller ones. Two-stage homogenization can be done by forcing the emulsion through two consecutive valves. The first provides the high pressure to break up the droplets and the second valve is at lower pressure to disrupt any flocs formed (McClements, 2004b).

### 2.1.4 Emulsion characterization

#### 2.1.4.1 Disperse phase volume fraction

The concentration of droplets in emulsion plays an important role in determining its appearance, texture and stability. Droplet concentration of an emulsion is usually described in terms of the disperse phase volume (or mass) fraction which is equal to the volume (or mass) of emulsion droplets divided by the total volume (or mass) of the emulsion (McClements, 1998). The dispersed phase volume fraction of an emulsion can be determined using many methods, including the proximate analysis methods for measuring fat content, measurements of emulsion density, and electrical conductivity (McClements, 2004b).

#### 2.1.4.2 Particle size distribution

Many of the properties of an emulsion, i.e., stability, appearance, and texture, depend on the droplet size (McClements, 2004b). Most emulsions are polydisperse with droplet sizes; typically between 0.1 and 100 μm (Dickinson, 1992; Dickinson & Stainsby,
Therefore, an emulsion should be characterized by a distribution of particle size by expressing the fraction of droplets in different size ranges, rather than a single number for droplet size. The fraction of droplets in different size ranges can be expressed in terms of number, mass, volume, or surface area. However, the size of the droplets in a polydisperse emulsion is often represented by a mean particle size rather than the full distribution (Walstra, 2003). For example, the area-weighed average diameter \( d_{32} \) is related to the average surface area of droplets exposed to the continuous phase per unit volume of emulsion. Another commonly used method of expressing the mean particle size is the volume-weighed average diameter \( d_{43} \) which is the sum of the volume ratio of droplets in each size class multiplied by the mid-point diameter of the size class (McClements, 2004b). The \( d_{43} \) is more sensitive to the presence of large particles in an emulsion than \( d_{32} \), hence it its more sensitive to phenomena such as flocculation (Walstra, 2003). The values of each mean can be calculated from the full distribution as follows:

\[
d_{32} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2}
\]  
(2.1)

\[
d_{43} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3}
\]  
(2.2)

where \( n_i \) is the number of particles in each size class per unit volume of emulsion and \( d_i \) is the diameter of the particles in each size class.

The width of the distribution should also be reported for an emulsion with a wide distribution of particle size. It can be conveniently expressed by the standard deviation of the size distribution weighed with the particles’ surface area, divided by \( d_{32} \). The width of
the distribution of food emulsion droplets generally ranges between 0.5 to 1 (Walstra, 2003). If the width of the distribution is large, the shape of the distribution curve should also be described (McClements, 2004b).

Several different instruments have been developed to measure particle size distribution of emulsions. The particle size analysis instrument used in the present work is based on laser diffraction light scattering technique in which a beam of monochromatic light (generated by a He-Ne gas laser, $\lambda=0.63 \text{ \mu m}$) is directed through an emulsion and the light scattered by the droplets of the emulsion is collected by an array of photosensitive detectors. The intensity of the scattered light is measured as a function of scattering angle and data is analyzed by a computer. The particle size distribution is determined by measuring, as function of scattering angle, the extent of light scattered by an emulsion and using a mathematical model, Mie theory, to relate the measured data to the particle characteristics (Krarup, 2004).

2.1.4.3 Microstructure

Microstructure is an important factor in determining the stability and properties of an emulsion. Particle size analysis can only give the information about the size of the droplets but not the structure and organization of the components within emulsions since the dilution and agitation involved in the measurement may disturb the microstructure of emulsions. Images of microstructure of emulsion systems can be acquired using optical microscopy, laser scanning confocal microscopy (LSCM), electron microscopy, or
atomic force microscopy due to the fact that many of the structural components in emulsions are so minute and cannot be observed directly by eye. Each technique works on different physicochemical principles and can be used to examine different levels and types of structural organization.

Conventional optical microscopy, due to its theoretical limit of resolution, provides valuable and reliable information only about the microstructure of emulsion containing relatively large droplets ($d>1$ μm). The natural contrast between the major components in emulsions is often fairly poor. This problem can be solved by using chemical stains that bind to a particular emulsion component or preferentially partition into a particular phase. Alternatively, differential interference contrast microscopy can also be used (McClements, 2004b).

LSCM provides higher clarity and better resolution images of the emulsions microstructure than conventional optical microscopy. It is a powerful method of studying the structure of emulsions, e.g., determining the size and organization of droplets, and following the changes in interfacial composition. The LSCM focuses a narrow laser beam at a particular point in the specimen and a detector measures the intensity of the resulting signal. It also allows the generation of three-dimensional images. Observation of the microstructure of emulsion systems is often facilitated by using fluorescence dyes that bind selectively to specific components.
Electron microscopy is widely used for emulsions containing structural component that are smaller than the resolution of optical microscope, e.g., protein aggregates, fat, or ice crystals. This type of microscope uses electron beam, rather than light beam, which is directed through the sample using a series of magnetic fields. The smaller wavelength of electron beam contributes to the fine resolution (~1 nm) of electron microscopy. There are two types of electron microscopes that are commonly used to examine the structure of food systems, i.e., transmission electron microscopy and scanning electron microscopy, which is more widely used due to its more three-dimensional image generated of the surface topography and the more convenient sample preparation steps.

2.1.4.4 Droplet charge

The droplets in emulsions have an appreciable electrical charge of which the magnitude and sign depend on the type of emulsifier used, since the origin of the charge is mainly the adsorption of emulsifier molecules that are ionized or ionizable (McClements, 2004b). Surfactants have hydrophilic head groups that may be neutral, positively charged, or negatively charged. The electrical charge of proteins depends on the pH of the solution compared to their isoelectric point. Droplets in an emulsion are usually coated with the same type of emulsifier so they have the same electrical charge. In addition to the charge of emulsifiers, the surface charge of emulsion droplets is also determined by adsorption of other types of ionic substances, e.g., multivalent mineral ions or polyelectrolytes. The magnitude and the electrical charge on the droplets
contribute to the bulk physicochemical properties of emulsions. When the charge is sufficiently large, the droplets are prevented from aggregating due to electrostatic repulsion.

Most particles possess a surface charge in a polar medium and attract ions of the opposite charge (counter-ion) in the dispersant, forming a strongly bound layer close to the surface of the particle (i.e., Stern layer). Those ions further away from the core particle make up a diffuse layer, more loosely bound to the particle. The attached counter-ions in the Stern layer and the charged atmosphere in the diffuse layer are together referred to as the double layer. As we move away from the surface of colloid, the potential drops off roughly linearly in the Stern layer, then exponentially through diffuse layer, and thus approaching zero at the boundary of the double layer. Within the diffuse layer is a notional boundary known as surface of shear and the potential at this boundary is known as the ζ-potential, which better describes the electrostatic interaction between colloidal particles than surface potential (Hunter, 1981; Stachurski & Michalek, 1996) (Figure 2.1).

The ζ-potential is a function of surface charge of a droplet suspended in a medium (Hunter, 1993), which takes into account that charged species in the surrounding medium may adsorb to the surface of the droplet and alter its net charge. The ζ-potential of emulsion droplets is reduced by the presence of indifferent ions that do not specifically bind to the droplet surface but increase the ionic strength of the surrounding liquid. In this case, the thickness of the counter-ion cloud that surrounds the charged surface (i.e.,
Figure 2.1: Schematic presentation of the structure of the electric double layer (Source: http://www.bic.com/WhatIsZetaPotential.html).
Debye screening length) becomes shorter due to the screening of electrostatic interaction by the added electrolytes (McClements, 2004b). It was reported that the magnitude of $\zeta$-potentials of at least 20 mV is sufficient for preventing droplet coalescence (Wiącek & Chibowski, 1999).

Surface charge directly is often estimated indirectly by electrophoresis. A sample of the emulsion is placed into a measurement cell and an electrical field is applied across it via a pair of electrodes (Hunter, 1981). The charged emulsion droplets move toward the oppositely charged electrode and the velocity depends on the size, shape, and charge of the particle. Droplet motion can be monitored using a light scattering technique or optical microscopy.

A more sophisticated particle tracking system is used in phase analysis light scattering, which has been used in this work. In this method, a laser beam is split, a frequency modulation is applied to a portion of the light and it is then used to generate a scattering pattern from a suspension of particles. The scattered light is recombined with the original (unmodulated) beam. Light scattered from even a stationary particle by a phase modulated laser beam will have a finite, measurable, Doppler frequency which is solely due to the Brownian motion of the particles and equal to the frequency of the modulation. The net motion vector of the particle that moves in the applied voltage gradient is a sum of coherent (charge dependant) and incoherent movements (Brownian motion). The relative phase shifted by an amount depending on the speed and direction of the movement. Phase analysis detects the change as a phase shift that is measured over
many cycles. The phase shift is related to speed, which in turn is related to the charge on the particles (Vanapalli & Coupland, 2000).

2.1.4.5 Interfacial composition

The interface is a narrow region that surrounds each emulsion droplet which contains a mixture of oil, water, and surface-active molecules (Hunter, 1993; 2001). The interface plays a critical role in determining many important bulk properties of food emulsions. The interfacial composition is determined by the type and concentration of surface-active species initially present in the system, as well as by the events that occur during and after emulsion formation (McClements, 2004b).

If an emulsion is prepared using a single type of emulsifier, the interfacial membrane will be comprised of only this emulsifier. The amount of surface-active material adsorbed per unit area of the droplet surface, i.e., surface load, is typically 1 to 2 mg m\(^{-2}\), depending on the initial emulsifier concentration, homogenization condition, pH, ionic strength, and temperature. However, many food emulsions contain a mixture of different surface-active components which results in the interfacial membranes with more complex composition. In this case, the interfacial composition is determined by the concentrations of each surface-active substance, their relative adsorption rate to the interface, the method used to prepare the emulsion, the environmental conditions (e.g., temperature, pH, and ionic strength), and the history of the emulsion (e.g., the order in which the emulsifiers were added) (McClements, 2004b).
In food emulsions the surfaces will tend to be initially coated by those surface-active molecules that adsorb most rapidly under turbulent condition in the homogenizer. The relative adsorption rate of the emulsifiers depends mostly on their molecular characteristics, e.g., size, shape, and polarity. However, the interfacial composition may change during storage due to the displacement of the initially adsorbed molecules by molecules in the aqueous phase that are more surface active (Dickinson, 1992; Walstra, 2003). Moreover, the surface-active components, especially small-molecule surfactants, that are added to the emulsions after homogenization can also displace some of the original emulsifier molecules, usually proteins, from the droplet surface (Dalgleish, Euston, Hunt & Dickinson, 1991; Damodaran, 2004; Dickinson, 1991; Dickinson & Woskett, 1989; Dickinson & Tanai, 1992; Dickinson, Euston & Woskett, 1990). The relative affinities of emulsifiers for an interface have been demonstrated to be influenced by solution and environmental conditions, e.g., temperature, pH, and ionic strength (Dickinson & Tanai, 1992; Hunt & Dalgleish, 1996). Moreover, the interfacial composition of an emulsion containing different types of emulsifiers also depends on droplet size, since this determines the total interfacial area available for the adsorption of the emulsifier molecules (McClements, 2004b).

The interfacial composition of an emulsion can be conveniently determined from the difference between the aqueous phase and total concentration of emulsifier in the system (Dalgleish, 1996; Dickinson, 1992). If the total surfactant concentration is unknown the droplets can be separated from the aqueous phase by centrifugation, collected, and repeatedly washed by redispersing in an appropriate buffer solution and
recentrifuging to remove any residual aqueous phase (Hunt & Dalgleish, 1994b). The emulsifier molecules are then displaced from the droplet surface; and their concentration and identity could be established using appropriate analytical methods, e.g., electrophoresis, chromatography, or nuclear magnetic resonance (McClements, 2004b).

2.1.5 Emulsion stability

Emulsions are thermodynamically unstable and tend to separate into an oil-water two phase system due to their large excess surface energy (Friberg, 1997; Israelachvili, 1992). Emulsion stability is therefore the capacity of a system to kinetically resist changes in structure over time and depends on the ingredients used and the environmental conditions and history (McClements, 2004b). There are various mechanisms by which an emulsion can destabilize, i.e., creaming, flocculation, coalescence, partial coalescence, and Ostwald ripening (Dickinson, 1992; Dickinson & Stainsby, 1982). In practice, these instability mechanisms operate in concert causing destabilization in food emulsions. The present work is exclusively concerned with physical instability and therefore some of the mechanisms of emulsion destabilization are discussed.

2.1.5.1 Creaming

Creaming is the upward movement of oil droplets, under gravity or in a centrifuge, to form a cream layer at the top of an oil-in-water emulsion, with no accompanying change in the droplet size distribution (Dickinson, 1992) (Figure 2.2). The
The driving force for creaming is the density difference between the continuous and dispersed phases. In the initial stage of creaming, the oil droplets move upward and a droplet-depleted layer is observed at the bottom of the container. The droplets pack at the top of the emulsion to form an optically opaque droplet-rich (cream) layer. The final thickness of the cream layer depends on the droplet concentration of the emulsion and the effectiveness of droplet packing. Droplets may pack tightly or loosely into different structures depending on their polydispersity (Dukhin & Sjöblom, 1996) and the nature of the interactions between them (Dickinson, Ma & Povey, 1994). Tightly packed droplets tend to form a relatively thin cream layer, whereas loosely packed droplets form a thick cream layer. The droplets in a creamed emulsion can often be redispersed by mild agitation. However, prolonged interdroplet contact in the cream layer can lead to flocculation or coalescence, and eventually oiling off.

The creaming rate of an emulsion depends on droplet size of the emulsion. Larger droplets tend to cream faster than smaller ones. The rate of creaming for isolated particles in a Newtonian fluid is given by Stoke’s law:

\[ v = \frac{2gr^2(\rho_2 - \rho_1)}{9\eta} \]  

(2.3)

where \( v \) is the creaming velocity, \( g \) is the acceleration due to gravity, \( r \) is the radius of the droplet, \( \rho_2 \) is the density of oil phase, \( \rho_1 \) and \( \eta \) are the density and viscosity of aqueous phase, respectively. According to the above equation, the creaming rate of an emulsion therefore can be controlled by reducing the droplet size, minimizing the density.
Figure 2.2: Schematic presentations of physical changes in oil-in-water emulsions. Oil droplets are shown as shaded circles.
difference between two phases, and increasing the droplet concentration and the viscosity of the continuous phase (Dickinson, 1992).

Stability of an emulsion to creaming can be simply determined by measuring the height of the interface between the opaque cream layer and the transparent serum layer of the emulsion stored for a given time or centrifuged under a set protocol (McClements, 1998).

### 2.1.5.2 Flocculation

Flocculation is a reversible phenomenon occurred when two or more droplets come together to form an aggregate, but retain their individual integrity (Dickinson, 1992) (Figure 2.2). The droplets in an emulsion are in continual movement due to Brownian motion, gravity, or applied mechanical forces, and so frequently collide. Under Brownian condition, the collision frequency \( (F) \) is given by Smoluchowski equation:

\[
F = \frac{8kTn^2}{3\eta} = \frac{3kT\phi^2}{2\eta r^6}
\]

where \( k \) is the Boltzmann constant, \( T \) is the temperature, \( n \) is the number of droplet per unit volume of emulsion, \( \eta \) is the continuous phase viscosity, \( \phi \) is the dispersed phase volume fraction, and \( r \) is the droplet radius (Dickinson & Stainsby, 1982).

Thus, the collision frequency in an emulsion can be reduced by decreasing the dispersed phase volume fraction, increasing the droplet size, or increasing the viscosity of
the continuous phase. After a collision, droplets may associate or separate depending on the relative magnitude of the attractive and repulsive interdroplet forces. As flocculation occurs, the total number of particle decreases. The structure of the floc is influenced by the difference between interdroplet attractive forces and the thermal energy. More open structure is formed when the attractive forces are stronger since the droplets firmly stick together at the point they collide and are unable to undergo any subsequent structural rearrangements.

The rate of flocculation in an emulsion is also influenced by changes in the properties of its interfacial layer. Flocculation in emulsions stabilized by proteins can be induced by (i) increasing ionic strength which reduces the electrostatic repulsion due to the adsorbed molecules at the interface (Agboola & Dalgleish, 1995; 1997a; Demetriades, Coupland & McClements, 1997b); (ii) changing the pH of emulsion to approach the isoelectric point of the proteins (Demetriades et al., 1997a; Hunt & Dalgleish, 1994a; McClements, 2004a); (iii) heating and high pressure treatment which unfold the surface-bound protein resulting in the exposure of hydrophobic groups (Demetriades & McClements, 1998; Demetriades et al., 1997b; Dickinson & James, 1998; Hunt & Dalgleish, 1995).

Other common mechanisms of flocculation found in emulsions are bridging and depletion flocculation. Bridging flocculation occurs when there is insufficient concentration of biopolymer emulsifier to provide complete surface coverage to the oil-water interface (Dickinson, 1992). A single emulsifier molecule is therefore shared
between multiple droplets and forms a bridge between them (Dickinson, 1992). Bridging flocculation can also occur when the electrical charge of a biopolymer in the continuous phase is opposite to that on the droplets (Dickinson, 2003). Depletion flocculation occurs when non-adsorbing colloidal particles, which may be surfactant micelles, individual polymer molecules, or aggregated polymers in the continuous phase of emulsions, are excluded from a narrow region closed to the droplet surfaces (McClements, 2004b). The movement of those colloidal particles from the depletion zone into the bulk liquid is associated with an osmotic effect that causes an increase in attraction between emulsion droplets. The attractive force increases as the concentration of the non-adsorbed polymer increases until it becomes large enough to overcome the repulsive interactions between the droplets, thus lead to flocculation (Jenkins & Snowden, 1996; Tuinier & de Kruijf, 1999).

The presence of and structure of flocs is one of the important factors influencing creaming in emulsions (Pinfield, Dickinson & Povey, 1997). At low or intermediate droplet concentrations, flocculation tends to increase the creaming velocity because the flocs have a larger effective size than the isolate droplets (Chanamai & McClements, 2000). In concentrated emulsions, flocculation retards creaming because a three-dimensional network of interconnected flocs is formed, thus inhibit the movement of individual droplets (Dickinson et al., 1994). A network can also form at lower droplet concentrations when the flocs are more openly packed due to a strong attraction between droplets. In flocculated emulsions, a substantial delay period is observed prior to rapid
creaming (Manoj, Fillery-Travis, Watson, Hibberd & Robins, 1998; Tuinier & de Kruif, 1999).

Flocculation can be monitored using variety of techniques based on particle size measurement or inferred from an increase in the viscosity of the emulsion (McClements, 2004b). To determine whether the increase in droplet size is caused by flocculation or coalescence (see below), the emulsion is usually altered in a way that would be expected to break down any flocs that are present. This can be achieved by altering solvent conditions (e.g., pH, ionic strength, polarity, or temperature), applying mechanical agitation, or adding small-molecule surfactants which can break up the floc into its constituent droplets. The measured droplet size would be decreased if there were flocs present because they were dissociated by the additional treatment step (Demetriades et al., 1997b; Tomas, Paquet, Courthaudon & Lorient, 1994).

2.1.5.3 Coalescence

Coalescence is an irreversible process in which two or more droplets merge together to form a single larger droplet (McClements, 2004b) (Figure 2.2). This mechanism involves breakage of the interfacial film separating two droplets in close proximity and the merging of their contents. Coalescence reduces the contact area between oil and water phases (van Aken, 2004). The increase in droplet size due to coalescence causes emulsions to cream rapidly and can lead to accumulation of a separate oil layer at the surface which is known as oiling off.
There are two different conditions which can lead to coalescence in food emulsions. First, coalescence occurs immediately after two or more droplets collide due to Brownian motion, gravitational force, or applied mechanical shear. The rate at which this type of coalescence occurs is dominated by many of the same factors as flocculation. Second, in emulsions containing a high concentration of droplets due to creaming or flocculation, coalescence may follow prolonged contact between droplets. The coalescence rate therefore strongly depends on the nature of emulsifier and interfacial layer of droplet surface. The interfacial layer formed due to adsorption of small-molecule emulsifiers is thinner and more dynamic (lower interfacial tension and viscosity) as compared to that of protein-stabilized emulsions. Therefore, protein emulsifiers have been found to strongly provide protection against droplet rupture and coalescence due to their electrostatic and steric interactions that provide strong repulsive forces between droplets. Protection against flocculation and subsequent coalescence may be achieved by two main mechanisms. The droplet may be prevented from reaching each other because of an energy barrier between them or because of their slow movement due to increased viscosity of the continuous medium (McClements, 2004b; Walstra, 2003).

Coalescence can be determined by measuring the change in droplet size or observing an emulsion under a microscope (McClements, 2004b). Coalescence can also be measured indirectly by determining the amount of free oil separated from an oil-in-water emulsion. The amount of free oil can be quantified by solvent extraction of emulsion (Ghosh, Cramp & Coupland, 2006) or by adding an oil soluble dye to the emulsion which will be diluted only if free oil is present (Palanuwech, Potineni, Roberts
& Coupland, 2003). Coalescence can be distinguished from flocculation by adding small-molecule surfactant to the emulsion. The measured size of coalesced sample will not be affected because the coalesced droplets will remain intact, whereas the measured size of flocculated sample will decrease, as described earlier (Demetriades et al., 1997b).

2.2 Coconut milk

2.2.1 Coconuts

Coconuts (Cocos nucifera L.) are monocotyledon palms in Palmaceae family (Ohler, 1999). They are the most cultivated palms and by far the most economically important palm grown in all tropical regions around the world where the temperature, humidity, soil, and elevation are suitable (Green, 1991; Woodroof, 1979). Asia is the biggest coconut producer in the world (Maneepun, Varangoon & Phithakpol, 1988), and ninety percent of the world’s total coconuts are cultivated in Indonesia, Philippines, India, Sri Lanka and Thailand (Table 2.1). In the United States, coconuts can grow in the wild in southern Florida (Green, 1991).

It is estimated that about 70% of the coconuts are used for domestic consumption in the producing countries, of which just over half the produce is consumed fresh (Green, 1991). Coconut is also largely consumed in the form of coconut oil, either edible or industrial. The edible coconut products are mostly obtained from meat (solid endosperm) and water (liquid endosperm), which account about 29% to 30% and 21% to 26% of
coconut total weight, respectively (Grimwood, 1975). Coconut meat can be consumed fresh; or grated, mixed with hot water and pressed to extract the coconut milk (Cancel, 1979).

**Table 2.1**: World coconut production between 1997 and 2001 (FAO, 2002).

<table>
<thead>
<tr>
<th>Continent</th>
<th>Production quantity per year (1000 tons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>1822</td>
</tr>
<tr>
<td>Asia</td>
<td>42848</td>
</tr>
<tr>
<td>North America</td>
<td>1813</td>
</tr>
<tr>
<td>Oceania</td>
<td>1876</td>
</tr>
<tr>
<td>South America</td>
<td>995</td>
</tr>
<tr>
<td>Total</td>
<td>49354</td>
</tr>
</tbody>
</table>

2.2.2 General characteristics of coconut milk

The term coconut milk sometimes is used interchangeably to refer to coconut water, i.e., the liquid endosperm of coconut fruits, which appears to lead to considerable confusion in the literature. I, therefore, refer to coconut milk as “the white, opaque liquid obtained from by pressing grated or comminuted solid endosperm of coconut with or without addition of water” (Cancel, 1979; Gonzalez, 1990). Coconut milk has been used as an important ingredient for Asian cuisine as well as in other parts of the world due to its unique flavor and other desirable sensory characteristics. It has been estimated that
25% of the world’s output of coconuts is consumed as coconut milk (Seow & Gwee, 1997).

2.2.3 Coconut milk extraction

Coconut milk is generally produced from mature nuts of about twelve months in age of which the meat is hard and thick. The process begins with deshelling of fully mature coconuts following by removing the thin brown layer that covers the kernel, i.e., testa, which, if present, will impart a brown color and a slight bitter taste to the extracted milk. The coconut meat is then washed, drained, and grated by machine prior to mixing with water. The milk is manually or mechanically extracted from the comminuted endosperm. This method is sometime referred to as the wet process. Extraction may be repeated twice or thrice by adding water to maximize the extraction of soluble material from endosperm. In addition to the differences in variety, extraction yield depends on coconut maturity, particle size of the comminuted meat, extraction pressure, water-to-meat ratio, and pre-treatments of fresh coconut kernels to be extracted (Cancel, 1979; Waisundara, Perera & Barlow, 2007).

2.2.4 Composition and properties

The composition of coconut milk is much dependent on that of the coconut meat used for extraction. The solid endosperm of coconut is an abundant source of fat (Table 2.2). The composition of the coconut meat shows considerable difference...
depending on variety, maturity, and growing condition (Ohler, 1999; Woodroof, 1979).
The constituents of coconut endosperm cells at the region next to the testa and those at
the region next to cavity were found to be different (de Mason & Chandra Sekhar, 1990).
A marked concentration gradient in the increasing order was also observed for proteins
from the inner to the outer regions of coconut endosperm (Balachandran & Arumughan,

**Table 2.2**: Proximate composition of fresh coconut meat (Ohler, 1999).

<table>
<thead>
<tr>
<th>Composition</th>
<th>Range, % (wet weight basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>44-52</td>
</tr>
<tr>
<td>Fat</td>
<td>35-38</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>9-11</td>
</tr>
<tr>
<td>Protein</td>
<td>3-4</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>2-4</td>
</tr>
<tr>
<td>Ash</td>
<td>1</td>
</tr>
</tbody>
</table>

Published data for the composition and physical properties of coconut milk
extracted with the added water-to-shredded coconut meat ratio of 1:1 is shown in
**Table 2.3**. However, composition and quality of coconut milk varies according to the
cocoanut (Grimwood, 1975) as well as the method of preparation used in extraction, e.g.,
equipment, water amount, and temperature (Cancel, 1979; Gonzalez, 1990; Mepba,
2002).
The composition, especially fat content, is the important criteria in categorizing coconut milk products. According to the Codex Standard for Aqueous Coconut Products (CODEX STAN 240-2003), coconut milk should contain at least 10% fat, 2.7% non-fat solids, and 12.7% to 25.3% total solids (Table 2.4).

Table 2.3: Physical properties and chemical composition of coconut milk (Gonzalez, 1990).

<table>
<thead>
<tr>
<th>Physical properties</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity</td>
<td>1.0029-1.0080</td>
</tr>
<tr>
<td>Surface tension, dyne cm(^{-2})</td>
<td>97.76-125.43</td>
</tr>
<tr>
<td>Viscosity, mPa s</td>
<td>1.61-2.02</td>
</tr>
<tr>
<td>Refractive index</td>
<td>1.3412-1.3446</td>
</tr>
<tr>
<td>pH</td>
<td>5.95-6.30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture, %</td>
<td>73.47-76.84</td>
</tr>
<tr>
<td>Fat, %</td>
<td>18.83-21.09</td>
</tr>
<tr>
<td>Protein, %</td>
<td>2.14-2.97</td>
</tr>
<tr>
<td>Ash, %</td>
<td>0.63-0.96</td>
</tr>
<tr>
<td>Total sugars, %</td>
<td>0.82-1.62</td>
</tr>
</tbody>
</table>
Coconut milk is a poor source of vitamins and minerals. The major minerals found in raw coconut milk are phosphorous, calcium and potassium (Seow & Gwee, 1997). It is rich in digestible carbohydrates as most of the indigestible fibrous material is removed during the process of preparing the milk (Cancel, 1979). The main carbohydrates present are sugars (primarily sucrose) and some starch. Besides water, coconut oil and coconut protein are the two major components of coconut milk.

### 2.2.3.1 Coconut oil

The triglycerides in coconut oil contain mostly medium chain saturated fatty acids (as high as 90%), of which mainly are those with medium chain lengths, including lauric, myristic, and palmitic acids (Table 2.5). The fatty acid composition of coconut oil has been reported to be varied among different coconut cultivars (Kumar, Champakam & Rajagopal, 2004; Laureles, Rodgriguez, Reano, Santos, Laurena & Mendoza, 2002), maturity (Azeez, 2007), and even across different regions of the endosperm in the same
fruit (Balachandran, Arumughan & Mathew, 1985). It has been reported that the milk from younger nuts has a high content of linoleic acid (Banzon & Escada, 1985; Padua-Resurreccion & Banzon, 1979).

Table 2.5: Fatty acid composition of coconut oil (White, 1992).

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>% of total fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6:0 Caproic</td>
<td>0.6</td>
</tr>
<tr>
<td>C8:0 Caprylic</td>
<td>8.0</td>
</tr>
<tr>
<td>C10:0 Capric</td>
<td>6.4</td>
</tr>
<tr>
<td>C12:0 Lauric</td>
<td>48.5</td>
</tr>
<tr>
<td>C14:0 Myristic</td>
<td>17.6</td>
</tr>
<tr>
<td>C16:0 Palmitic</td>
<td>8.4</td>
</tr>
<tr>
<td>C18:0 Stearic</td>
<td>2.5</td>
</tr>
<tr>
<td>C18:1 Linoleic</td>
<td>6.5</td>
</tr>
<tr>
<td>C18:2 Linolenic</td>
<td>1.5</td>
</tr>
</tbody>
</table>

The major triacylglycerols of coconut oil, as analyzed by HPLC, are di- and triglycerides of lauric acid and either caproic or myristic acid (Tan & Che Man, 2002).

The high degree of saturation in coconut oil is responsible for its high melting and crystallization temperatures (Dia, Garcia, Mabesa & Tecson-Mendoza, 2005; Reyes-Hernandez, Dibildox-Alvarado, Charo-Alonso & Toro-Vazquez, 2007; Tan & Che Man, 2002).
The medium chain triglycerides in coconut oil are known for their nutritional advantages as they are easily digested and quickly adsorbed (Moffatt, 2006; Suyitno, 2003). The health impact of the coconut oil is still controversial. It has been reported over the past decades that coconut oil, similar to other saturated fats, may contribute to several chronic diseases, e.g., hypertension, hypercholesterolemia, and obesity (Gardey, Burstyn & Taylor, 1978; Pehowich, Gomes & Bames, 2000; Zulet, Barber, Garcin, Higueret & Martinez, 1999). However, some of the recent studies have demonstrated the hypolipidemic effect of lauric acid and coconut oil, either in animals or human subjects (Kris-Etherton & Yu, 1997; Mohamed, Hussein, Bhathena & Hafez, 2002; Müller, Lindman, Blomfeldt, Seljeflot & Pedersen, 2003; Talavera, Zafra, Gil-Villarino, Perez, Alvarez-Pez & Garcia-Peregrin, 1997).

2.2.3.2 Coconut proteins

Most of the total protein in coconut is in coconut skim milk (Balasubramaniam & Sihotang, 1979; Capulso, Gonzales & Celestino, 1981). Many studies reported that coconut proteins provide good nutritional value with a relatively balanced amino acid profile (Chakraborty, 1985; Gonzalez & Tanchuco, 1977; Gunetileke & Laurentius, 1974; Kwon, Park & Rhee, 1996; Rasyid, Manullang & Hansen, 1992). Those proteins contain high amount of essential amino acids (Table 2.6) with a high biological value of 71% to 77% and a digestibility of 86% to 94% (Hagenmaier, Mattil & Cator, 1974; Lachance & Molina, 1974; Mepba & Achinewhu, 2003). The most limiting amino acids
of coconut skim milk are isoleucine, methionine, threonine, and tryptophan (Hagenmaier, Loptakwong & Verasestakul, 1975).

Table 2.6: Amino acid composition of coconut proteins (Rasyid et al., 1992).

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Mole %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>8.00</td>
</tr>
<tr>
<td>Arginine</td>
<td>13.78</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>9.46</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.37</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>23.96</td>
</tr>
<tr>
<td>Glycine</td>
<td>7.28</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.74</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.26</td>
</tr>
<tr>
<td>Leucine</td>
<td>6.04</td>
</tr>
<tr>
<td>Lysine</td>
<td>4.67</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.20</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.46</td>
</tr>
<tr>
<td>Proline</td>
<td>4.49</td>
</tr>
<tr>
<td>Serine</td>
<td>3.35</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.90</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.01</td>
</tr>
<tr>
<td>Valine</td>
<td>6.04</td>
</tr>
</tbody>
</table>
Coconut proteins are usually classified according to their solubility behavior and amino acid analysis (Rasyid et al., 1992). Based on their solubility characteristics, the predominant proteins in coconut endosperm are classified as globulins (salt-soluble) and albumins (water-soluble), which account for 40% and 21% of the total protein, respectively (Balachandran & Arumughan, 1992; Kwon et al., 1996). Of the protein content in coconut skim milk, 75% is accounted for globulin while the remaining 25% is albumin (Garcia, Arocena, Laurena & Tecson-Mendoza, 2005). The large fraction of globulin in coconut proteins is mainly due to their high level of charged amino acids, i.e., glutamic acid, arginine, aspartic acid, and lysine (de Mason & Chandra Sekhar, 1990; Kwon et al., 1996). The coconut globulin contains higher content of essential amino acids including phenylalanine and valine but contains less glutamic acid, lysine, and arginine than the albumin (Kwon et al., 1996). The albumin fraction therefore contains higher proportions of polar side chains, while globulin is more hydrophobic.

Coconut globulins are consist of two major types, i.e., 11S and 7S globulins. The majority of the globulins in coconut endosperm are cocosin, i.e., one of a large class of seed storage proteins known as 11S globulin, which contributes about 86% of the total globulins (Balasundaresan, Sugadev & Ponnuswamy, 2002). Cocosin is generally hexameric quaternary structures of which the molecular weight about 300 to 360 kDa, with each subunit of 55 kDa (Figure 2.3). The subunits consist of the acidic (32 to 34 kDa) and basic (22 to 24 kDa) polypeptides linked by a disulfide bridge. Under reducing conditions, the acidic and basic chains that comprise each cocosin subunit are cleaved (Carr, Plumb, Parker & Lambert, 1990; Garcia et al., 2005). Cocosin is unique among
other 11S globulins due to the presence of a carbohydrate group in the basic polypeptide of its subunits. The basic band is also N-glycosylated which is unusual for legumin type of proteins. The 7S coconut globulin is a type of vicilins which are characterized by a trimeric organization with an oligomeric molecular weight of 150 to 190 kDa, with each single chain subunit of about 55 kDa (Garcia et al., 2005). The coconut 7S globulin is unglycosylated and lack of sulfur-containing amino acids, hence has no disulfide linkages (Carr et al., 1990).

![Model of 11S coconut globulin](image)

**Figure 2.3**: Model of 11S coconut globulin. A and B represent acidic and basic subunits, respectively.

Functional properties of coconut proteins depend strongly on their solubility (Chakraborty, 1985). The solubility of coconut proteins is generally low (Gonzalez & Tanchuco, 1977). The proteins from different regions of coconut endosperm have been reported to have different solubility (Balachandran & Arumughan, 1992), which is the result of different amino acid profile. The minimum solubility of major protein components of coconut skim milk, coconut protein isolate and the extracts of coconut
endosperm was observed between pH 4 and 5 which is the range of isoelectric point of the protein (Balasubramaniam & Sihotang, 1979; Gonzalez & Tanchuco, 1977; Hagenmaier, Cater & Mattil, 1972; Hagenmaier et al., 1974; Kwon & Rhee, 1996) while the maximum solubility was reported at pH 10.3 (Balasubramaniam & Sihotang, 1979).

Coconut proteins have been shown to be highly sensitive to temperature. They denature and coagulate upon heating to 80°C (Gonzalez, 1990; Kwon et al., 1996). Differential scanning calorimetric studies of raw undiluted coconut milk revealed several endothermic transitions over the temperature range of 80 °C to 120°C, which reflects the complex protein composition and thermal denaturation behavior of the coconut proteins (Kwon et al., 1996; Seow & Goh, 1994). The exposure to high temperatures for long time results in denaturation and precipitation of proteins in the coconut milk. The process of coconut protein denaturation by heat is accelerated at the acidic and basic pH regions (Onsaard, Vittayanont, Sringam & McClements, 2005). It has been reported that coconut protein is more resistant to heat denaturation in the presence of sugars, polyols, and salts (Seow & Goh, 1994).

2.2.4 Coconut milk emulsion

Coconut milk is an oil-in-water emulsion naturally stabilized by the coconut proteins (mainly cocosin) and phospholipids (Birosel, Gonzales & Santos, 1963; Monera & del Rosario, 1982). As with all emulsions, coconut milk is not physically stable and is prone to phase separation. Within 5 to 10 h of manufacture, coconut milk will separate
into cream and serum layers, known as coconut cream and coconut skim milk, respectively (Seow & Gwee, 1997). The separated milk, however, can be easily rehomogenized by shaking (Escueta, 1980).

The major reason for the instability of coconut milk emulsion is the poor emulsifying properties and low surface activity of coconut proteins (Monera & del Rosario, 1982). Onsaard, Vittayanont, Sringam & McClements (2005) reported that proteins isolated from coconut skim milk are effective at stabilizing emulsions that are fairly viscous, when droplet flocculation and creaming are not major problems. They also reported the lower efficacy of the proteins extracted from coconut cream, compared to whey protein isolate, in either creating small droplets within the homogenizer or preventing droplet aggregation during or after homogenization to obtain a stable emulsion (Onsaard, Vittayanont, Sringam & McClements, 2006). Moreover, the emulsifying properties of coconut proteins, similarly to other proteins, can be altered by pH, ionic strength, and especially temperature, as discussed in the earlier section (Gonzalez & Tanchuco, 1977; Kwon & Rhee, 1996; Onsaard et al., 2005; 2006; Rhee & Sringam, 1997). Coconut milk thus coagulates to produce unacceptable product and exhibits poor stability after being treated at high temperatures (Agrawal, Choudhary & Sharma, 1991; Seow & Gwee, 1997). The milk was also found to be poorly stable over the pH range of 3.5 to 5 and to exhibit two stability maxima at pH 1.5 to 2 and pH 6.5 (Monera & del Rosario, 1982).
In order to improve the emulsion stability, various types and amounts of additives have been used, together with homogenization in many coconut milk products. The selection is mainly influenced by the fat content and the processing condition of the products. Sodium stearoyl lactylate in the range between 0.5% and 2.5% has been used to improve the creaming stability of unheated coconut milk homogenized at 14/7 MPa (stage I/stage II pressure) (del Rosario & Punzalan, 1977). In another study, sodium caseinate (0.5%) or glyceryl monostearate (1%) was mixed with the fresh coconut milk to produce a stable emulsion after being heated at 95°C to 100°C for 1 h (Genato & Gonzalez, 1985). Modified starch, at the level of 0.4%, was also used to minimize coagulation in canned coconut milk (Timmins & Kramer, 1977). Combinations of emulsifier and thickening agent are sometime applied to several products, especially to those treated by heat. The addition of polyoxyethylene sorbitan monooleate (0.3%) and carboxymethyl cellulose (CMC, 0.4%) to coconut milk prior to homogenizing at 30 MPa has been reported to inhibit the visible separation in the sterilized (115°C for 45 min) product (Martin, Uboldi-Eiroa, Kato, Angelucci, Silva & Leital, 1974). Coconut milk homogenized at the stage I/stage II pressure in the range of 11/4 to 23/4 MPa with 0.6% polyoxyethylene sorbitan monostearate and 0.6% CMC also showed good stability after sterilizing at commercial sterilizing condition (109.3°C to 121.1°C under pressure to obtain $F_0$ value of 5 min) (Chiewchan, Phungamnogoen & Siriwattanayothin, 2006). Recently, different ratios of maltodextrin and gum acacia were used together with sonication to produce a stable coconut milk (Jena & Das, 2006).
References


containing trans-free and partially hydrogenated soybean oil. *Journal of the American Oil Chemists’ Society*, 84(12), 1081-1093.


Chapter 3

Effect of Homogenization Process and Heat Treatment on the Stability of Coconut Milk Emulsions

Abstract

The effects of homogenization and heat treatment on the colloidal stability of coconut milk were studied. Fresh coconut milk (15% to 17% fat, 1.5% to 2% protein) was extracted and stored at 30°C prior to homogenization at 40/4 MPa (stage I/stage II pressure). Both homogenized and non-homogenized samples were heated at 50°C, 60°C, 70°C, 80°C, and 90°C for 1 h. Homogenization reduced the size of the primary emulsion droplets from 10.9 to 3.0 μm, but increased the degree of flocculation presumably via a bridging mechanism. This flocculation was also responsible for increased viscosity of the homogenized samples. Heating increased the degree of flocculation in both non- and homogenized samples. A slight amount of coalescence was also observed after heating above 80°C. All samples creamed after 24 h storage but the heated samples formed a larger cream layer, presumably because the flocculated droplets packed together less efficiently. Optical microscopy was used to confirm the combination of flocculation and creaming responsible for changes in coconut milk quality. The information obtained from this study provides a better understanding of the emulsion science important in controlling coconut milk functionality.
3.1 Introduction

Coconut milk is the white opaque liquid obtained from shredded coconut (Cocos nucifera L.) meat made by comminuting or grating the flesh of the nut (with or without the addition of water) and pressing or dewatering the comminuted pulp. It is an important ingredient for Asian cuisine as well as in other parts of the world. The composition of coconut milk varies according to variety, age, growing environment of the coconut, cultural practices, method of preparation, and the process conditions used in extraction (e.g., the amount of added water and temperature used for extraction) (Cancel, 1979; Gonzalez, 1990). Typical compositions of the coconut milk directly expelled from coconut kernel (without added water) are protein, 2.6% to 4.4%; water, 50% to 54%; lipids, 32% to 40%; and ash, 1% to 1.5% (Seow & Gwee, 1997).

Coconut milk is essentially an oil-in-water emulsion, stabilized by the naturally occurring proteins (i.e., globulins and albumins), and phospholipids (e.g., lecithin and cephalin) (Birosel, Gonzales & Santos, 1963). As with all emulsions, coconut milk is not physically stable and is prone to phase separation. Natural coconut milk will separate into a cream and serum layer within 5 to 10 h of manufacture.

Thermal processing is an effective means of extending the shelf life of coconut milk. The processing of canned coconut milk starts with the extraction of coconut milk, which is then heated to a temperature of about 92°C to 95°C for 5 to 20 min (a process often referred to in the coconut industry as pasteurization) and often mixed with
emulsifiers and/or stabilizers prior to a homogenization process. The homogenized milk is either hot-filled in cans or passed through an exhaust box before can sealing. Since the pH of coconut milk is about 6, it is considered as a low acid food and the cans must be retorted (Arumughan, Balachandran & Sundaresan, 1993; Timmins & Kramer, 1977).

The physical properties of coconut emulsions have not been well studied. It is common practical knowledge that heating and homogenization affect the stability of coconut milk emulsions. However, the mechanisms of such stability alterations and the underlying emulsion science are still unclear. In this work we determine the effects and mechanisms of homogenization and heat treatment on the colloidal stability of freshly-manufactured coconut milk.

### 3.2 Materials and methods

#### 3.2.1 Materials

Whole fresh coconuts were purchased from a local retailer and kept chilled in the cold storage (4°C) until being used for extraction. Thimerosal, and petroleum ether were purchased from Sigma-Aldrich (St. Louis, MO). Sodium dodecyl sulfate (SDS), was purchased from Fisher Scientific (Fairlawn, NJ).
3.2.2 Sample preparation

Coconuts were deshelled and shredded using a traditional coconut grater. Coconut milk was produced by mixing the shredded pulp with an equal weight of warm distilled water (60°C) at the meat-to-water ratio of 2:1 (w/w) in a blender (I120, Waring, Winstel, CT), filtered through a double-layered cheese cloth, and manually squeezed with a twisting motion to extract most of the milk. Thimerosal (0.02 wt%) was added as an antimicrobial agent. The extracted emulsion was stored at 30°C prior to analysis and used within 24 h of manufacture. The crude protein content of coconut milk was measured using the nitrogen combustion method by an automatic nitrogen analyzer (FP-528, Leco, St. Joseph, MI). The fat content was determined using a modified Majonnier ether extraction method (AOAC Official Method 989.05, AOAC, 2000). Homogenized samples were prepared by recirculating fresh coconut milk through a twin-stage valve homogenizer (Panda, GEA Niro Soavi, Hudson, WI) at a pressure of 40/4 MPa (stage I/stage II pressure) for 4 min to achieve multiple passes through the valves. Both non- and homogenized samples were heated in a temperature-controlled water bath set at 50°C, 60°C, 70°C, 80°C, and 90°C for 1 h and then cooled to 30°C in another water bath prior to analysis.

3.2.3 Particle size analysis

The size distribution (volume fraction as a function of particle size) of the coconut milk emulsion droplets was measured using a laser diffraction particle analyzer (LA-920, Horiba, Irvine, CA) using a relative refractive index of 1.09, i.e., the ratio of the
refractive index of coconut oil (1.45) and that of the water as dispersion medium (1.33). Coconut milk samples were diluted to approximately 0.001% fat prior to analysis to minimize multiple scattering effects. The droplet size was reported in terms of the volume-weighed average diameter: \( d_{43} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \), where \( n_i \) is the number of droplets of diameter \( d_i \). In some experiments, the coconut milk was diluted in 1 wt% SDS solution rather than water. SDS is an anionic surfactant which effectively displaces protein from the surface of emulsion droplets and disrupts droplet flocs formed due to interdroplet protein-protein interactions. Particle size measured in water is referred to in this work as an “effective” particle size and includes the presence of flocs, while measurement in SDS solution is referred to as the “primary” particle size. Emulsion coalescence will be seen as a change in both primary and effective particle diameter, while flocculation will increase the effective diameter but the primary diameter will remain unchanged.

3.2.4 Microscopy

Samples of coconut milk (~25 μl) were placed on a microscope slide, gently covered with a cover slip, and observed at 200x magnification using an optical microscope (BX40, Olympus America, Melville, NY) equipped with a color video camera (DXC-970MD, Sony, New York, NY). The optical micrographs were analyzed using image analysis software (PAX-it, MIS, Villa Park, IL). Pictures were taken from three different fields on each slide and representative images are presented.
3.2.5 Rheological study

Rheological measurements were carried out using a controlled strain rheometer (Ares, TA Instruments, New Castle, DE) operating with a cone and plate geometry (50 mm cone diameter, 0.04 radian cone angle). Samples were equilibrated at 30°C, gently mixed, and then portions (~1.5 ml) were transferred to the instrument. The instrument had previously been equilibrated at 30°C and the test was run immediately. The shear rate was increased from 0 to 100 s⁻¹ over 7 min and the required stress used to calculate the apparent viscosity.

3.2.6 Creaming stability measurement

Portions (10 g) of coconut milk samples were transferred into flat-bottomed test tubes (15 mm internal diameter, 125 mm height), covered, and allowed to stand for 24 h at 30°C in a temperature-controlled water bath. All samples separated into the opaque layer at the top and the transparent aqueous phase at the bottom during storage. The extent of the phase separation was assessed by creaming index, which is the percentage ratio between the height of the transparent layer and total height of the emulsion in the test tube.

3.2.7 Determination of free fat

The degree of emulsion destabilization was measured as the amount of solvent-extractable oil. Samples of coconut milk (10 g) were transferred to Majonnier flasks and
extracted with petroleum ether at the ratio of 3:1 (v/v). The organic extracts were evaporated to dryness in a Soxtec extraction unit and the extractable oil weighed. The extraction was repeated five times and the cumulative value of extracted fat was calculated.

3.2.8 Statistical analysis

Most experiments were conducted in triplicate with freshly prepared coconut milk used on each occasion. Data were analyzed using statistical software (SPSS 11.5, SPSS, Chicago, IL). One-way analysis of variance (ANOVA) and Duncan’s multiple range tests were used to evaluate the significance of differences (p≤0.05) between the samples. Only significantly different results are discussed in the text. Data are presented as the mean and standard deviation.

3.3 Results and discussion

The droplet size distribution in the fresh milk had an approximately log-normal form (Figure 3.1a). The effective droplet diameter of the fresh coconut milks was 13.1 μm with a standard deviation of the distributions of approximately 2 μm. Surprisingly, the homogenized milk had only slightly different effective particle size from the non-homogenized milk (Figure 3.1b). However, when the milk was dispersed in SDS solution rather than distilled water prior to laser diffraction particle sizing, the
Figure 3.1: Representative particle size distribution of non-homogenized (a) and homogenized (b) coconut milks dispersed in distilled water (solid line) or SDS solution (dashed line).
homogenized samples were much smaller than the non-homogenized samples. SDS displaces the protein from the oil-water interface, thus, disrupting any flocculation caused by interdroplet protein-protein interactions and allows the instrument to measure the primary particle size rather than the apparent size of flocs present. The non-homogenized coconut milk particle size was not markedly affected by dilution in SDS, suggesting the particles present were less significantly flocculated.

This suggested that homogenization significantly reduced the mean diameter of the droplets, but the fine droplets formed quickly flocculated to approximately the same effective size as was present before homogenization. The non-homogenized milk had large droplets but these were largely non-flocculated. Optical micrographs of the homogenized and non-homogenized samples revealed more large droplets in the former and more flocculation in the latter (Figure 3.2). A likely explanation for this is the amount of protein capable of stabilizing the emulsion is limited in coconut milk (del Rosario & Punzalan, 1977). As the particle size is reduced, the interfacial area increased and a single protein molecules originally adsorbed to one droplet ended up simultaneously adsorbing to the surface of two droplets leading to bridging flocculation (McClements, 2004).

In other experimental works, the effect of homogenization pressure on the effective and primary particle diameter of coconut milk was measured (Figure 3.3). Samples of freshly extracted coconut milks were homogenized at stage I/stage II pressures of 20/2 MPa, 40/4 MPa, and 60/6 MPa for 1 to 5 passes through the
Figure 3.2: Micrographs taken of non-homogenized (a, c, e) and homogenized (at 40/4 MPa) (b, d, f) coconut milks either unheated (a, b) or heated to 50°C (c, d) or 90°C (e, f). Scale bar is 50 μm.
Figure 3.3: Mean particle size of coconut milks homogenized at (●, ○) 20/2, (■, □) 40/4, and (▲, ∆) 60/6 MPa. Filled points represent emulsions dispersed in water, open points represent emulsions dispersed in SDS solution.
homogenizer. Homogenization reduced both the effective and primary particle sizes of coconut milk by about 50% to 75%. Increasing the homogenization pressure marginally decreased the effective particle size, and after the third pass through the homogenizer subsequent passes had no further effect on particle size. This supports our hypothesis that the amount and quantity of protein limits the effectiveness of homogenization on coconut milk.

Figure 3.4 shows the mean droplet diameter of both non- and homogenized coconut milks heated at different temperatures. The effective particle size of homogenized coconut milk increased dramatically (from about 10 μm to more than 22.7 μm) after heating at above 70°C for 1 h while the primary particle size only increased after heating at 90°C. For non-homogenized samples, effective particle size changed from 12.2 μm to 30.5 μm when the heating temperature increased. Significant changes in the primary particle size are also detected at higher heating temperatures. Both the effective and primary particle size increased in non- and homogenized coconut milks heated at temperatures above 70°C, suggesting that both flocculation and possibly a slight degree of coalescence occurred in heated coconut milk emulsions. This is supported by observations of the microstructure of the heated emulsion samples (Figure 3.2). Just as solutions of globular proteins sometimes gel if thermally denatured, protein-stabilized emulsions have been shown to flocculate following heating as the protein-protein associations formed bind the droplets together in a network (Sliwinski, Roubos, Zoet, van Boekel & Wouters, 2003). Coconut proteins have been shown to denature and coagulate at 80°C and higher (Gonzalez, 1990; Kwon, Park & Rhee, 1996); it seems likely that the
Figure 3.4: Mean particle size of (●, ○) non-homogenized and (■, □) homogenized (at 40/4 MPa) coconut milks heated at different temperatures. Filled points represent emulsions dispersed in water, open points represent emulsion dispersed in SDS solution.
denaturation and aggregation of surface-bound proteins is responsible for the thermally-induced flocculation seen here. Coalescence (seen as a change in primary particle size) may then result due to the breakdown of the lamella separating the flocculated droplets as the effectiveness of the coconut proteins as stabilizing agents is reduced.

Rheological measurements showed that both the non-homogenized and homogenized coconut milk samples were shear-thinning fluids of which the apparent viscosity decreased with increasing shear rate. Similar flow behavior was reported in previous studies (Simuang, Chiewchan & Tansakul, 2004; Vitali, Soler & Rao, 1986). The flow curves were modeled using a power law equation ($\tau = K\gamma^n$; where $\tau$ is the shear stress, $\gamma$ is the shear rate, $K$ is the consistency coefficient, and $n$ is the flow behavior index, respectively). Power-law equations are frequently used to describe emulsion rheology and low shear apparent viscosity (here reported at 10 s$^{-1}$) and the $n$ parameter is often used to describe the inherent structure of whatever weak network in present and how readily it is disrupted by shear respectively (McClements, 2004). In all cases, the power-law equation described the data well ($r^2>0.98$) and values of $K$ and $n$ are reported in Figure 3.5.

The homogenized samples were more viscous than the non-homogenized coconut milks, consistent with the presence of flocculated droplets. Emulsion flocculation leads to a higher effective volume fraction, and thus higher viscosity (McClements, 2004). The consistency coefficient increased with temperature for both homogenized and non-
Figure 3.5: Flow behavior index (a), and consistency coefficient (b) of (●) non-homogenized and (○) homogenized (at 40/4 MPa) coconut milks heated at different temperatures. Measurements were taken at a shear rate of 10 s⁻¹.
homogenized samples, probably due to additional thermally induced flocculation. The flow behavior index decreased with temperature as the structures formed could be readily disrupted by applied shear.

The creaming indices for the non-homogenized and homogenized coconut milk samples are shown as a function of thermal history in Figure 3.6. All samples creamed after 24 h storage, but the heated samples separated less as indicated by the lower serum heights, and thus the lower creaming indices. Non-homogenized coconut milk is more prone to creaming than homogenized coconut milk because of its larger globule size (Monera & del Rosario, 1982). The small but flocculated particles present in homogenized milk creamed more slowly because the density contrast of a floc is smaller than that of a droplet, and secondly because large flocs can be extensively interconnected and trap the droplets in a network (Dickinson, Golding & Povey, 1997). The large network necessary to inhibit creaming may not be seen in light scattering measurements from a diluted emulsion as the process of sample preparation disrupted the fragile structures. Better creaming stability was found in both non-homogenized and homogenized coconut milks after heating at temperature above 80°C for 1 h, probably due to the higher viscosity in these samples slowing the creaming rate (McClements, 2004) or differences in the structure of the creamed layer (Chanamai & McClements, 2000).
Figure 3.6: Creaming index after 24 h storage of (●) non-homogenized and (○) homogenized (at 40/4 MPa) coconut milks heated at different temperatures.
Fink and Kessler (1985) argued that oil extraction by organic solvent (in native milk fat globules) is a measure of the ability of the interfacial layer to protect the fat destabilization process. They also argued that degradation of this interfacial layer could be inferred from changes in the amount of extractable oil. In our experiments, more than 80% of oil in fresh coconut milk could be extracted by petroleum ether, while only about 10% of the oil from similar homogenized milks could be extracted (Figure 3.7). Homogenization reduced the primary particle size of the coconut milk and led to extensive flocculation (Figure 3.1b, 3.2, and 3.4) and it seemed likely that the droplets in the core of the flocs were more protected from the extracting solvent.

Thermal processing progressively decreased the amount of solvent-extractable oil in the non-homogenized samples while increasing it in the homogenized samples. The markedly changes were found at the heating temperature between 60 ºC and 70 ºC. When heated at 90 ºC, the amount of oil that can be extracted from the coconut milks was independent of the homogenization process. We hypothesize that, on heating the homogenized samples, the protein involved in bridging flocculation denatured. It thus rearranged and was pulled away from one of the bridged droplets to expose some lipid surface and allowed more fat to be solvent extracted. In the non-homogenized samples the protein was not involved in bridging flocculation so it was not necessarily pulled away from the droplet surface on denaturation. The resultant flocculation of the non-homogenized samples was induced by protein-protein hydrophobic attractions and might served to decrease the amount of solvent-extractable oil.
Figure 3.7: Free oil solvent-extracted from (●) non-homogenized and (○) homogenized (at 40/4 MPa) coconut milks heated at different temperatures.
3.4 Conclusions

Coconut milk is an emulsion extracted with minimal processing from fresh coconuts. The emulsion particle size is naturally of the order of 13.1 μm and can only be slightly reduced on homogenization as the quality and quantity of emulsifiers (probably protein) naturally present is low. The homogenized emulsions tended to be highly flocculated, probably by bridging flocculation, and this increased the product viscosity. Thermal treatment increased the degree of flocculation, probably because of protein-protein hydrophobic attractions following denaturation, and led to increased effective particle size and apparent viscosity. The creaming stability of the emulsion improved on heating since the larger flocs can form a network in the more viscous emulsion. Solvent-extractable oil of non-homogenized coconut milk decreased with increasing heating temperature, while those of homogenized samples increased due to the differences in accessibility of the extracting solvent to the fat globules. The information obtained from the study provides a better understanding of the changes in stability of coconut milk emulsion during processing important in controlling coconut milk functionality.

References


Chapter 4

Effect of pH and Ionic Strength on the Physicochemical Properties of Coconut Milk Emulsions

Abstract

Coconut milk (16% to 17% fat, 1.8% to 2% protein) was extracted from coconut (Cocos nucifera L.) endosperm and diluted in buffer to produce natural oil-in-water emulsions (10 wt% oil). The effect of pH (3 to 7) and NaCl (0 to 200 mM) on the properties and stability, namely, mean particle size, $\zeta$-potential, viscosity, microstructure, and creaming stability, of the natural coconut milk emulsions was investigated. At pH values close to the isoelectric point (pI) of the coconut proteins (pH 3.5 to 4) and in the absence of NaCl, coconut milk flocculated but did not coalesce. Flocculation corresponded to low surface charges and was accompanied by an increase in emulsion viscosity. Adding up to 200 mM NaCl to those flocculated emulsions did not change the apparent degree of flocculation. Coconut milk emulsion at pH 6 was negatively-charged and non-flocculated. Upon addition of salt the $\zeta$-potential decreased from -16 to -6 mV (at 200 mM NaCl) but this was not sufficient to induce flocculation in coconut milk emulsions. At low pH (< pI), the positively-charged droplets of coconut milk emulsions only flocculated when the NaCl concentration exceeded 50 mM, as the $\zeta$-potential approached zero.
4.1 Introduction

Coconut milk is the oil-in-water emulsion obtained from the aqueous extraction of coconut (*Cocos nucifera* L.) endosperm. It is widely used as a food ingredient to provide creaminess and unique aroma in many cuisines, especially in Asia and Pacific regions. However, coconut milk is not thermodynamically stable and readily separates into two distinct layers - a cream layer and a more dense aqueous serum (Seow & Gwee, 1997). The reason for the instability is that the coconut protein content and quality is not sufficient to stabilize the emulsion (Monera & del Rosario, 1982). Moreover, coconut milk composition, including protein, varies according to variety, age, and growing environment of the coconut, and the method and condition used in the extraction process (Cancel, 1979).

The coconut oil globules in the emulsion are naturally stabilized by coconut proteins (i.e., globulins and albumins) and phospholipids (Birosel, Gonzales & Santos, 1963; Monera & del Rosario, 1982). The largest amount of protein in the coconut endosperm is a storage 11S globulin known as cocosin which is a hexamer of 55 kDa subunits. The protein is believed to govern the emulsion stability of coconut milk (Garcia, Arocena, Laurena & Tecson-Mendoza, 2005; Seow & Gwee, 1997). Proteins are charged and surface-active molecules that are able to facilitate the formation and improve the stability of an oil-in-water emulsion. They adsorb at the surface of the droplets and provide repulsive interactions (e.g., electrostatic and steric) that help prevent droplet aggregation (McClements, 2004b). The emulsifying properties of proteins are influenced
by their structures, which are in turn affected by environmental factors, i.e., pH, temperature, and ionic strength of the medium (Das & Kinsella, 1990). Protein-stabilized emulsions tend to flocculate at pH values close to their isoelectric point (pI) due to a loss of the electrostatic repulsion forces between the droplets. Electrolytes can affect the stability of protein-stabilized emulsions either by reducing the electrostatic repulsion through a screening effect or by binding to oppositely charged groups on the droplet surface, which can lead to droplet flocculation (Hunter, 2001; McClements, 2004a). Flocculation can be detrimental to the quality and shelf life of emulsions as it affects the rate of gravitational separation, and the viscosity of the products (McClements, 2004b). For coconut milk emulsions, rapid phase separation and high viscosity are undesirable as they diminish the products’ sensory attributes (Cancel, 1979).

The effect of pH and NaCl on particle size and creaming stability of oil-in-water emulsions stabilized by proteins extracted from coconut skim milk have been reported by Onsaard, Vittayanont, Sringam a McClements (2005). Their study was conducted in a model emulsion that did not contain the non-protein components of the coconut milk. The goal of this study was to examine the influence of pH and concentration of sodium chloride on the stability and physicochemical properties of natural coconut milk emulsions.
4.2 Materials and methods

4.2.1 Materials

Frozen grated coconut meat (35% fat, 3% protein, 45% moisture) was purchased from a local grocery store and kept in the freezer until being used for extraction. Thimerosal, and sodium dodecyl sulfate (SDS) were purchased from Sigma-Aldrich (St. Louis, MO). Sodium chloride, citrate buffer ingredients (citric acid monohydrate and sodium citrate), standard solutions of hydrochloric acid, and sodium hydroxide (0.1 and 1 N) were purchased from Fisher Scientific (Fairlawn, NJ).

4.2.2 Sample preparation

Coconut milk was produced by mixing the thawed coconut meat with distilled water (2:1 w/w), in a Waring blender (1120, Waring, Winstel, CT). The slurry was then pressed and filtered through cheesecloth to remove the solid residue. Thimerosal was added to a final concentration of 0.02 wt% to prevent microbial spoilage of the emulsion. Nitrogen content of the extracted coconut milk was analyzed using a combustion method (FP-528, Leco, St. Joseph, MI) from which the protein content was calculated by using a conversion factor of 6.25. The fat content was determined using a modified Mojonnier ether extraction method (AOAC Official Method 989.05, AOAC, 2000).

To determine the effect of pH on the coconut milk emulsions, the pH of the milk was adjusted by titrating with 0.1 or 1 N HCl or NaOH solutions prior to dilution in 20
mM citrate buffer at an appropriate pH value (3 to 7) to obtain a final fat content of 10 wt%. Sodium chloride (0 to 200 mM) was added to samples with pH values close to, below, and above the pI of coconut proteins to investigate the changes in emulsion properties due to ionic strength.

4.2.3 Particle size analysis

Particle size distributions of the samples were measured using a laser diffraction particle analyzer (LA-920, Horiba, Irvine, CA). To avoid multiple scattering effects during measurement, samples were diluted to approximately 0.001 wt% fat in buffer of the same pH and salt concentration. The particle size of the samples was also determined by diluting the droplets 5 folds in SDS solution (1.25 wt%) and stirring for 30 min. The surfactant displaced the protein from the droplet interfaces and thus dispersed the aggregates. Preliminary experiments showed that SDS only acts as a dissociating agent of aggregates without affecting the droplet size of non-flocculated emulsions. Particle size measured in buffer is referred to as an “effective” particle size which includes the presence of any flocs, while measurement in SDS solution is referred to as the “primary” particle size which is the actual size of the droplets. To identify if the emulsion is flocculated, effective and primary particle sizes were compared. Both measured particle sizes of coalesced samples increased, while flocculated samples had larger effective diameter but unchanged primary diameter.
The scattering pattern was used by the internal software of the instrument to calculate the particle size of the droplets using a relative refractive index of 1.09, which is the ratio of the refractive index of coconut oil, 1.45, and that of the dispersion medium, 1.33. The mean particle size was reported as the volume fraction-length mean diameter,

\[ d_{43} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3}, \]

where \( n_i \) is the number of droplets of diameter \( d_i \).

Samples were stored at 30°C in a water bath until they were analyzed. All analyses were completed within 8 h after manufacture, except the creaming stability measurement which was determined after 24 h storage in order to allow phase separation. The experiment was repeated 3 times with freshly prepared samples used on each run.

### 4.2.4 Microscopy

Samples of coconut milk were observed under 400x magnification using an optical microscope (BX41-TF, Olympus, Tokyo, Japan) equipped with a color digital video camera (SPOT Insight QE 4.2, Diagnostic Instruments, Sterling Heights, MI).

Laser scanning confocal microscopy (LSCM) was also used to observe the microstructure of coconut milk at pH 6 (not flocculated), and pH 4 (flocculated). The coconut protein was stained with rhodamine isothiocyanate (RITC) dissolved in distilled water with a dye concentration of 0.25 mg g\(^{-1}\) protein. Dispersions were incubated overnight in the dark at 30°C prior to dialyzing against buffer to remove excess free marker. Dialyzed dispersions were observed using a LSCM (FV1000, Olympus America,
Melville, NY) with a 60x 1.42 N.A. oil-immersion objective. The dye was excited by a
green He-Ne laser at its excitation wavelength of 543 nm, and the fluorescence emission
between 555 to 578 nm was recorded. Differential interference contrast (DIC) transmitted
light images were collected simultaneously.

The optical and confocal micrographs were analyzed using image analysis
softwares (SPOT Advanced, Version 4.6, Diagnostic Instruments and FV10-ASW,
Version 1.6a, Olympus). Pictures were taken from three different fields on each slide and
representative images are presented.

4.2.5 ζ-potential measurement

The electrical charge of the oil droplets in the emulsions was determined using a
particle electrophoresis instrument (ZetaPALS, Brookhaven Instruments, Holtsville, NY).
The ζ-potential is determined by measuring the velocity of the droplet in an applied
electrical field. Emulsions were diluted in buffer solutions of the appropriate pH and salt
concentration to 0.001 wt% to avoid multiple scattering effects. The diluted emulsions
were mixed thoroughly prior to being placed in a standard four-sided, 1 cm polystyrene
cuvette. A parallel plate electrode (0.45 cm² square platinum plates with a 0.4 cm gap)
was inserted and the cuvette was placed in a temperature-controlled holder at 30°C. The
electrophoretic mobility was measured by phase analysis light scattering. Each
measurement was the average of 120 (6 sets of 20) measurements and the entire
experiment was conducted in triplicate. The $\zeta$-potential was calculated from the electrophoretic mobility using the Smoulokowski model.

### 4.2.6 Rheological study

Viscosity was determined using a temperature controlled Brookfield viscometer (Cannon Instrument Company, LV-2020, State College, PA) fitted with an UL adapter (Brookfield Engineering Laboratories, Middleboro, MA) which consisted of a cylindrical spindle (25 mm diameter, 90 mm height) rotating inside a machined tube (28 mm internal diameter, 135 mm height). Sixteen milliliters of sample was placed in the tube and allowed to equilibrate to 30°C for 5 min prior to measurement. The shear rate was increased from 12.24 to 122.4 s$^{-1}$ over 210 s and the apparent viscosity was recorded every 30 s.

### 4.2.7 Creaming stability measurement

Immediately after preparation, coconut milk samples (10 g) were transferred into a flat-bottomed test tube (15 mm internal diameter, 125 mm height), capped, and stored for 24 h at 30°C in a temperature-controlled water bath. During storage, all samples separated into an opaque cream layer at the top and a transparent serum layer at the bottom. The total height of the emulsions ($H_E$) and the height of the serum layer ($H_S$) were measured. The extent of the phase separation was assessed by a creaming index (CI = 100 × $H_S / H_E$) as described by Demetriades & McClements (1998). The CI provides
indirect information about the extent of droplet aggregation in an emulsion: typically the more the aggregation, the larger the effective particle size and the faster the creaming. However, a direct causative link between flocculation and creaming is not always justified. Sometimes flocculated emulsions cream more slowly because either the density contrast of a floc is smaller than that of a droplet or because large flocs can be extensively interconnected and trap the droplets in a network (Dickinson, Golding & Povey, 1997).

4.2.8 Statistical analysis

Data were analyzed using statistical software (SPSS 11.5, SPSS, Chicago, IL). One-way analysis of variance (ANOVA) and Duncan’s multiple range tests were used to determine the significance of differences between the samples. Only significantly different results ($p \leq 0.05$) are discussed in the text. Data are presented as the mean and standard deviation.

4.3 Results and discussion

The extracted coconut milk (67% to 70% yield) contained 17% to 18% fat and 1.8% to 2% protein and had an initial pH of 6.1 to 6.3, which is similar to the pH of fiber-free coconut milk extract reported elsewhere (Balasubramaniam & Sihotang, 1979). The mean particle size ($d_{43}$) of coconut oil droplets in the natural emulsion was 11 to 12 µm with a bimodal distribution. There was no difference between the particle size
distribution of the samples diluted in buffer and in SDS solution (data not shown),
indicating that the emulsion droplets were relatively non-flocculated. This is similar to
what we have reported previously (Tangsuphoom & Coupland, 2008). The surface charge
of coconut milk droplets at its native pH was negative (-16 mV) which reflected the
amino acid composition of the coconut proteins (Rasyid, Manullang & Hansen, 1992).
The most abundant (~20%) amino acid in coconut proteins is glutamic acid (Gonzalez &
Tanchuco, 1977; Kwon, Park & Rhee, 1996) which has a pKₐ value around 4, and is
therefore negatively charged at neutral pH values.

There was no significant change in the primary particle size in any of these
experiments suggesting the important instability mechanism was always flocculation
rather than coalescence.

4.3.1 Effect of pH

At pH 3, the effective droplet diameter was only slightly greater than the primary
donut size (13 and 11 μm, respectively; Figure 4.1) suggesting the droplets did not
flocculate and indeed those unflocculated droplets were seen by optical microscopy
(Figure 4.2a). The droplets were positively charged (Figure 4.3) because the amino
groups of the amino acid residues on the interfacial coconut proteins were positively
charged while the carboxyl groups were neutral. At neutral pH (~7) the coconut milk
droplets developed negative charges due to the dissociation of the carboxylic side chains
of the amino acid residues of coconut proteins (Figure 4.3) and were again non-
Figure 4.1: Mean particle size of coconut milk emulsions adjusted to different pH values. Filled points represent emulsions dispersed in water; open points represent emulsions dispersed in SDS solution.
**Figure 4.2:** Optical micrographs taken of coconut milk emulsions adjusted to pH 3 (a), 3.5 (b), 4 (c), and 6 (d). Scale bar represents 50 μm. Arrows indicate possible insoluble protein clumps.
Figure 4.3: ζ-potential of coconut milk emulsions adjusted to different pH values.
flocculated (Figure 4.1 and 4.2d). When the pH was between 3.5 and 4.5, which is close to the reported pI of coconut proteins (Gonzalez, 1993), the effective droplet size of the emulsions increased to larger than 20 μm while the primary particle size remained unchanged, ranging between 11 and 12 μm, which suggested extensive droplet flocculation (Figure 4.1). This hypothesis is supported by the microscopy (Figure 4.2). However, some caution should be exercised in definitively describing the various emulsions as flocculated and non-flocculated. We identified flocculated emulsions as those with an effective diameter larger than the primary diameter when measured by the light scattering instrument. Nevertheless, the process of diluting a flocculated emulsion in water and pumping it through the optical cell may be enough to disrupt weak aggregates. Indeed, when the undiluted emulsions were observed by optical microscopy there was some flocculation in all samples albeit larger ones at pH close to pI.

The coconut milk emulsion presumably flocculated when the stabilizing proteins approached their pI and the magnitude of droplet surface charge was too small to overcome the attractive interactions between droplets (McClements, 2004a). Similar behavior has been seen in oil-in-water emulsions stabilized by coconut skim milk proteins at the pH close to the pI (Onsaard et al., 2005), and also in a variety of other protein-stabilized emulsions, e.g., whey proteins (de Wit & van Kessel, 1996; Demetriades, Coupland & McClements, 1997; Kulmyrzaev, Chanamai & McClements, 2000; Kulmyrzaev, Sivestre & McClements, 2000), caseins (Agboola & Dalgleish, 1996; Surh, Decker & McClements, 2006), and soy protein (Comas, Wagner & Tomas, 2006). The instability of natural coconut milk emulsions close to the pI of coconut protein
corresponds to the minimum solubility of the proteins themselves (Gonzalez, 1993; Kwon & Rhee, 1996; Onsaard et al., 2005; Samson, Khaund, Cater & Mattil, 1971), and their minimum effectiveness as emulsifiers (Capulso, Gonzales & Celestino, 1981; Gonzalez & Tanchuco, 1977). Interestingly, the samples close to the pI contained non-spherical clumps of material, often at the center of flocs (see arrows in Figure 4.2). These clumps are less prevalent in the samples of pH higher and lower than pI, and may correspond to isoelectric precipitation of coconut proteins acting as a “seed” for droplet flocculation. To confirm this finding, we labeled the coconut proteins and observed the microstructure of the coconut milk emulsions at pH 4 and 6 under the LSCM (Figure 4.4). The stained proteins appeared red on the micrographs. In Figure 4.4a we can see that the proteins in the emulsions at pH 4 clumped together with droplets attached as flocs while the proteins in the control emulsion (pH 6) distributed evenly (Figure 4.4b).

The viscosity of the coconut milk emulsions is shown as a function of shear rate and pH in Figure 4.5, in which the data was fitted to the power-law rheological model. The flocculated emulsions (pH=pI) were both more viscous and more shear thinning than the non-flocculated emulsion. Measurements of apparent viscosity are sensitive to changes in the degree of flocculation in an emulsion (McClements, 2004b). Flocculation increases the effect of volume fraction of particles because the aggregates trap some of the continuous phase within their structure, and thus increases the viscosity. The breakdown of flocculated structures under applied shear leads to shear thinning behavior.
Figure 4.4: LSCM images overlaid with corresponding images from DIC transmitted light of coconut milk emulsions adjusted to pH 4 (a), and 6 (b). Coconut proteins were labeled red with RITC ($\lambda_{exc}=543$ nm) while the emulsion droplets are seen grey under DIC. Scale bar represents 20 $\mu$m. Arrows indicate possible insoluble protein clumps.
Figure 4.5: Steady shear viscosity of coconut milk emulsions adjusted to pH (●) 3, (♦) 3.5, (■) 4, and (▲) 6. The lines fit the power-law rheological model.
Several studies have reported shear-thinning behavior in coconut milk (Simuang, Chiewchan & Tansakul, 2004; Tangsuphoon & Coupland, 2005; Vitali, Soler & Rao, 1986) as well as an increase in viscosity of coconut milk at acidic conditions where pH≤pI (Arumughan, Balachandran & Sundaresan, 1993).

Coconut milk emulsions creamed extensively (CI>70%) and formed 2 distinct layers of cream and serum after 24 h storage (Figure 4.6). The extensive creaming was due to the relatively large particle size and the low viscosity (2 mPa s; Figure 4.5) of the unflocculated coconut milk emulsions. Creaming in coconut milk emulsion was retarded when pH was close to the pI (Figure 4.6) perhaps because the flocculated network slowed the upward movement of the droplets (Dickinson et al., 1997). It should be noted that although coconut milk emulsions at different pH values separated into two distinctive phases, the aqueous phase was transparent without any apparent precipitation of the proteins. This suggested that the coagulated proteins in the coconut milk remained with the cream phase which was an indication of the affinity between the coconut proteins and the coconut oil globules (Birosel et al., 1963; Gonzalez, 1993).
Figure 4.6: Creaming index after 24 h storage of coconut milk emulsions adjusted to different pH values.
4.3.2 Effect of NaCl

The influence of salt concentration on coconut milk emulsion stability was investigated at pH values below the pI (pH 3), close to the pI (pH 4), and above the pI (pH 6). The addition of NaCl (>50 mM) to the coconut milk emulsion at pH 3 resulted in an increase in effective droplet diameter, indicating flocculation (Figure 4.7 and 4.8). The ζ-potential of the emulsions decreased from a net positive charge (+12 mV) to approximately zero as the salt concentration was increased to 100 mM (Figure 4.9). The alteration in ζ-potential of the droplets might have occurred due to either screening the electrostatic interactions (reducing the Debye screening length) and/or ion binding of the added chloride ions to the amino groups on the proteins (Hunter, 2001). The presence of NaCl at pH 3 was also reported to decrease the solubility of coconut proteins (Kwon & Rhee, 1996; Samson et al., 1971) which may have contributed to the instability of the emulsions. Interestingly non-spherical clumps were seen in the samples containing higher salt that may correspond to precipitated protein (see arrows in Figure 4.8).
Figure 4.7: Mean particle size of coconut milk emulsions adjusted to pH (●) 3, (■) 4, and (▲) 6 as affected by the addition of NaCl. Data points represent emulsions dispersed in water. Mean particle size of the emulsions dispersed in SDS solution range between 11 and 13 μm.
Figure 4.8: Optical micrographs of coconut milk emulsions adjusted to pH 3 (a to c), 4 (d to f), and 6 (g to i) as affected by the addition of 50 (a, d, g) 100 (b, e, h), and 200 (c, f, i) mM NaCl. Scale bar represents 50 μm. Arrows indicate possible insoluble protein clumps.
Figure 4.9: $\zeta$-potential of coconut milk emulsions adjusted to pH (●) 3, (■) 4, and (▲) 6 as affected by the addition of NaCl.
The flocculation of emulsion droplets by added salt at pH 3 resulted in a decrease in creaming index (data not shown) and an increase in viscosity of the emulsion when the concentration of NaCl reached 100 mM (Figure 4.10a). Flocculated samples were more viscous and exhibited more shear-thinning behavior than the no salt added coconut milk emulsion at pH 3, or even than the flocculated emulsions of pH 4 (Figure 4.10). The viscosity data also confirmed that the degree of flocculation in the emulsion did not markedly increase when the NaCl concentration increased from 100 to 200 mM.

At pH 4, the droplets were flocculated whatever the concentration of added salt (Figure 4.7; see also micrographs in Figure 4.8). The $\zeta$-potential of these droplets was low even in the absence of added salt (Figure 4.9), so the changing ionic strength had no effect on the intrinsically unstable dispersion. No change in rheological properties was observed (Figure 4.10b). Coconut milk emulsions at pH 6 remained non-flocculated at all NaCl concentrations. In this case, the magnitude of the $\zeta$-potential was still high enough (-6 mV) to inhibit flocculation in coconut milk emulsions even as it decreased with added salt (Figure 4.9). Thus, samples with NaCl showed similar rheological behavior to the sample without added salt (Figure 4.10c). It has been reported that emulsion stabilized by proteins extracted from coconut skim milk was relatively stable to droplet aggregation and creaming in the presence of NaCl (Onsaard et al., 2005). The solubility of coconut proteins at pH $\geq$6 was also found to be enhanced by the addition of NaCl (Capulso et al., 1981; Kwon & Rhee, 1996; Samson et al., 1971) which in turn enhance the emulsifying properties of the proteins.
Figure 4.10: Steady shear viscosity of coconut milk emulsions adjusted to pH 3 (a), 4 (b), and 6 (c) as affected by the addition of (●) 0, (♦) 50, (■) 100, and (▲) 200 mM NaCl. The lines fit the power-law rheological model.
Figure 4.10 (Continued)
4.4 Conclusions

Coconut milk emulsions were extensively flocculated at pH values close to their pI where they had no net surface charge. Flocculation resulted in a more viscous, shear-thinning product with greater resistance to creaming. The impact of NaCl on the stability of coconut milk varied with the pH of the emulsions. Coconut milk emulsions with natural pH (>pI) were stable to NaCl addition up to 200 mM, possibly because the ζ-potential was still sufficient to prevent aggregation. Addition of NaCl to the flocculated coconut milks also did not affect their stability. However, adding NaCl to the emulsions at pH<pI led to extensive flocculation of the droplets when the salt concentration exceeded 50 mM, which resulted from the screening effect of the added electrolyte.

References


Chapter 5

Effect of Surface-Active Stabilizers on the Microstructure and Stability of Coconut Milk Emulsions

Abstract

The effect of surface-active stabilizers (0 to 1 wt% sodium caseinate, whey protein isolate, sodium dodecyl sulfate, and polyoxyethylene sorbitan monolaurate) added before or after a homogenization step on the microstructure and colloidal stability of coconut milk was determined using measurements of particle size, creaming, and microscopy. The freshly prepared coconut milk emulsions (1.8% to 2% protein, 17 to 18% fat) had large ($d_{43}$~10 µm) but non-flocculated droplets. Homogenization reduced the primary droplet size but induced flocculation. Adding small-molecule surfactants after the homogenization step can displace coconut proteins from the interface and break up these flocs but adding them before homogenization increases the efficiency of the homogenization step and produces stable, submicron-sized emulsion droplets. Protein stabilizers did not break up the flocs of coconut milk droplets when added after homogenization but did increase the efficacy of the homogenization step if added prior to it. Adding stabilizers to non-homogenized coconut milk had no effect on the structure or properties of the emulsions.
5.1 Introduction

An emulsion is a mixture of two immiscible liquids, with one (a dispersed phase) present as small spherical droplets in another (a continuous phase). Emulsions are thermodynamically unstable due to the unfavorable contact between oil and water molecules (Friberg, 1997) and, as a consequence, their physical structure will tend to change over time by various mechanisms (e.g., creaming, flocculation, and coalescence) eventually leading to complete phase separation (McClements, 2004). Kinetically stability over the lifetime of the product is usually achieved by the addition of amphiphilic proteins and/or small-molecule surfactants and/or thickening agents.

Coconut milk is the oil-in-water emulsion extracted from the endosperm of mature coconut (Cocos nucifera L.) either with or without the addition of water (Seow & Gwee, 1997). The white, opaque emulsion has been used widely as an important food ingredient, especially in Asia and Pacific regions. The emulsion is known to be naturally stabilized by coconut proteins (i.e., globulins and albumins) and phospholipids (Birosel, Gonzales & Santos, 1963). However, the coconut milk emulsion is unstable and readily separates into two distinct phases – a heavy aqueous phase and a lighter cream phase (Cancel, 1979; Gonzalez, 1990). The reason for the instability is that the protein content and quality in coconut milk is not sufficient to stabilize the fat globules (Monera & del Rosario, 1982).
The emulsifying properties of coconut proteins are affected by pH, ionic strength, and temperature (Gonzalez & Tanchuco, 1977; Kwon & Rhee, 1996; Onsaard, Vittayanont, Sringam & McClements, 2005; Onsaard, Vittayanont, Sringam & McClements, 2006). They are generally poorly-soluble, particularly at pH values close to their isoelectric points (pI~4) (Kwon & Rhee, 1996; Monera & del Rosario, 1982; Samson, Khaund, Cater & Mattil, 1971). Coconut proteins have been shown to denature and coagulate upon heating to 80°C (Gonzalez, 1990; Kwon, Park & Rhee, 1996).

Proteins extracted from coconut cream are less surface active than whey protein isolate (WPI) and are not particularly effective at either creating small droplets within the homogenizer or preventing droplet aggregation during or after homogenization, thus resulting in less stable emulsions (Onsaard et al., 2006).

In order to improve the stability of coconut milk, various types and amounts of stabilizers are often added during processing. Several studies have been conducted to investigate the effect of stabilizers on the quality of coconut milk products, but generally only in terms of changes in bulk properties, and in particular susceptibility to creaming (del Rosario & Punzalan, 1977; Genato & Gonzalez, 1985; Martin, Ubaldi-Eiroa, Kato, Angelucci, Silva & Leital, 1974; Timmins & Kramer, 1977). To best of our knowledge, none of these studies have focused on elucidating a microstructural basis for the functionality of the additives. This study aims to determine the effect of the addition of proteins and surfactants as examples of surface-active stabilizers on the microstructure and bulk properties of coconut milk emulsions.
5.2 Materials and methods

5.2.1 Materials

Frozen grated coconut meat (35% fat, 3% protein, 45% moisture) was purchased from a local retailer and kept in the freezer until being used for extraction. Thimerosal, sodium caseinate (92% protein), polyoxyethylene sorbitan monolaurate (Tween 20), and phosphate buffer ingredients (disodium hydrogen phosphate heptahydrate and sodium dihydrogen phosphate monohydrate) were purchased from Sigma-Aldrich (St. Louis, MO). Sodium dodecyl sulfate (SDS), and 1 N hydrochloric acid and sodium hydroxide standard solutions were purchased from Fisher Scientific (Fairlawn, NJ). A commercial WPI sample (BiPro, 98% protein) was provided by Davisco Foods International (Le Sueur, MN).

5.2.2 Sample preparation

Coconut milk was produced by mixing the thawed grated pulp with distilled water at a weight ratio of 2:1 in a Waring blender (I120, Waring, Winstel, CT). The slurry was then filtered through a double-layered cheese cloth and pressed to extract the milk. Thimerosal (0.02 wt%) was added as an antimicrobial agent. The nitrogen content of coconut milk was measured using an automatic nitrogen analyzer (FP-528, Leco, St. Joseph, MI) from which the protein content was calculated by using the conversion factor of 6.25. The fat content was determined using a modified Mojonnier ether extraction
method for determination of the fat content in milk (AOAC Official Method 989.05, AOAC, 2000).

Appropriate amounts of buffer (20 mM phosphate buffer pH 6.2) or a stabilizer solution in buffer were mixed, either prior to or after homogenization, into aliquots of the extracted milk to obtain the final fat content of 10 wt% and stabilizer concentration of 0.1, 0.25, 0.5, or 1 wt%. Homogenized samples were prepared by recirculating the emulsions through a twin-stage valve homogenizer (Panda, GEA Niro Soavi, Hudson, WI) at a stage I/stage II pressure of 20/2 MPa for several minutes to achieve 4 to 5 passes through the valves. The pH of the milks was adjusted to 6.2, i.e., close to the pH of many coconut milk-based products, by adding 1 N hydrochloric acid, or sodium hydroxide standard solution. The addition of small amounts of acid or alkali needed to adjust the pH caused only small (<0.3 wt%) changes in fat content. Samples were stored at 30°C in a water bath and analyzed 24±2 h after manufacture. The experiment was repeated three times with freshly prepared coconut milk samples used for each trial.

5.2.3 Particle size analysis

The particle size distributions (volume fraction as a function of particle size) of the samples were measured using a laser diffraction particle analyzer (LA-920, Horiba, Irvine, CA). The scattering pattern was used to calculate the particle size of the droplets by the internal software of the instrument using a relative refractive index of 1.09, i.e., the ratio of the refractive index of coconut oil (1.45) and that of the dispersion medium
Coconut milk samples were diluted using distilled water to about 0.001% fat concentrations to avoid multiple scattering effects. The mean particle size was reported in terms of the volume-weighted mean diameter: \( d_{43} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \), where \( n_i \) is the number of droplets of diameter \( d_i \).

Samples were also diluted in 1 wt% SDS solution rather than water and measured for their particle sizes. SDS is an anionic small-molecular surfactant which effectively displaces other molecules from the surface of emulsion droplets and thereby disrupts droplet flocs formed due to interactions between the surfactant layers. Particle size measured in water is referred to in this work as an “effective” particle size and includes the presence of any flocs, while measurement in SDS solution is referred to as the “primary” particle size. Emulsion coalescence will be seen as a change in both primary and effective particle diameter, while flocculation will increase the effective diameter but the primary diameter will remain unchanged.

### 5.2.4 Creaming behavior

Ten grams of coconut milk samples were transferred into a flat-bottomed test tube (internal diameter 15 mm, height 125 mm), covered, and stored for 168 h at 30°C in a temperature-controlled water bath. During storage, most samples separated into an opaque cream layer at the top and a transparent serum layer at the bottom. The total height of the emulsions (\( H_E \)) and the height of the serum layer (\( H_S \)) were measured. The
extent of the phase separation was assessed by a creaming index (CI=\(100 \times \frac{H_s}{H_E}\)).

The CI provides indirect information about the extent of droplet aggregation in an emulsion: typically the more aggregation, the larger the effective particle size and the faster the creaming. However, a direct causative link between flocculation and creaming is not always justified and sometimes flocculated emulsions cream more slowly because either the density contrast of a floc is smaller than that of a droplet or because large flocs can be extensively interconnected and trap the droplets in a network (Dickinson, Golding & Povey, 1997).

### 5.2.5 Microscopy

Samples of coconut milk (~15 μl) were placed on a microscope slide, gently covered with a cover slip, and observed under 400x magnification using an optical microscope (BX41-TF, Olympus, Tokyo, Japan) equipped with a color digital video camera (SPOT Insight QE 4.2, Diagnostic Instruments, Sterling Heights, MI).

For non-homogenized and homogenized coconut milks, images from laser scanning confocal microscopy (LSCM) and cryo-scanning electron microscopy (cryo-SEM) were also obtained. In LSCM, the coconut protein was stained with rhodamine isothiocyanate (RITC) dissolved in distilled water with a dye concentration of 0.25 mg g\(^{-1}\) protein. Dispersions were incubated overnight in the dark at 30°C prior to dialyzing against buffer to remove excess free marker. Dialyzed dispersions were observed using a LSCM (FV1000, Olympus America, Melville, NY) with a 60x 1.42 N.A. oil-immersion
objective. The dye was excited by a green He-Ne laser at its excitation wavelength of 543
nm, and the fluorescence emission between 555 to 578 nm was recorded. Differential
interference contrast (DIC) transmitted light images were collected simultaneously.

For cryo-SEM, samples were diluted in buffer to the final droplet concentration of
2.5 wt% and then rapidly frozen using liquid nitrogen slush (-210°C) under vacuum. The
samples were then transferred to a Cryotrans system (C1500C, Oxford Instruments,
Oxford, United Kingdom) and sublimated at -90°C for 15 min to remove the top layers of
water molecules. Finally, the samples were sputter coated with a 10 nm thick layer of
gold. Images were taken at -196°C using an SEM (JSM 5400, JEOL, Peabody, MA) with
5 kV acceleration voltage and analyzed with a prism light element detector (IMIX-PC,

The optical, confocal, and electron micrographs were analyzed using image
analysis softwares (SPOT Advanced, Version 4.6, Diagnostic Instruments; FV10-ASW,
Version 1.6a, Olympus; and IMIX, Version 10, Princeton Gamma-Tech, respectively).
Pictures were taken from three different fields on each slide and representative images
are presented.

5.2.6 Statistical analysis

One-way analysis of variance (ANOVA) and Duncan’s multiple range tests were
used to evaluate the significance of differences (p≤0.05) between the samples. Analyses
were conducted using statistical software (SPSS 11.5, SPSS, Chicago, IL). Data are presented as the mean and standard deviation of the triplicates and only significant effects are discussed in the text.

5.3 Results and discussion

Samples of homogenized or non-homogenized coconut milk were prepared varying in type (sodium caseinate, WPI, SDS, or Tween 20) and concentration (0 to 1 wt%) of stabilizer as well as the process step of addition (i.e., prior to and after homogenization) and their physical properties compared.

5.3.1 Effect of processing on coconut milk

The extracted coconut milk contained 17% to 18% fat and 1.8% to 2% protein. The particle size distributions of coconut milks were bimodal and the effective particle size (12.1 µm) was only slightly greater than the primary droplet size (11.5 µm), suggesting the freshly prepared emulsion was not flocculated (Figure 5.1, see also microscopic images in Figure 5.2). The emulsion creamed rapidly and extensively due to its large droplet size (Figure 5.3).

Homogenization decreased the primary particle size of the coconut milk emulsion but slightly increased the effective droplet size (Figure 5.1). This suggested that the fine droplets formed during homogenization quickly flocculated to approximately same
Figure 5.1: Representative particle size distribution of (●, ○) non-homogenized and (■, □) homogenized coconut milks. Filled points represent emulsions dispersed in water; open points represent emulsions dispersed in SDS solution.
Figure 5.2: Representative images from optical microscopy (a, b), LSCM overlaid with corresponding images from DIC transmitted light (c, d), and cryo-SEM (e, f) taken of non-homogenized (a, c, e), and homogenized (b, d, f) coconut milks. In (c) and (d), coconut proteins were labeled red with RITC ($\lambda_{\text{exc}}=543$ nm) while the emulsion droplets are seen grey under DIC. Arrows point the flocculated droplets. $P$ shows some of the background protein.
Figure 5.3: Creaming index after 24 h storage of (●) non-homogenized and (■) homogenized coconut milks.
effective size (also seen in the micrographs in Figure 5.2), presumably by a bridging mechanism due to the limited amount and poor surface activity of coconut protein (Tangsuphoom & Coupland, 2005). The extensive flocculation of the homogenized samples reduced the degree of creaming (Figure 5.3).

**5.3.2 Addition of stabilizers to non-homogenized coconut milk**

Adding any of the stabilizers to non-homogenized coconut milk had minimal effects on either the particle size (Figure 5.4a), microstructure (Figure 5.5), or creaming behavior (Figure 5.6). Even if the stabilizers were able to displace the coconut proteins at the interface, non-homogenized coconut milk is largely non-flocculated and without the additional energy of a homogenization step, there will be no associated change in droplet diameter or microstructure.

One intriguing exception to this rule is SDS which at the 1 wt% and particularly the 0.5 wt% level increased the mean effective droplet diameter but not the primary droplet diameter indicating some degree of surfactant-induced flocculation (see also microstructural evidence in Figure 5.5g). All added concentrations of SDS were higher than 8.1 mM which is its critical micelle concentration in water (Robins, Watson & Wilde, 2002). SDS micelles have been shown to promote flocculation in emulsions via depletion mechanism (Dickinson & Ritzoulis, 2000). However, depletion flocculation is typically reversible to dilution, so it seems unlikely this can be responsible for the higher effective droplet sizes seen in the samples diluted in water for droplet size analysis.
Figure 5.4: Mean particle size of coconut milk emulsions with (●, ○) sodium caseinate, (■, □) WPI, (▲, Δ) SDS, or (♦, ◊) Tween 20 added without (a), after (b), or prior to (c) homogenization. Filled points represent emulsions dispersed in water; open points represent emulsions dispersed in SDS solution.
Figure 5.4 (Continued)
Figure 5.5: Representative micrographs taken of coconut milk emulsions with 1 wt% sodium caseinate (a to c), WPI (d to f), SDS (g to i), or Tween 20 (j to l) added without (a, d, g, j), after (b, e, h, k), or prior to homogenization (c, f, l, i). Scale bar is 50 μm.
Figure 5.6: Creaming index after 24 h storage of coconut milk emulsions stabilized with sodium caseinate (a), WPI (b), SDS (c) or Tween 20 (d) added (●) without, (▲) after, or (■) prior to homogenization.
Figure 5.6 (Continued)
Alternatively, SDS has been shown to bind to certain proteins and either induce
denaturation (Dickinson & Ritzoulis, 2000) or solubilize otherwise insoluble protein
that the adsorbed micelle-like structures can promote bridging flocculation between
emulsion droplets and promote creaming and perhaps a similar mechanism is responsible
for the flocculation seen in these coconut milk samples.

5.3.3 Addition of stabilizers to homogenized coconut milk

Adding proteins (sodium caseinate or WPI) to homogenized coconut milk (after
homogenization) caused minimal changes to the effective or primary droplet diameter
(Figure 5.4a and 5.4b), microstructure (Figure 5.5b and 5.5e), or creaming behavior
(Figure 5.6a and 5.6b). It seems likely that the added protein was not able to accumulate
at the oil-water interface and had only minor effects as a component of the aqueous
phase.

Adding small-molecule surfactants (SDS or Tween 20) to homogenized coconut
milk reduced the effective droplet diameter to the same value as the primary droplet
diameter while having no effect on the primary droplet size (Figure 5.4c and 5.4d).
Small-molecule surfactants are known to be able to displace proteins from the surface of
emulsion droplets (Dickinson & Woskett, 1989) and thereby disrupt flocs induced the
protein (see also microstructure in Figure 5.5h and 5.5k). Again, SDS at the 0.5 wt% level proved an exception as it increased the effective droplet size of the coconut milk
emulsions. The deflocculation caused by the small-molecule surfactants had no effect on the creaming behavior of the emulsions (Figure 5.6c and 5.6d). SDS samples were typically less stable to creaming than the corresponding Tween 20 samples.

### 5.3.4 Addition of stabilizers to coconut milk prior to homogenization

When stabilizers were added to emulsions and the mixture subsequently homogenized the effects were more dramatic. Homogenizing coconut milk in the presence of 1 wt% of any of the added stabilizers decreased the effective and primary droplet size down to submicron range because there was sufficient added protein or surfactant to cover the newly divided droplet interface created (Figure 5.4). The difference in droplet size and flocculation of samples was confirmed by optical microscopy (Figure 5.5), and the fine, non-flocculated emulsions formed were stable to creaming (Figure 5.6).

Lower concentrations (≤0.5 wt%) of added stabilizer reduced the magnitude of all of these effects as there was less material available to coat the new surfaces generated during homogenization. At low levels of stabilizer addition, the effective droplet size was larger than the primary droplet size (Figure 5.4c), suggesting the emulsions were still somewhat flocculated. In these cases, the coconut milk emulsions had bimodal particle size distributions with the large droplet peak corresponding to the presence of residual flocs and the small droplet peak the fine droplets (Figure 5.7).
Figure 5.7: Representative particle size distribution of coconut milk emulsions with sodium caseinate (a), WPI (b), SDS (c) or Tween 20 (d) added at concentrations of (●) 0, (△) 0.1, (■) 0.25, (◊) 0.5, or (▲) 1 wt% prior to homogenization.
Figure 5.7 (Continued)
While most of these samples were stable to creaming over 24 h (Figure 5.6), analysis of the kinetics of creaming over a longer period revealed differences in their properties (Figure 5.8). Stability to creaming increased with added stabilizer concentration but also depended on the type of stabilizer used. Low levels of SDS (0.1 wt%) made no difference to the kinetics or extent of creaming, but similar levels of the other additives tended to delay the onset of creaming to about 60 h but also had no effect on the final height of the cream layer (~70% CI, although the height of the cream layer in the Tween 20-containing samples was still changing at the end of the experiment). Similarly, low levels of SDS had minimal effect on the droplet size distribution, while the other additives tended to either skew the distribution to smaller sizes (Tween 20) or form a bimodal distribution with a significant population of much smaller droplets (sodium caseinate and WPI). Low levels of SDS are inefficient in preventing creaming when homogenized with coconut milk as they provide little improvement to the homogenization process.

All samples were more stable to creaming when homogenized with 0.25 wt% stabilizers, although samples containing SDS creamed more substantially than the others. The SDS-containing samples still had a single peak in the distribution close to the surfactant-free samples while most of the droplets in the samples with the other stabilizers were in the submicron range and hence more stable to creaming. Interestingly, the sodium caseinate-containing samples at the 0.25 and 0.5 wt% levels formed a small but stable cream layer (~30% CI) late in the experiment and perhaps some alternative mechanism is contribution to creaming in these systems. The addition of ≥0.5 wt%
Figure 5.8: Creaming index of coconut milk emulsions with sodium caseinate (a), WPI (b), SDS (c) or Tween 20 (d) added at concentrations of (●) 0, (▼) 0.1, (■) 0.25, (◊) 0.5, or (▲) 1 wt% prior to homogenization.
Figure 5.8 (Continued)
stabilizers before homogenization produced reasonably stable emulsions (<25% CI over the period of analysis), especially those stabilized with small-molecule surfactants. At the highest stabilizer concentration (1 wt%), all samples were stable to creaming because of their submicron range particle sizes.

### 5.4 Conclusions

This work reveals some of the ways that surface active stabilizers can affect the properties of coconut milk and the main findings are summarized in Table 5.1 and 5.2. In the absence of added stabilizer, homogenized coconut milk is highly flocculated. Adding small-molecule surfactants after the homogenization step can displace coconut proteins from the interface and break up these flocs but adding them before homogenization increases the efficiency of the homogenization step and produces stable, submicron-sized emulsion droplets. Additional flocculation was seen at certain levels of SDS addition which was tentatively ascribed to effects of the coconut protein-surfactant adduct. Adding protein stabilizer did not break up the flocs of coconut milk droplets when added after homogenization but also increased the efficacy of the homogenization step if added previously. Adding stabilizers to non-homogenized coconut milk had no effect.
Table 5.1: Mean effective and primary particle sizes of coconut milk emulsions with 1 wt% surface-active stabilizers added without, after, or prior to homogenization.

<table>
<thead>
<tr>
<th>Stabilizer</th>
<th>Effective $d_{43}$ (µm)*</th>
<th>Primary $d_{43}$ (µm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-homogenized</td>
<td>After homogenization</td>
</tr>
<tr>
<td>Caseinate</td>
<td>12.81 ± 0.99AB</td>
<td>13.45 ± 0.46aA</td>
</tr>
<tr>
<td>WPI</td>
<td>12.86 ± 1.46A</td>
<td>13.43 ± 1.23AA</td>
</tr>
<tr>
<td>SDS</td>
<td>14.56 ± 2.02A</td>
<td>11.60 ± 3.00AA</td>
</tr>
<tr>
<td>Tween 20</td>
<td>11.77 ± 0.90A</td>
<td>6.61 ± 1.13bB</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation of three replicates.

a,b,c Means within the same column having the same or without superscript are not significantly different (p>0.05).

A,B,C Means within the same row having the same or without superscript are not significantly different (p>0.05).
Table 5.2: Creaming index after 24 h storage of coconut milk emulsions with 1 wt% surface-active stabilizers added without, after, or prior to homogenization.

<table>
<thead>
<tr>
<th>Stabilizer</th>
<th>Non-Homogenized*</th>
<th>After homogenization*</th>
<th>Prior to homogenization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caseinate</td>
<td>73.69 ± 0.74cA</td>
<td>69.56 ± 0.44bB</td>
<td>ND</td>
</tr>
<tr>
<td>WPI</td>
<td>75.00 ± 0.71cA</td>
<td>66.99 ± 0.75cB</td>
<td>ND</td>
</tr>
<tr>
<td>SDS</td>
<td>80.79 ± 0.16b</td>
<td>82.53 ± 1.90a</td>
<td>ND</td>
</tr>
<tr>
<td>Tween 20</td>
<td>77.20 ± 1.29cA</td>
<td>71.52 ± 1.15bB</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Mean ± standard deviation of three replicates. 

Means within the same column having the same or without superscript are not significantly different (p>0.05).

Means within the same row having the same or without superscript are not significantly different (p>0.05).

ND Not detected.

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Chapter 6

Effect of Surface-Active Stabilizers on the Surface Properties of Coconut Milk Emulsions

Abstract

Recently we have shown how surface-active stabilizers (≤1 wt% sodium dodecyl sulfate, polyoxyethylene sorbitan monolaurate, whey protein isolate, and sodium caseinate) added either before or after homogenization affect the stability and properties of coconut milk emulsions. In this work, we proposed a mechanism to explain these observations based on changes in surface composition and properties. Coconut milk had a protein high surface load (~7 mg m⁻²), the major component of which is cocosin. Small-molecule surfactants (>0.25%) added to the homogenized coconut milk displaced the coconut proteins from the surface resulting in a change in \( \zeta \)-potential. Dairy proteins added to the coconut milk after homogenization did not accumulate at the surface. Addition of small-molecule surfactant to the coconut milk prior to homogenization completely displaced coconut proteins at interface. Homogenization of coconut milk with proteins resulted in a decrease in total protein surface concentration (to about 2 mg m⁻²) and a significant replacement of coconut proteins by the dairy proteins. The change in \( \zeta \)-potential also reflected the change in protein and surface composition as the values moved close to the value of coconut oil emulsions stabilized solely with the corresponding added stabilizer.
6.1 Introduction

Coconut milk is the aqueous extract of coconut (Cocos nucifera L.) endosperm and is widely used as an ingredient in many tropical countries. It is an oil-in-water emulsion of coconut oil droplets naturally stabilized by coconut proteins and phospholipids (Monera & del Rosario, 1982). As with all emulsions, coconut milk is not physically stable and is prone to separating into cream (coconut cream) and serum (coconut skim milk) layers (Seow & Gwee, 1997). The particular instability in coconut milk arises from the fact that the droplets are large and relatively aggregated, which is the result of the poor quality of coconut proteins in stabilizing the emulsion (Tangsuphoom & Coupland, 2005). The major (~65%) protein in coconut endosperm is a 11S globulin known as cocosin (Balasundaresan, Sugadev & Ponnuswamy, 2002; Garcia, Arocena, Laurena & Tecson-Mendoza, 2005), a hexamer of 55 kDa subunits with each subunit comprising an acidic (32 to 34 kDa) and basic (22 to 24 kDa) polypeptides linked by a disulfide bridge (Carr, Plumb, Parker & Lambert, 1990; Garcia et al., 2005). Cocosin is believed to play a more important role in governing the stability of coconut milk than either the albumin or the 7S globulin fraction (Kwon, Park & Rhee, 1996; Monera & del Rosario, 1982).

In our previous work, we investigated the influence of the addition of various types and levels of surface-active stabilizer on the stability of coconut milk emulsions (Tangsuphoom & Coupland, 2008). We showed that the stability of coconut milk emulsions can be improved by the addition of sufficient alternative proteins prior to a
homogenization step, or small-molecule surfactants added either before or after a homogenization step. We concluded that work by hypothesizing the added small-molecule surfactant can displace coconut proteins from the interface and stabilize the emulsion, while the added proteins could not displace the coconut proteins and were not functional unless homogenized, in which case the added protein accumulates at the newly-divided oil-water interface. In this work, the composition and properties of the interfacial layer of similar coconut milk emulsions are investigated to test these hypotheses.

6.2 Materials and methods

6.2.1 Materials

Frozen grated coconut meat (35% fat, 3% protein, 45% moisture) was purchased from a local retailer and kept in the freezer until required for extraction. Thimerosal, sodium caseinate (92% protein), polyoxyethylene sorbitan monolaurate (Tween 20), and phosphate buffer ingredients (disodium hydrogen phosphate heptahydrate and sodium dihydrogen phosphate monohydrate) were purchased from Sigma-Aldrich (St. Louis, MO). Sodium dodecyl sulfate (SDS), and 1 N hydrochloric acid and sodium hydroxide standard solutions were purchased from Fisher Scientific (Fairlawn, NJ). A commercial WPI sample (BiPro, 98% protein) was purchased from Davisco Foods International (Le Sueur, MN). Coconut oil was purchased from a local retailer and used without further
preparation. All sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) reagents were purchased from Bio-Rad Laboratories (Hercules, CA).

6.2.2 Sample preparation

Coconut milk was extracted from coconut meat and mixed with SDS, Tween 20, WPI, or sodium caseinate either before or after homogenization as described by Tangsuphoom and Coupland (2008). Briefly, coconut milk was produced by mixing the thawed coconut meat with distilled water (2:1 w/w), in a Waring blender. The slurry was then pressed and filtered through cheesecloth to remove the solid residue. Thimerosal was added to a final concentration of 0.02 wt% to prevent the emulsion from microbial spoilage. The nitrogen content of the extracted coconut milk was analyzed using a combustion method (FP-528, Leco, St. Joseph, MI), and the fat content was determined using a modified Mojonnier ether extraction method for determination of fat content in milk (AOAC Official Method 989.05, AOAC, 2000).

Two hours after extraction, appropriate amounts of buffer (20 mM phosphate buffer pH 6.2) and a surface-active stabilizer (SDS, Tween 20, WPI, or sodium caseinate) solution in buffer were mixed, either prior to or after homogenization, into aliquots of the extracted milk to obtain a final fat content of 10 wt% and stabilizer concentration of 0.1, 0.25, 0.5 or 1 wt%. Samples were homogenized by recirculating through a twin-stage valve homogenizer (Panda, GEA Niro Soavi, Hudson, WI) at a stage I/stage II pressure of 20/2 MPa for several minutes to achieve 4 to 5 passes through the valves. The pH of
the final emulsions was adjusted to 6.2, which is close to the pH of many coconut milk-
based products, by adding small volumes of 1 N hydrochloric acid or sodium hydroxide
solution. Samples were incubated at 30°C for 2 h in a water bath prior to analysis, which
was completed within 24 h after manufacture. The experiment was repeated three times
with freshly prepared coconut milk samples used on each run.

6.2.3 Specific surface area and particle size determination

Particle size distributions and specific surface area of the samples were measured
using a laser diffraction particle analyzer (LA-920, Horiba, Irvine, CA). To avoid
multiple scattering effects during measurement, samples were diluted to approximately
0.001% fat in the measuring chamber. Samples were also diluted 5 fold in SDS solution
(1 wt%) rather than water and stirred for 30 min prior to measuring their particle sizes.
The surfactant displaces the protein from the droplet interfaces and thus disperses the
aggregates (Courthaudon, Dickinson, Matsumura & Williams, 1991). Preliminary
experiments showed that SDS only acts as a dissociating agent of aggregates without
affecting the droplet size of unflocculated emulsions. Particle size measured in buffer is
referred to as an “effective” particle size which includes the presence of any flocs, while
measurement in SDS solution is referred to as the “primary” particle size which is the
actual size of the droplets. A change in both primary and effective particle diameter will
be seen in coalesced samples while flocculated samples will have larger effective
diameter but unchanged primary diameter (Tomas, Paquet, Courthaudon & Lorient,
1994).
The scattering pattern was used by the internal software of the instrument to calculate the particle size of the droplets using a relative refractive index of 1.09, which is the ratio of the refractive index of coconut oil, 1.45, and that of the dispersion medium, 1.33. The mean particle size of the emulsion droplets was characterized using the volume fraction-length mean diameter, \[ d_{4,3} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \]; where \( n_i \) is the number of droplets of diameter \( d_i \).

### 6.2.4 ζ-potential measurement

The ζ-potential of the droplets was measured by phase analysis light scattering instrument (ZetaPALS, Brookhaven Instruments, Holtsville, NY) (Vanapalli & Coupland, 2000). Emulsions were diluted to a fat concentration of about 0.001 wt% in buffer to avoid multiple scattering effects. The diluted emulsions were mixed thoroughly prior to being placed in a standard four-sided, 1 cm polystyrene cuvette. A parallel plate electrode (0.45 cm\(^2\) square platinum plates with a 0.4 cm gap) was inserted and the cuvette was placed in a temperature-controlled holder at 30°C. Each measurement was the average of 120 (6 sets of 20) measurements and the entire experiment was conducted in triplicate. The ζ-potential was calculated from the electrophoretic mobility using the Smolukowski model (Hunter, 1981).
6.2.5 Determination of surface protein concentration and composition

Surface protein load was determined using a minor modification of the method of Hunt & Dalgleish (1994). Emulsions were centrifuged at 15000 x g for 30 min at 22°C; the cream phase was removed and resuspended in buffer to maintain the original weight fraction of the two phases in the emulsion. The resuspended cream was recentrifuged under the same conditions and the resulted cream phase was spread onto filter paper (Whatman 541) to drain out any contaminating buffer. Aliquots of the cream were diluted in buffer to obtain a washed cream dispersion with droplet concentration similar to that of the original emulsion. The protein contents of the emulsions and washed cream dispersions were determined as described above. Surface protein concentration was calculated from the protein content of washed cream dispersion and the specific area of the droplets: $\Gamma = \frac{e_c}{a \cdot v_m}$; where $\Gamma$ is the surface protein load (mg m$^{-2}$), $e_c$ is protein content of the washed cream dispersion (mg ml$^{-1}$), $a$ is the surface area (m$^2$) of 1 ml emulsion, and $v_m$ is fat fraction of the emulsion (by volume).

The composition of the protein adsorbed at the surface of the emulsion droplets was determined directly by analyzing the washed cream phase using SDS-PAGE (Mini-PROTEAN 3 system, Bio-Rad, Hercules, CA). A small known amount of dried cream was dispersed in sample buffer (0.12 M tris, 3.84% SDS, 19.2% glycerol, 9.6% β-mercaptoethanol, and 0.024% bromophenol blue pH 6.8). The mixture was heated in a water bath at 95°C for 5 min then cooled to room temperature. Approximately 15 µg of protein (10 to 20 µl of solution) was loaded into each well of 12% (w/v) precast
polyacrylamide gels. Electrophoresis was carried out at 110 V for 1 h 40 min in a Mini-
PROTEAN 3 cell filled with tris-glycine-SDS buffer (0.049 M tris, 0.366 M glycine, and
0.1% SDS pH 8.3). The gels were then stained for 1 h with 0.1% Coomassie brilliant blue
R-250 in 10% acetic acid and 40% methanol and destained for 8 h in 10% acetic acid and
7% methanol with two buffer changes. Identification of the main proteins was done on
the basis of the relative migration of known markers consisting of the following proteins:
phosphorylase b (97.4 kDa), serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic
anhydrase (31 kDa), trypsin inhibitor (21.5 kDa), and lysozyme (14.4 kDa). The gels
from the samples along with gels of known protein standards were scanned using a
calibrated densitometer (GS-800, Bio-Rad). The molecular weights, peak density, and
area of each protein bands were estimated by matching with the migration patterns of the
markers using computer software (Quantity One, Version 4.4, Bio-Rad).

6.3 Results and discussion

6.3.1 Homogenized coconut milk without addition of stabilizers

The homogenized coconut milk contained 0.8% to 1% total protein (i.e., surface
bound and aqueous) about 25% of which was adsorbed at the oil water interface.
Balasubramaniam and Sihotang (1979) reported the same proportion of protein in the
cream layer obtained after centrifugation of coconut milk. The interfacial protein load in
homogenized coconut milk was 7 mg m⁻² (Figure 6.1), which is considerably higher than
Figure 6.1: Surface protein load of coconut milk emulsions stabilized with SDS (a), Tween 20 (b), WPI (c), or sodium caseinate (d) added (■) after, or (●) prior to homogenization. Total protein content is constant at 1 wt% for (a) and (b), and ranges from 1 to 2 wt% for (c) and (d). The dashed line in (c) and (d) represents the value of 10% coconut oil emulsion stabilized with 1 wt% WPI, and sodium caseinate, respectively. The lines are drawn to simply guide the eyes.
Figure 6.1 (Continued)
the 1 to 2 mg m\(^{-2}\) expected for most proteins (de Feijter, Benjamins & Tamboer, 1987; Euston, Singh, Munro & Dalgleish, 1995; Fang & Dalgleish, 1993; Srinivasan, Singh & Munro, 1996; Srinivasan, Singh & Munro, 1999). High surface protein loads have also been reported in the emulsion stabilized by other plant proteins, i.e., soy (Dickinson & Matsumura, 1991), sunflower (Gonzalez-Perez, van Koningsveld, Vereijken, Merck, Gruppen & Voragen, 2005), and African locust bean proteins (Lawal, Adebowale, Ogunsanwo, Sosanwo & Bankole, 2005), and may be due to multi-layer adsorption or the persistence of quaternary structure at the interface.

The coconut proteins separated electrophoretically under reducing conditions into seven bands spanning a molecular weight range from 18 to 50 kDa, i.e., two major bands of molecular weight 18 and 50 kDa, a set of three bands at 22, 24, and 26 kDa, and another set of two bands at approximately 31 and 33 kDa (Figure 6.2, lane A). This is similar to previously reported studies on the protein composition of coconut endosperm (de Mason & Chandra Sekhar, 1990; Kwon et al., 1996; Rasyid, Manullang & Hansen, 1992). The bands at 26 and 18 kDa were identified as the albumin fraction, the major band of molecular weight 50 kDa is probably cocosin monomer, and two pairs of bands, at approximately 33 and 24 kDa correspond to the acidic-, basic polypeptide subunits reported previously (Balachandran & Arumughan, 1992; Carr et al., 1990; de Mason & Chandra Sekhar, 1990; Garcia et al., 2005; Kwon et al., 1996).

The SDS-PAGE pattern of the interfacial proteins of the homogenized coconut milk suggested that the oil-water interface of coconut milk emulsion was dominated by
Figure 6.2: SDS-PAGE gel and the corresponding densitometric profiles of total (lane A) and interfacial proteins (lane B) in homogenized coconut milk. The left-hand lane is the molecular weight standard proteins containing phosphorylase b (97.4 kDa), serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), trypsin inhibitor (21.5 kDa), and lysozyme (14.4 kDa).
coconut globulins, as the two bands corresponding to the albumin fraction were not present (Figure 6.2, lane B). Rasyid et al. (1992) also reported similar molecular weights of the globulin precipitated from the cream fraction of coconut milk. Globulins also found dominate at the interfaces in emulsions stabilized by other plant proteins (Dickinson & Matsumura, 1991; Gonzalez-Perez et al., 2005; Lawal et al., 2005).

The $\zeta$-potential of the homogenized coconut milk (-16 mV, Figure 6.3) reflected negative charge on coconut proteins at pH 6.2 which have isoelectric point around pH 4 (Gonzalez, 1993).

### 6.3.2 Coconut milk emulsions stabilized with surfactants

We previously reported changes in stability of coconut milk emulsions due to the addition of surfactant either before or after homogenization (Tangsuphoom & Coupland, 2008). The mean droplet size of samples is summarized in Table 6.1. Mixing the surfactant solution with homogenized coconut milk reduced the degree of flocculation (i.e., the effective particle size decreased while primary particle size was unchanged). When small-molecule surfactants (>0.25 wt%) were added to coconut milk emulsions before homogenization the droplets produced were fine (~0.3 μm), not flocculated (Table 6.1), and very stable (Tangsuphoom & Coupland, 2008). At lower levels of surfactant addition prior to homogenization, the emulsions were still somewhat flocculated.
Figure 6.3: ζ-potential of coconut milk emulsions stabilized with SDS (a), Tween 20 (b), WPI (c), or sodium caseinate (d) added (■) after, or (●) prior to homogenization. The dashed line represents the value of 10% coconut oil emulsion stabilized with 1 wt% corresponding stabilizer. The lines are drawn to simply guide the eyes.
Figure 6.3 (Continued)
Table 6.1: Mean effective and primary (in parentheses) particle sizes of coconut milk emulsions with surface-active stabilizers added after or prior to homogenization. Data reproduced from Tangsuphoom & Coupland (2008).

<table>
<thead>
<tr>
<th>Addition level (wt%)</th>
<th>Mean droplet size, $d_{43}$ (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SDS</td>
</tr>
<tr>
<td></td>
<td>After</td>
</tr>
<tr>
<td>0</td>
<td>15.2 (6.2)</td>
</tr>
<tr>
<td>0.1</td>
<td>8.0 (5.8)</td>
</tr>
<tr>
<td>0.25</td>
<td>8.0 (5.6)</td>
</tr>
<tr>
<td>0.5</td>
<td>14.6 (6.0)</td>
</tr>
<tr>
<td>1</td>
<td>11.6 (6.4)</td>
</tr>
</tbody>
</table>
Table 6.2: Molar ratio of surface-active stabilizer and coconut proteins in coconut milk emulsions stabilized with different surface-active stabilizers.

<table>
<thead>
<tr>
<th>Addition level (wt%)</th>
<th>SDS$^2$</th>
<th>Tween 20$^3$</th>
<th>WPI$^4$</th>
<th>Caseinate$^5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>8.3</td>
<td>2.0</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>0.25</td>
<td>20.8</td>
<td>4.9</td>
<td>0.3</td>
<td>3.0</td>
</tr>
<tr>
<td>0.5</td>
<td>41.6</td>
<td>9.8</td>
<td>0.6</td>
<td>6.0</td>
</tr>
<tr>
<td>1.0</td>
<td>83.2</td>
<td>19.6</td>
<td>1.3</td>
<td>1.2</td>
</tr>
</tbody>
</table>

$^1$ 1 wt%; average molecular weight of polypeptide subunit of cocosin, 24 kDa.
$^2$ Molecular weight 288.38 Da
$^3$ Molecular weight 1227.54 Da.
$^4$ Average molecular weight of $\beta$-lactoglobulin, 18.4 kDa.
$^5$ Average molecular weight of $\beta$-casein, 20 kDa.
The molar ratio of added small-molecule surfactant to coconut proteins, $R$, in each sample (Table 6.2) was calculated based on the average protein content in coconut milk (1%), and molecular weight of the major component of coconut proteins (polypeptide subunits of 11S globulin, 24 kDa) (Kwon et al., 1996), SDS (288 Da), and Tween 20 (1227 Da). In case of SDS, $R$ was relatively high (up to 80, Table 6.2) and the concentrations exceed its critical micelle concentration in water (8.1 mM) at all levels of addition (Robins, Watson & Wilde, 2002). For Tween 20, the maximum $R$ value was about 20. A molar excess of surfactant such as this has been previously shown to displace the protein from the interface by competitive adsorption (Courthaudon et al., 1991; Dickinson & Gelin, 1992; Dickinson & Tanai, 1992). We therefore hypothesized that the changes in the emulsions seen are due to the surfactants displacing the coconut proteins from the interface.

The amount of interfacial coconut proteins decreased to zero when the coconut milk was mixed or homogenized with small-molecule surfactants (SDS or Tween 20) (Figure 6.1) and there was no significant difference in the protein surface load when the surfactant was added before or after homogenization (Figure 6.3), despite the large difference in interfacial area. As the amount of surfactant added increased, the $\zeta$-potential of emulsion droplets approached the value of coconut oil emulsion solely stabilized by the corresponding surfactant (see the dashed line in Figure 6.3) which also reflected the change in at the droplet surface composition and supporting our observation that the surfactants displaced the proteins from the interface.
6.3.3 Coconut milk emulsions stabilized with added proteins

There was no change in the effective and primary droplet diameters of the coconut milk when WPI or sodium caseinate was added after homogenization (Table 6.1).

The $R$ values of the emulsions with added surface-active proteins (Table 6.2) were calculated using molecular weight of major components of coconut proteins (24 kDa), WPI ($\beta$-lactoglobulin, 18.4 kDa), and sodium caseinate ($\beta$-casein, 20 kDa) (Fox & McSweeney, 1998). The displacement of interfacial globular proteins by added aqueous proteins is limited as globular protein, e.g., coconut globulin, once adsorbed, unfolds and changes its conformation to anchor its polypeptide chain to the interface (Damodaran, 1997). Thus, the protein which is likely to predominate the droplet surface is the one which was first introduced to the interface, irrespective of whether or not it is the more surface active of the two proteins (Dickinson, Rolfe & Dalgleish, 1989). We hypothesized that the surface protein concentration and/or composition were also not affected by the added proteins (Tangsuphoom & Coupland, 2008).

Indeed, when protein (WPI or sodium caseinate) was added to coconut milk after homogenization, there was minimal change to the amount of protein adsorbed at the surface of emulsion droplets despite the increase in total protein content of the system (Figure 6.1). No change was observed in the $\zeta$-potential of the emulsion (Figure 6.3), suggesting that the composition of the interface was not affected by the addition of
protein to a quiescent emulsion. The SDS-PAGE gels of the interfacial proteins from samples with WPI or caseinate added after homogenization suggested qualitatively that there was no change in the interfacial protein composition (Figure 6.4).

We attempted to deconvolute the surface protein peaks by comparing the results from our established data for coconut proteins (Figure 6.2) and gel patterns for the added proteins (Figure 6.5). The WPI resolved into bands for β-lactoglobulin (18 kDa) and α-lactalbumin (14 kDa) (Figure 6.5) which did not overlap with any of the coconut protein bands (Figure 6.4), so the proportion at the interface could be readily calculated and indeed did not change with level of addition (Figure 6.6). The caseinate appeared as three bands between 27 and 32 kDa (Figure 6.5), similar to gels presented in several works (Alim, Fondrini, Bonizzi, Feligini & Enne, 2005; Hunt & Dalgleish, 1994; Kauf & Kensinger, 2002). Unfortunately, the larger fraction overlapped with the acidic subunit of cocosin (33 kDa, see Figure 6.4) and it was not possible to deconvolute the peaks. To get a measurement of any displacement of coconut proteins we selected the other fractions (i.e., 50, 24, and 22 kDa) as indicators to monitor the change in interfacial composition and found that the summed peak areas of the selected indicator proteins were not affected by the increase in caseinate concentration (Figure 6.6).

For the coconut milk homogenized with surface-active proteins, both effective and primary droplet sizes decreased to about 0.4 μm when the level of addition increased (Table 6.1), which is similar to the samples with added surfactants. In this case, the protein which is likely to predominate at the droplet surface is the one that adsorbs more
Figure 6.4: SDS-PAGE patterns of coconut milk emulsions with WPI (a, c), and sodium caseinate (b, d) at 0.1, 0.25, 0.5, and 1 wt% added after (a, b) or prior to (c, d) homogenization. Marker proteins in the left lane are the same as Figure 6.2. Asterisks indicate bands of indicative proteins.
Figure 6.4 (Continued)
Figure 6.5: SDS-PAGE patterns and the corresponding densitometric profiles of WPI (a), and sodium caseinate (b). Marker proteins in the left lane are the same as Figure 6.2.
Figure 6.6: Peak area of selected protein fractions in coconut milk emulsion stabilized with WPI (a), and sodium caseinate (b) added (■) after, or (●) prior to homogenization. The lines are drawn to simply guide the eyes.
readily to the interface created during homogenization. We therefore hypothesized that
the added proteins, which are more surface active than the coconut proteins (Onsaard,
Vittayanont, Sringam & McClements, 2006), out-competed the coconut proteins to
adsorb at the surface of newly divided droplets (Tangsuphoom & Coupland, 2008).

The surface properties data showed that homogenization of coconut milk together
with caseinate or WPI resulted in a reduction in interfacial protein concentration from 6
mg m\(^{-2}\) to about 2.5 mg m\(^{-2}\) at the added protein concentrations higher than 0.25 wt%
(Figure 6.1). This value is similar to the surface protein load in 10 wt% coconut oil
emulsions prepared with 1 wt% caseinate or WPI (1.5 to 1.8 mg m\(^{-2}\), see dashed line in
Figure 6.1c and d) and similar to the reported values for monolayer adsorption of the
dairy proteins (Hunt & Dalgleish, 1994; Shimizu, Kamiya & Yamauchi, 1981;
Srinivasan, Singh & Munro, 1996; 1999). The \(\zeta\)-potential of the coconut milk emulsions
stabilized with surface-active proteins added before homogenization approached the
value of coconut oil emulsions solely stabilized with sodium caseinate or WPI
(Figure 6.3), suggesting the added protein dominated the interface.

Changes in the band intensities of the SDS-PAGE gels suggested qualitatively
that as the added protein concentration increased, they tended to replace the coconut
proteins at the interface (Figure 6.4). This was supported by the increase in the WPI band
intensities and peak area at higher levels of addition (Figure 6.4 and 6.6). For the
samples emulsified with caseinate, the peak area of the indicator coconut protein
decreased when increased the added concentration (Figure 6.6). There was still a small
amount of interfacial coconut proteins at the maximum level of caseinate or WPI addition (Figure 6.4) which may be responsible for the slightly higher than expected total protein load (Figure 6.1).

### 6.4 Conclusions

The key hypotheses from our previous work (Tangsuphoom & Coupland, 2008) on the improved stability of the coconut milk emulsions by the addition of surface-active stabilizers were supported by the determination of surface properties of the emulsions. Small-molecule surfactants completely displaced the interfacial coconut proteins when added to the coconut milk emulsion either after or before homogenization, and thus resulted in a decrease in surface protein load as well as a change in \( \zeta \)-potential of the emulsion droplets. Addition of dairy proteins to the homogenized coconut milk did not affect the interface as the added protein remained mostly in the aqueous phase. However, the stabilizing protein was added to the coconut milk prior to emulsification, they were able to out-compete the coconut proteins to adsorb at the newly-created interface.

### References


Chapter 7

Effect of Thermal Treatments on the Properties of Coconut Milk Emulsions Prepared with Surface-Active Stabilizers

Abstract

Previously we have demonstrated improved stability of coconut milk emulsions homogenized with various surface-active stabilizers, i.e., 1 wt% sodium caseinate, whey protein isolate (WPI), sodium dodecyl sulfate (SDS), and polyoxyethylene sorbitan monolaurate (Tween 20). This study aims to examine the changes in bulk and microstructural properties of those emulsions following thermal treatments normally used to preserve coconut milk products. Homogenized coconut milk prepared without additives was destabilized by freeze-thaw (-20°C and -10°C) but not by chilling (5°C). Samples homogenized with proteins were not affected by any low temperature treatments while those prepared with surfactants were stable to chilling but partially or fully coalesced upon following freeze-thaw. Homogenized coconut milk prepared without additives coalesced and flocculated after being heated at 90°C or 120°C for 1 h in due to the denaturation and subsequent aggregation of coconut proteins. Samples emulsified with caseinate samples were not affected by heat treatments while those prepared with WPI showed extensive coalescence and phase separation after being treated at temperatures higher than 70°C. Samples prepared with SDS were stable to heating but those prepared with Tween 20 sample completely destabilized by heating at 120°C.
7.1 Introduction

Coconut milk is an oil-in-water emulsion formed during the aqueous extract of coconut solid endosperm. The emulsion is relatively unstable because the globulin and albumin proteins adsorbed at the oil-water interface are poor emulsifiers (Monera & del Rosario, 1982). To make more stable products, other emulsifiers are usually added during manufacturing and frequently the stabilized coconut milks are subsequently preserved by chilling, freezing, pasteurization, or sterilization (Seow & Gwee, 1997), which can provide additional stresses on the emulsion structure. We have recently studied the effects of heating process on the stability of homogenized coconut milk (Tangsuphoom & Coupland, 2005) and the improved stability obtained by homogenizing the coconut milk with surface-active proteins or small-molecule surfactants of sufficient concentration (Tangsuphoom & Coupland, 2005; 2008).

When emulsions are cooled to temperatures where the fat phase becomes semi-crystalline, fat crystals from one droplet may penetrate into another droplet leading to emulsion destabilization by partial coalescence (Walstra, 2003). It has been reported that proteins provide better protection against droplet coalescence than small-molecule surfactants due to their ability to form thick interfacial membranes (Palanuwech & Coupland, 2003; Thanasukarn, Pongsawatmanit & McClements, 2004; Vanapalli, Palanuwech & Coupland, 2002). When both oil and water phases are crystallized, the resultant destabilization is often more severe and the emulsion will frequently completely phase separate after thawing. Again, emulsions stabilized by proteins are usually more
stable to freeze-thawing than those using small molecular surfactants (Cramp, Docking, Ghosh & Coupland, 2004). Freezing is known to be a way to break the emulsion of fresh coconut milk and it has been used in a process of cold extraction of coconut oil from the emulsion (Cancel, 1979; Gonzalez, 1990).

The effects of heating on the stability of emulsions have been widely studied. Globular protein adsorbed to the oil-water interface undergo conformational changes at their denaturation temperature which can lead to instability of the emulsions (de Wit, 1990; Demetriades, Coupland & McClements, 1997; Hunt & Dalgleish, 1995; Kim, Decker & McClements, 2002; Monahan, McClements & German, 1996; Sliwinski, Roubos, Zoet, van Boekel & Wouters, 2003). The effect of heating is less severe in the systems stabilized by caseins or surfactants which do not denature in a similar manner. The effect of heating on the bulk properties of coconut milk has been reported earlier by our group and the others (Chiewchan, Phungamngoen & Siriwattanayothin, 2006; Peamprasart & Chiewchan, 2006; Simuang, Chiewchan & Tansakul, 2004).

In this work, coconut milk stabilized by different emulsifiers was subjected to various cooling and heating treatments and the changes in bulk properties and microstructure were measured.
7.2 Materials and methods

7.2.1 Materials

Frozen grated coconut meat (35% fat, 3% protein, 45% moisture) was purchased from a local retailer and stored at -20°C until needed. Coconut oil was purchased from a local retailer and used without further preparation. Thimerosal, sodium caseinate (92% protein), polyoxyethylene sorbitan monolaurate (Tween 20), and phosphate buffer ingredients (disodium hydrogen phosphate heptahydrate and sodium dihydrogen phosphate monohydrate) were purchased from Sigma-Aldrich (St. Louis, MO). Sodium dodecyl sulfate (SDS) and standard solutions of hydrochloric acid and sodium hydroxide (1 N) were purchased from Fisher Scientific (Fairlawn, NJ). A commercial WPI sample (BiPro, 98% protein) was purchased from Davisco Foods International (Le Sueur, MN).

7.2.2 Sample preparation

Coconut milk was produced according to the method of Tangsuphoom and Coupland (2008). Briefly, thawed coconut meat was mixed with distilled water (2:1 w/w) in a Waring blender. The slurry was then pressed and filtered through cheesecloth to remove the solid residue. Thimerosal (0.02 wt%) was added to prevent microbial spoilage. The nitrogen content of the extracted coconut milk was analyzed using a combustion method (FP-528, Leco, St. Joseph, MI) from which the protein content was calculated by using a conversion factor of 6.25. The fat content was determined using a
modified Mojonnier ether extraction method for determination of fat content in milk
(AOAC Official Method 989.05, AOAC, 2000).

The extracted milk was mixed with a solution of sodium caseinate, WPI, SDS, or
Tween 20 in buffer (20 mM phosphate buffer pH 6.2) to obtain a final lipid concentration
of 10 wt% and surface-active stabilizer concentration of 0 or 1 wt%. Samples were then
homogenized by recirculating through a twin-stage valve homogenizer (Panda, GEA Niro
Soavi, Hudson, WI) at a stage I/stage II pressure of 20/2 MPa for several minutes to
achieve 4 to 5 passes through the valves. The pH of the final emulsions was adjusted to
6.2, i.e., close to the pH of many coconut milk-based products, by titrating with 1 N
hydrochloric acid or sodium hydroxide standard solution. The emulsions were sealed in
glass bottles then subjected to thermal treatments as described in Table 7.1. After the
thermal treatments, the samples were stored at 30°C for 24 h prior to analysis. The
experiment was repeated three times with freshly prepared coconut milk samples used
each time.
7.2.3 Particle size determination

Particle size distributions and specific surface area of the samples were measured using a laser diffraction particle analyzer (LA-920, Horiba, Irvine, CA). Prior to analysis, emulsions were gently mixed to disperse any cream layers. Samples with visible surface oil could not be reliably characterized by this method and were not analyzed. Samples were diluted to approximately 0.001% fat in the measuring chamber to avoid multiple scattering effects during measurement. The scattering pattern was used by the internal software of the instrument to calculate the particle size of the droplets using a relative refractive index of 1.09, which is the ratio of the refractive index of coconut oil, 1.45, and that of the dispersion medium, 1.33. The mean particle size of the emulsion droplets was characterized using the volume-weighed average diameter, $d_{43} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3}$; where $n_i$ is the number of droplets of diameter $d_i$.  

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Thermal history</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep freezing</td>
<td>Freezer (-20°C), 24 h</td>
</tr>
<tr>
<td>Freezing</td>
<td>Refrigerated waterbath (-10°C), 24 h</td>
</tr>
<tr>
<td>Chilling</td>
<td>Refrigerator (5°C), 24 h</td>
</tr>
<tr>
<td>Control</td>
<td>Waterbath (30°C), 24 h</td>
</tr>
<tr>
<td>Moderate heating</td>
<td>Waterbath (70°C), 1 h</td>
</tr>
<tr>
<td>Intense heating</td>
<td>Waterbath (90°C), 1 h</td>
</tr>
<tr>
<td>Autoclaving</td>
<td>Autoclave (120°C), 1 h</td>
</tr>
</tbody>
</table>

Table 7.1: Temperature protocols.
The particle size of the samples was also determined by diluting the droplets five fold in SDS solution (1.25 wt%) and stirring for 30 min. The surfactant displaces the protein from the droplet interfaces and thus disperses the aggregates (Courthaudon, Dickinson, Matsumura & Williams, 1991). Preliminary experiments showed that SDS will dissociate flocs without affecting the droplet size of unflocculated emulsions. Particle size measured in buffer is referred to as an “effective” particle size which includes the presence of any flocs, while measurement in SDS solution is referred to as the “primary” particle size which is the actual size of the droplets. A change in both primary and effective particle diameter will be seen in coalesced samples while flocculated samples will have larger effective diameter but unchanged primary diameter.

It should be stressed that the measurement of effective droplet size is only an indication of the actual floc size in the undiluted emulsion because the process of diluting and circulating the droplets will disrupt the structure to some extent. However, changes in effective droplet size have previously been used by several groups as an indication of flocculation (Agboola, Singh, Munro, Dalgleish & Singh, 1998; Demetriades et al., 1997; Euston, Finnigan & Hirst, 2001; Monahan et al., 1996; Tomas, Paquet, Courthaudon & Lorient, 1994).

7.2.4 Visual appearance

Ten grams of emulsions were poured into flat bottomed glass tubes (15 mm internal diameter, 125 mm height), covered, and subjected to different temperature
treatments as described in Table 7.1. The heights of any visible layers were measured with a ruler.

7.2.5 Thermal analysis

To identify any changes in the emulsions induced by low temperature treatments, cooling and heating thermograms were measured using differential scanning calorimetry (DSC, Perkin Elmer, DSC-7, Shelton, CT). The instrument was calibrated against indium and equilibrated at 30°C for 1 h. Samples of emulsions (10 to 15 mg) were weighed, sealed into aluminum pans, and placed inside the DSC alongside an empty reference pan. Samples were cooled from 30°C to either -15°C or -40°C at 1.5°C min⁻¹ then reheated to 30°C at the same rate. Water nucleates to form ice at about -20°C in a DSC pan so the shallower cooling allowed the observation of phase transitions in the lipid without freezing the aqueous phase while the deeper cooling also allowed the observation of the freezing of water. The cool-heat cycles were repeated three times and the heat flow was recorded as a function of temperature. The freezing and melting points of the continuous and dispersed phases were taken from the onset and end point temperatures of peaks on the heating and cooling thermograms, respectively.

The thermal denaturation properties of proteins associated with heating treatments were examined with a differential scanning microcalorimeter (VP-DSC, MicroCal, Northampton, MA). Degassed samples (513.1 μl) were run against a similar reference cell filled with degassed buffer. Samples were held at 30°C for 15 min and heated to
120°C at 10°C h⁻¹ then cooled down to 30°C. The samples were immediately rescanned and the heat flux data from the second scan was subtracted from the first to eliminate any reversible phase transitions occurring in the oil. Thus, the only thermal transitions visible in the thermograms are due to irreversible transitions, presumably in the proteins. Data were collected and analyzed using the software provided with the instrument (Origin, MicroCal). All thermal analyses were conducted at least in duplicate.

7.3 Results and discussion

7.3.1 Changes in emulsion structure

7.3.1.1 Coconut milk

As noted previously, homogenized coconut milk was a coarse emulsion and somewhat flocculated (i.e., effective diameter greater than primary particle size, Table 7.2) (Tangsuphoom & Coupland, 2005). The mean particle size of homogenized coconut milk remained unchanged after storage at 5°C for 24 h but both primary and effective diameter increased after freeze-thaw (-10°C and -20°C) indicative of coalescence and flocculation (Table 7.2). However, none of these changes resulted in changes in the bulk appearance of the samples which all creamed to a similar extent (Figure 7.1). This result is somewhat surprising as freezing has been reported to be a way to break the emulsion of fresh coconut milk (Cancel, 1979; Gonzalez, 1990).
Table 7.2: Mean effective and primary particle sizes after thermal treatments of coconut milk emulsions homogenized with 1 wt% stabilizer.

<table>
<thead>
<tr>
<th>Treatment temperature (°C)</th>
<th>Effective $d_{43}$ (μm)*</th>
<th>Primary $d_{43}$ (μm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Caseinate</td>
</tr>
<tr>
<td>-20</td>
<td>66.2 ± 9.6dH</td>
<td>0.5 ± 0.0k*</td>
</tr>
<tr>
<td>-10</td>
<td>51.4 ± 4.0kA</td>
<td>0.5 ± 0.0kD</td>
</tr>
<tr>
<td>-5</td>
<td>16.6 ± 1.8kA</td>
<td>0.5 ± 0.0kC</td>
</tr>
<tr>
<td>30</td>
<td>16.0 ± 1.0kA</td>
<td>0.5 ± 0.0kC</td>
</tr>
<tr>
<td>70</td>
<td>16.7 ± 1.5kA</td>
<td>0.5 ± 0.0kC</td>
</tr>
<tr>
<td>90</td>
<td>33.2 ± 4.7kA</td>
<td>0.5 ± 0.0kC</td>
</tr>
<tr>
<td>120</td>
<td>47.3 ± 6.5kA</td>
<td>0.5 ± 0.0kD</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation of three replicates.

Means within the same column having the same or without superscript are not significantly different (p>0.05).

Means within the same row having the same or without superscript are not significantly different (p>0.05).

N/A Not analyzed.
Figure 7.1: Visual appearance after thermal treatments of homogenized coconut milk (a), coconut milk emulsions homogenized with 1 wt% sodium caseinate (b), WPI (c), SDS (d), and Tween 20 (e). Open bars represent serum layer, filled bars represent cream layer, diagonal striped bars represent emulsion layer, diagonal crisscrossed bars represent coagulated layer, and vertical striped bars represent free oil layer.
**Figure 7.1** (Continued)
Figure 7.1 (Continued)
Perhaps homogenization provided better stability of these coconut milk emulsions against freeze-thaw.

Homogenized coconut milk droplets coalesced and flocculated after being heated at 90°C or 120°C for 1 h while there was no change in particle size of the emulsion heated at 70°C (Table 7.2). The flocculated coconut milk (i.e., heated to 90°C) creamed less than the non-flocculated samples (30°C or 70°C, Figure 7.1) due to the smaller density contrast of a floc than that of a droplet; and the extensive interconnection of large flocs that can trap the droplets in a network (Dickinson, Golding & Povey, 1997). Similar effects of heating on the droplet size and the creaming stability of coconut milk have been reported previously (Tangsuphoom & Coupland, 2005). However, it should be noted that the sample autoclaved at 120°C did not simply cream but rather separated into a serum layer and another layer with large white aggregates dispersed in transparent liquid (Figure 7.1), which indicated the complete destabilization of emulsion. The thermally-induced changes observed likely resulted from the denaturation and subsequent aggregation of the coconut proteins at the surface of coconut oil emulsion droplets.

### 7.3.1.2 Coconut milk with added protein

The coconut milk emulsions homogenized with 1 wt% protein (sodium caseinate or WPI) had smaller droplets (0.5 μm compared to 6 μm in the absence of added protein, Table 7.2) and were stable to chilling. The caseinate sample was stable to freeze-thaw while the WPI emulsion showed some loss in stability (Table 7.2 and Figure 7.1).
The bulk properties and microstructure of coconut milk emulsions homogenized with sodium caseinate were not affected by heating (Table 7.2 and Figure 7.1). Other emulsions prepared with sodium caseinate have also been reported to be stable to heating at either 90°C for 30 min or 121°C for 15 min (Hunt & Dalgleish, 1995; Srinivasan, Singh & Munro, 2002); and sodium caseinate has been reported to improve the stability of coconut milk during the canning process (Genato & Gonzalez, 1985).

There was no change in the droplet size and the appearance after heating the coconut milk emulsified with WPI at 70°C while a slight decrease in the stability of WPI-stabilized coconut milk emulsions was observed after being treated at 90°C (Table 7.2 and Figure 7.1). Severe heating (120°C, 1 h) of the coconut milk emulsified with WPI, however, resulted in a massive change leading to extensive droplet coalescence and separation of emulsion into a free oil layer on top and a coagulated layer with large white aggregates dispersed in transparent fluid at the bottom.

### 7.3.1.3 Coconut milk with added surfactant

Coconut milk homogenized with small-molecule surfactant had smaller and non-flocculated droplets (about 0.3 μm compared to 6 μm, Table 7.2) and did not show any phase separation (Figure 7.1). They were stable to chilling but suffered significant destabilization upon following freeze-thaw (Table 7.2 and Figure 7.1). Increases in both effective and primary droplet size suggested that droplets were either partially or fully coalesced, which in turn led to the separation of the emulsion into distinct cream and
serum layers, or even a free oil layer in the Tween 20 sample stored at -10°C or -20°C. Small-molecule surfactants typically provide emulsions less protection than proteins to freeze-thaw destabilization (Palanuwech & Coupland, 2003; Thanasukarn et al., 2004; Vanapalli et al., 2002). The fact that samples with Tween 20 coalesced and destabilized more extensively than SDS samples was somewhat unexpected as the adsorbed layer of Tween 20 has been reported to be thicker than that of SDS (1.4 nm and 0.5 nm, respectively) (McClements, Dickinson, Dungan, Kinsella, Ma & Povey, 1993b) and thicker layers have been associated with better freeze-thaw stability (Thanasukarn et al., 2004).

Coconut milk emulsified with SDS was stable to all heating treatments (i.e., no change in droplet size and no phase separation, Table 7.2 and Figure 7.1). The Tween 20 stabilized emulsions were stable after being heated for 1 h at temperatures up to 90°C, despite the samples were treated at temperatures higher than the cloud point of Tween 20 (~76°C) (Mahajan, Chawla & Bakshi, 2004). The emulsions were completely destabilized by the treatment at 120°C (Table 7.2 and Figure 7.1, note that as a free oil layer was observed the particle size could not be usefully measured). Polysorbates such as Tween 20 and Tween 60 are widely used to improve the stability of sterilized coconut milk products, although typically at somewhat lower levels (~0.3 wt%) and in combination with gums (Seow & Gwee, 1997). However, coconut milk homogenized with Tween 20 in our study thus broke down, perhaps because the heat treatment used in our study (120°C, 1 h) is more severe than used in canned coconut milk production (Gonzalez, 1990). It is known that polysorbate-type emulsions typically have a limited
temperature range compared with those made with ionic emulsifiers (Cottrell & van Peij, 2004), which might be correspond to the reduced stability of coconut milk emulsions prepared with Tween 20.

The changes in stability for the processed coconut milk samples are summarized in Table 7.3 and in the following section we use calorimetry to investigate the thermal transitions underlying the differences seen.

**Table 7.3:** Summary of stability after thermal treatments of coconut milk emulsions homogenized with 1 wt% stabilizer.

<table>
<thead>
<tr>
<th>Treated temperature (°C)</th>
<th>Control</th>
<th>Caseinate</th>
<th>WPI</th>
<th>SDS</th>
<th>Tween 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>-20</td>
<td>●●</td>
<td>○</td>
<td>●</td>
<td>●</td>
<td>●●</td>
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<tr>
<td>-10</td>
<td>●●</td>
<td>○</td>
<td>○</td>
<td>●</td>
<td>●●</td>
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<tr>
<td>5</td>
<td>●</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
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<tr>
<td>30</td>
<td>●</td>
<td>○</td>
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<tr>
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<td>●</td>
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<td>○</td>
<td>○</td>
<td>○</td>
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<tr>
<td>90</td>
<td>●●</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>120</td>
<td>●●</td>
<td>○</td>
<td>●●</td>
<td>○</td>
<td>●●</td>
</tr>
</tbody>
</table>

* ○ stable, ● some destabilization, ●● extensive destabilization.
7.3.2 Thermal properties and discussion

7.3.2.1 Coconut milk

Unemulsified coconut oil exhibited a single crystallization exotherm on cooling (onset 15°C) and a single melting endotherm on heating (peak 22°C and end point 27°C) (Figure 7.2). These results are similar to reports in the literature for coconut oil (Reyes-Hernandez, Dibildox-Alvarado, Charo-Alonso & Toro-Vazquez, 2007; Tan & Che Man, 2002). In a similar experiment on coconut milk (homogenized without added stabilizers), there were two overlapping endothermic peaks with maxima at 5°C (minor peak), and 2°C (major peak) (Figure 7.3). In the samples cooled to -40°C (Figure 7.3b) there was a large exothermic peak with an onset of -20°C corresponding to the freezing of water (not shown in the figure), but the melting peak at 0°C is seen on melting peak and is absent in samples cooled to -15°C (Figure 7.3a). The melting thermograms of coconut milk emulsions were similar to the melting profile of the unemulsified coconut oil and the heating thermograms of subsequent cycles are omitted since they all were very similar to that of the first cycle. Indeed, throughout this work, all of the melting thermograms were very similar because melting does not require nucleation and is dependent only composition of the fat crystals. This suggests that whatever polymorphic transitions may occur in coconut oil the final crystal composition is similar in all cases.

The onset of crystallization in the emulsion was lower than in the bulk oil, presumably because the nucleation catalysts were divided amongst very many droplets (Walstra, 2003). The fact that there were two crystallization peaks in the emulsified state
Figure 7.2: Typical thermograms of coconut oil.
Figure 7.3: Successive cooling curves and a heating curve of homogenized coconut milk repeatedly cycled from 30°C to -15°C (a), and -40°C (b) at 1.5°C min⁻¹.
and only one in bulk could be either that there were two nucleation mechanisms occurring in the droplets: a higher temperature one in droplets containing a nucleation catalyst and a lower for droplets without a catalyst. Palanuwech and Coupland (2003) noted that when a series of emulsions were prepared from the same lipid to different final droplet sizes, the coarse emulsions crystallized at a high temperature (due to heterogeneous nucleation), the fine ones at a low temperature (due to homogeneous nucleation), and intermediate sizes showed two crystallization peaks. An alternative explanation is the emulsion structure changes the polymorphic phase transitions in the emulsified lipid, and thus gives rise to multiple peaks in the thermogram. The fat crystallization peaks were independent of phase transitions in the aqueous phase (i.e., cooling to -10°C or -20°C, Figure 7.3).

The onset of droplet crystallization (5°C) was the same temperature as used in the chilling regimen used in the bulk stability studies (Table 7.1) and several degrees below its melting point (10°C). However, an isothermal DSC trace (cooling to 5°C at 1.5°C min⁻¹) revealed no thermal transitions over 24 h suggesting that although the fat was supercooled by 5°C it did not crystallize over the course of the experiment (data not shown). Furthermore, an additional heating cycle after the completion of the isothermal hold showed no melting transition (data not shown), confirming that the oil droplets in homogenized coconut milk remain a supercooled liquid after lengthy storage under chilled conditions. All subsequent samples (see below) had lower nucleation temperatures than this and similar isothermal studies revealed that none of the emulsified coconut oil crystallized under the chilled conditions described in Table 7.1. The fact that
chilling did not induce droplet crystallization (which often in turn leads to partial coalescence) provides a reason that the chilled samples were so stable (Table 7.2 and Figure 7.1). Longer storage might allow the droplets to eventually crystallize but even if they did, we suspect the emulsion would still remain stable, as the emulsions were also stable to the much more severe freezing cycle.

Evidence of the denaturation of coconut proteins was gained from the microcalorimetry experiments. Thermograms of coconut milk emulsions previously heated to 30°C (unheated), 70°C, 90°C, and 120°C are shown in Figure 7.4a. Unheated homogenized coconut milk showed two separate peaks at about 85°C (peak I) and 100°C (peak II) which are comparable to the denaturation temperatures of coconut 7S and 11S globulins, respectively, as reported elsewhere (Kwon, Park & Rhee, 1996). A similar denaturation pattern was also found in the soymilk-the aqueous extract of soybean, which is a natural oil-in-water emulsion stabilized by soy proteins (Kwok & Niranjan, 1995). Soy protein is mainly (~70%) composed of 7S and 11S globulins (Utsumi, Matsumura & Mori, 1997), similar to the coconut proteins. It has been reported that the two endothermic peaks of soy milk at 70°C and 90°C presumably corresponded to the denaturation of the two soy protein fractions 7S and 11S globulins, respectively (Zhang, Takenaka & Isobe, 2004). It should be noted that these thermograms reflected the denaturation of both adsorbed proteins at the interfacial layer and the unadsorbed proteins in the aqueous phase of the emulsions.
Figure 7.4: Differential scanning microcalorimetric thermograms of coconut milk homogenized with 1 wt% no additive (a), sodium caseinate (b), and WPI (c) after heated different temperatures for 1 h. I and II indicate endothermic peaks due to thermal denaturation of coconut proteins whereas III indicates that of WPI.
Figure 7.4 (Continued)
The DSC heating curve of the homogenized coconut milk was unchanged by prior heating to 70°C for 1 h, suggesting that protein denaturation did not take place during this mild heat treatment and explaining why there were no changes in bulk quality of the heated milk (Table 7.3). Peak I disappeared in the sample previously treated at 90°C indicating that the 7S fraction was completely denatured during this heating process. When the heating temperature increased to 120°C both peaks disappeared which confirmed the denaturation of the 11S as well as the 7S fractions. The denaturation of the 7S and particularly the 11S coconut protein fractions are presumably responsible for the moderate destabilization of the homogenized coconut milk by heating to 90°C and extensive destabilization following heating to 120°C (Table 7.3).

7.3.2.2 Coconut milk with added protein

There were two crystallization peaks in the cooling thermograms of both emulsions prepared with added proteins; a larger one at -5°C and smaller peak at about -12°C (Figure 7.5 and 7.6), while the unmodified coconut milk emulsion also had two peaks but at higher temperatures (5°C and 2°C). Smaller droplets are more likely to nucleate homogeneously at a low temperature and the finer droplets here may be responsible for the change of crystallization pattern (Coupland, 2002). There was no change in the thermograms on repeated chilling to -15°C, while freezing to -40°C led to the development of a new exothermic peak at 0°C on the subsequent cooling scans (Figure 7.6b) for the WPI samples only. Changes in crystallization pattern of the dispersed phase
Figure 7.5: Successive cooling curves and a heating curve of coconut milk emulsions homogenized with 1 wt% sodium caseinate repeatedly cycled from 30°C to -15°C (a), and -40°C (b) at 1.5°C min⁻¹.
Figure 7.6: Successive cooling curves and a heating curve of coconut milk emulsions homogenized with 1 wt% WPI repeatedly cycled from 30°C to -15°C (a), and -40°C (b) at 1.5°C min⁻¹.
correspond to the changes in particle size seen resulting from freeze-thaw destabilization (Table 7.2).

No transition was observed in the thermograms of a 1 wt% sodium caseinate solution (Figure 7.4b) as the protein tends to be stable to heating at temperatures up to 140°C (Singh, 1995). The thermograms of the heated, caseinate-stabilized coconut milk were similar to those of the coconut milk itself (Figure 7.4b) although the modified emulsion was stable. We believe that the more surface active caseinate is the dominant protein at the interfaces of homogenized coconut milk so even when the coconut proteins are denatured the emulsion stability is unaffected.

Unheated WPI-stabilized coconut milk exhibited three exothermic peaks upon heating in microcalorimeter (Figure 7.4c). Peak I and peak II (85°C and 100°C) corresponding to coconut proteins and peak III (65°C) is similar to the reported denaturation temperature of whey proteins in an emulsion system (Corredig & Dalgleish, 1995). After heating the emulsion at 70°C or above, WPI was denatured, as peak III disappeared from the thermograms of the treated emulsions. The stability observed in the coconut milk prepared with WPI after being heated at 70°C might be due to the fact that there was sufficient free protein presented in the aqueous phase to adsorb to the heated droplet surfaces, and cover any non-polar patches of the heated-, unfolded interfacial WPI, thus minimized the hydrophobic interaction between the droplets (Kim, Decker & McClements, 2005). Although WPI-stabilized emulsions have been reported to be stable against heating at pH values other than the isoelectric point of the protein in the absence
of added salt (Demetriades et al., 1997; Hunt & Dalgleish, 1995; Kim et al., 2002; Kim et al., 2005; Sliwinski et al., 2003), coconut milk emulsions made with WPI showed some extent of destabilization after being treated at 90°C and 120°C. Perhaps the longer duration of treatment used in the present work was so extreme as to completely denature both free, and interfacial proteins, and thus inhibited the binding of the two.

7.3.2.3 Coconut milk with added surfactant

Coconut milk emulsions homogenized with added SDS exhibited two exothermic peaks upon cooling (-4°C and -8°C, Figure 7.7) while samples with added Tween 20 crystallized with a single peak (-6°C, Figure 7.8). In all cases, the phase transitions in the droplets occurred below the temperatures for similar protein-stabilized and unmodified coconut milk emulsions. The lower onset temperature may be due to the smaller droplet size and various workers have shown the composition of the interface can affect the crystallization properties of an emulsified lipid (Gülsener & Coupland, 2007; McClements, Dungan, German, Simoneau & Kinsella, 1993a; McClements et al., 1993b; Palanuwech & Coupland, 2003).

There was no change in the thermograms upon repeated cooling (to -15°C, no ice) which is not surprising as the emulsions were very stable. However, upon freeze-thaw cycling (to -40°C, ice), a dramatic change in cooling thermograms was observed in the coconut milks emulsified with SDS and Tween 20 (Figure 7.7 and 7.8), compared to
Figure 7.7: Successive cooling curves and a heating curve of coconut milk emulsions homogenized with 1 wt% SDS repeatedly cycled from 30 min$^{-1}$C to -15$^{\circ}$C (a), and -40$^{\circ}$C (b) at 1.5$^{\circ}$C min$^{-1}$.
Figure 7.8: Successive cooling curves and a heating curve of coconut milk emulsions homogenized with 1 wt% Tween 20 repeatedly cycled from 30°C to -15°C (a), and -40°C (b) at 1.5°C min⁻¹.
those homogenized with proteins (Figure 7.5 and 7.6). Indeed the later cooling thermograms, particularly those of SDS samples, began to resemble those of unemulsified coconut oil corresponding to the breakdown in structure described above. We believe the surfactant is the dominant species at the interface and although they allow the formation of very fine droplets they provide less protection than proteins against freeze-thaw destabilization (Palanuwech & Coupland, 2003; Thanasukarn et al., 2004; Vanapalli et al., 2002).

### 7.4 Conclusions

The stability of the coconut milk emulsions homogenized with different surface-active stabilizers after subjecting to different temperature treatments is summarized in Table 7.3. The homogenized coconut milk was unstable itself and its stability decreased either after freeze-thaw or after heating to temperatures sufficient to denature the coconut proteins. The samples emulsified with proteins were stable to low temperature treatments but only the caseinate samples were stable against heating. Coconut milk emulsion prepared with WPI was stable against heating at 70°C but extensively flocculated and coalesced after being treated at 90°C and 120°C. Coconut milk emulsions homogenized with small-molecule surfactants showed good stability against heating treatments but were completely destabilized upon freeze-thaw due to their thin interfacial layers which were less effective in protecting the droplets from either partial or full coalescence.
This work shows that the interfacial composition is critical to understanding the effects of processing on coconut milk emulsions. Therefore, the selection of surface-active stabilizer is important in producing coconut milk products that are able to maintain good stability and quality after being treated under processing conditions.

References


Chapter 8
Conclusions

The goal of this work was to investigate the effects of processing steps and additives on the properties, structure, and stability of the coconut milk emulsions.

Objective 1: Effect of heating and homogenization

The first objective of the study (Chapter 3) was to determine the changes in bulk quality of fresh coconut milk as affected by homogenization and heating, which are the fundamental and most commonly used process operations in coconut milk manufacture. It was found that coconut milk flocculated during homogenization via a bridging mechanism which is also responsible for increased viscosity in the homogenized sample. Heating caused extensive flocculation or even coalescence in coconut milk due to the denaturation of coconut proteins. Cream separation in coconut milk was retarded by flocculation due to the presence of a network of flocs. A homogenization condition (4 to 5 passes at stage I/stage II pressure of 20/2 MPa) was selected from this work and used throughout the remainder of the dissertation.
**Objective 2: Effect of pH and ionic strength**

Objective 2 (Chapter 4) was the study of the effects of addition of salt and modification of pH. The extracted coconut milk was diluted in buffer solution to achieve a better control in the composition of the samples. Like many protein-stabilized emulsions, the stability of coconut milk was determined by the electrostatic repulsion between emulsion droplets, and reduced either when the pH was adjusted to approach the isoelectric point of the coconut proteins or when the surface charge was screened by the added electrolyte.

**Objective 3: Effect of the addition of surface-active stabilizers**

The third objective was to examine the effect of surface-active stabilizers on the stability and properties of coconut milk emulsions. Stabilizers are often added in coconut milk processing and rather than consider the full set of industrially relevant additives. I selected WPI, caseinate, SDS, and Tween 20 as model examples of a globular protein, a disordered protein, a charged-, and an uncharged surfactant, respectively. I hoped by examining these model systems in detail to gain some general understanding of the behavior of the system. This was accomplished in Chapter 5 and 6, which focused on characterizing the changes in bulk and surface properties, respectively. More stable coconut milk emulsions can be produced by mixing the homogenized coconut milk with small-molecule surfactant or by emulsifying the fresh milk with any of the surface-active stabilizers (Chapter 5). I hypothesized that the improved stability resulted from the interfacial displacement of coconut proteins by the added surface-active
protein/surfactant. Those hypotheses were supported by the changes in surface properties of the emulsions (Chapter 6).

Objective 4: Susceptibility of coconut milk emulsions prepared with surface-active stabilizers to thermal treatments

The last objective (Chapter 7) was to investigate the effect of thermal treatments commonly used in coconut milk processing on the properties of the stabilized coconut milk emulsions obtained from the previous objective. Again, my approach was to select various model thermal treatments rather than attempt to replicate real coconut milk processing. I hope that the mechanistic understanding of the model processes will help explain the behavior of a wider range of real systems. Coconut milk emulsions prepared with sodium caseinate were stable to all of the thermal treatments used in this study; while those homogenized with whey protein isolate were not affected by low temperature treatments but coalesced extensively after being treated at 120°C for 1 h. Small-molecule surfactants were not effective in protecting destabilization of the coconut milk emulsion against freeze-thaw induced coalescence. However, the emulsions homogenized with those surfactants were more tolerant of heat treatments.
Overall, this work provides better understanding on the structure and properties of coconut milk by treating it as an oil-in-water emulsion. For example, it is common industrial experience that homogenization improves the stability to separation of coconut milk (Arumughan, Balachandran & Sundaresan, 1993). Objective 1 provides a mechanism for this observation, i.e., the presence of flocs in the emulsion that delay the separation. Similarly, changes in pH and salt concentration as the level of acidity is known to affect the quality of coconut milk and is often used by manufacturers as a measure of product quality. Data from Objective 2 shows this is due to changes in electrostatic repulsion between the droplets caused by the changes in pH and salt. Measurements of pH are useful for quality control in coconut milk manufacture because they indirectly give a measurement of droplet charge. Objective 3 reveals the mechanism of the added stabilizer in improving stability of coconut milk emulsion which gives better idea in producing stable coconut milk products. The last objective provides general information in selecting suitable surface-active stabilizers depending on the preservation process selected, which may sometimes be temperature-abused, e.g., canned coconut milk is heat-abused during processing and may be cold-abused during transportation, especially in winter. The dissertation also clearly shown that coconut proteins are poor in surface activity which resulted in bridging flocculation in fresh coconut milk after homogenization, and displacement of interfacial coconut protein by the added surface-active stabilizers (Objective 1 and 3).
Future work

**Effect of protein-to-fat ratio.** The fat to protein ratio has been reported to be important in determining the stability of dairy protein-stabilized emulsions (Tomas, Paquet, Courthaudon & Lorient, 1994). It is well established that the composition of coconut milk depends on both the extraction conditions and the composition of the coconut itself (Gonzalez, 1990). Although the coconut milk used in the present work was prepared in-house from fresh coconut or frozen grated coconut meat obtained from the same suppliers under a single extraction method that has been developed and used throughout the study, the variation in coconut meat composition was not well controlled. It thus might be interesting to determine the influence of protein-to-fat ratio on the stability and properties of coconut milk emulsions.

**Organoleptic properties.** In the present work, the quality and stability of coconut milk was determined based on the improved bulk and surface properties of the emulsions. However, the organoleptic properties are also important as the unique flavor and creaminess are the key characteristics that impart the quality and acceptability of coconut milk and its related products (Cancel, 1979; Seow & Gwee, 1997). The composition and physicochemical properties of food emulsions have been found to affect their organoleptic properties, e.g., flavor (Giroux, Perreault & Britten, 2007; Malone, Appelqvist & Norton, 2003; Relkin, Fabre & Guichard, 2004), color (Chantrapornchai, Clydesdale & McClements, 2001; McClements, 2002), and mouthfeel (Akhtar, Stenzel, Murray & Dickinson, 2005; Vingerhoeds, de Wijk, Zoet, Nixdorf & van Aken, 2008).
Therefore, it would be useful to investigate the changes in organoleptic properties of coconut milk emulsions due to processing steps and additives.

**Cooking test.** Coconut milk is normally used as an ingredient, and thus the properties of coconut milk should directly affect the quality of the dishes. The use of coconut milk in foods is highly diverse in terms of cooking method and the desirable characteristics of each dish, e.g., several dishes require the partial breakdown of the coconut emulsion to form a layer of free oil. How can an emulsion remain stable during processing and storage yet quickly and partially break down during food preparation? Tests to simulate the behavior of the coconut milk emulsion during simulated domestic cooking would be interesting.

**References**


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