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Effect of thermal treatments on the properties of coconut milk emulsions prepared with surface-active stabilizers

Nattapol Tangsuphoom [1], John N. Coupland*

Department of Food Science, The Pennsylvania State University, 337 Food Science Building, University Park, PA 16802-2504, USA

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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), or polyoxyethylene sorbitan monolaurate (Tween 20) [Tangsuphoom, N., & Coupland, J. N. (2008). Effect of surface-active stabilizers on the microstructure and stability of coconut milk emulsions. *Food Hydrocolloids*, *22*(7), 1233–1242]. This study examines the changes in bulk and microstructural properties of those emulsions following thermal treatments normally used to preserve coconut milk products (i.e., −20 °C, −10 °C, 5 °C, 70 °C, 90 °C, and 120 °C). Calorimetric methods were used to determine the destabilization of emulsions and the denaturation of coconut and surface-active proteins. 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 low temperature treatments while those prepared with surfactants were stable to chilling but partially or fully coalesced 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 were not affected by heat treatments while those prepared with WPI showed extensive coalescence and phase separation after being treated at 90 °C or 120 °C. Samples prepared with SDS were stable to heating but those prepared with Tween 20 completely destabilized by heating at 120 °C.

1. Introduction

Coconut milk is an oil-in-water emulsion formed from the aqueous extract of coconut solid endosperm. The emulsion is relatively unstable because of the large droplet size (Tangsuphoom & Coupland, 2008) and the poor emulsifying properties of coconut proteins adsorbed at the oil–water interface (Monera & del Rosario, 1982; Onsaard, Vittayanont, Sringam, & McClements, 2006). 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 on the stability of homogenized coconut milk (Tangsuphoom & Coupland, 2005) and demonstrated the improved stability obtained by homogenizing the coconut milk with surface-active proteins or small-molecule surfactants of sufficient concentration (Tangsuphoom & Coupland, 2008).

When emulsions are cooled to temperatures where part of the fat phase becomes 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–thaw than those stabilized by small-molecule surfactants (Cramp, Docking, Ghosh, & Coupland, 2004). Freezing is used as a method to break the fresh coconut milk emulsion and extract coconut oil (Cancel, 1979; Gonzalez, 1990).

The effects of heating on the stability of emulsions have been widely studied. Emulsions stabilized by globular proteins often

* Corresponding author. Tel.: +1 814 865 2636; fax: +1 814 863 6132.
E-mail address: jnc3@psu.edu (J.N. Coupland).
[1] Present address: Food Science and Technology Division, Institute of Nutrition, Mahidol University, Nakhon Pathom 73170, Thailand.

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destabilize to some degree when heated above the denaturation temperature of the proteins (Demetriades, Coupland, & McClements, 1997; Hunt & Dalgleish, 1995; Kim, Decker, & McClements, 2002; Monahan, McClements, & German, 1996; Sliwinski, Roubos, Zoet, van Boekel, & Wouters, 2003; de Wit, 1990). The effect of heating is less severe in emulsions 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 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.

2. Materials and methods

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. Coconut oil is largely a mixture of triglycerides but may contain a small fraction of unquantified surface-active lipids (Bezard, Bugaut, & Clement, 1971). 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 whey protein isolate (WPI) sample (BiPro, 98% protein) was purchased from Davisco Foods International (Le Sueur, MN).

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 fat 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 4–5 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 and then subjected to thermal treatments as described in Table 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.

Table 1
Temperature protocols.

Protocol	Thermal history
Deep freezing	Freezer (−20 °C), 24 h
Freezing	Refrigerated waterbath (−10 °C), 24 h
Chilling	Refrigerator (5 °C), 24 h
Control	Waterbath (30 °C), 24 h
Moderate heating	Waterbath (70 °C), 1 h
Intense heating	Waterbath (90 °C), 1 h
Autoclaving	Autoclave (120 °C), 1 h

2.3. Particle size determination

Particle size distributions and specific oil–water interface areas of the emulsions 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, $d_{43} = \sum n_i d_i^4 / \sum n_i d_i^3$; where n_i is the number of droplets of diameter d_i.

The particle size of the samples was also determined after diluting them 5-fold in SDS solution (1.25 wt%) and stirring for 30 min. The surfactant displaces the protein from the droplet surfaces and thus disperses the aggregates (Courthaudon, Dickinson, Matsumura, & Williams, 1991). Preliminary experiments showed that the SDS dissociates flocs 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. A change in both primary and effective particle diameter is seen in coalesced samples while flocculated samples 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).

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 1. The heights of any visible layers were measured with a ruler.

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 (10–15 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} and 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 to 30 °C. The samples were immediately rescanned and the heat flux data from the second scan were 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.

3. Results and discussion

3.1. Changes in emulsion structure

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, Tables 2 and 3) (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), which indicated coalescence and flocculation (Tables 2 and 3). However, none of these changes resulted in changes in the bulk appearance of the samples which all creamed to a similar extent (Fig. 1a). 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). Possibly the homogenization provided some protection against freeze–thaw.

Table 2
Mean effective particle sizes of 10 wt% coconut milk emulsions prepared with 1 wt% stabilizer after thermal treatments.

Treatment temperature (°C)	Effective d_{43} (μm)[f]				
	Control[g]	Caseinate	WPI	SDS	Tween 20
−20	66.2 ± 9.6^{aA}	0.5 ± 0.0^{B}	1.4 ± 0.2^{bB}	5.6 ± 2.9^{aB}	78.6 ± 14.2^{aA}
−10	51.4 ± 4.0^{bA}	0.5 ± 0.0^{B}	0.8 ± 0.1^{cB}	4.7 ± 1.2^{aB}	56.9 ± 17.4^{bA}
5	16.6 ± 1.8^{eA}	0.5 ± 0.0^{B}	0.6 ± 0.1^{cB}	0.3 ± 0.0^{bB}	0.4 ± 0.1^{cB}
30	16.0 ± 1.0^{eA}	0.5 ± 0.0^{B}	0.5 ± 0.0^{cB}	0.4 ± 0.0^{bB}	0.4 ± 0.0^{cB}
70	16.7 ± 1.5^{eA}	0.5 ± 0.0^{B}	0.6 ± 0.1^{cB}	0.4 ± 0.0^{bB}	0.6 ± 0.1^{cB}
90	33.2 ± 4.7^{dA}	0.5 ± 0.0^{B}	1.0 ± 0.3^{bcB}	0.4 ± 0.0^{bB}	0.7 ± 0.2^{cB}
120	47.3 ± 6.5^{cA}	0.5 ± 0.0^{C}	16.2 ± 1.0^{aB}	0.3 ± 0.0^{bC}	N/A

[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 ($p > 0.05$).
N/A Not analyzed.
[f] Mean ± standard deviation of three replicates. Droplets were diluted in water prior to analysis.
[g] Coconut milk emulsion homogenized without addition of surface-active stabilizer.

Table 3
Mean primary particle sizes of 10 wt% coconut milk emulsions prepared with 1 wt% stabilizer after thermal treatments.

Treatment temperature (°C)	Primary d_{43} (μm)[f]				
	Control[g]	Caseinate	WPI	SDS	Tween 20
−20	22.7 ± 3.0^{aB}	0.4 ± 0.1^{C}	0.5 ± 0.0^{bC}	2.8 ± 0.5^{aC}	44.0 ± 1.3^{aA}
−10	18.5 ± 2.4^{bB}	0.4 ± 0.0^{C}	0.4 ± 0.1^{bC}	1.2 ± 0.0^{bC}	31.1 ± 0.3^{bA}
5	9.5 ± 1.7^{dA}	0.4 ± 0.1^{B}	0.5 ± 0.0^{bB}	0.4 ± 0.0^{cB}	0.4 ± 0.0^{cB}
30	6.3 ± 0.8^{eA}	0.4 ± 0.1^{B}	0.4 ± 0.1^{bB}	0.4 ± 0.0^{cB}	0.4 ± 0.1^{cB}
70	6.2 ± 0.5^{eA}	0.5 ± 0.1^{B}	0.4 ± 0.2^{bB}	0.3 ± 0.0^{cB}	0.4 ± 0.1^{cB}
90	9.7 ± 1.2^{dA}	0.5 ± 0.0^{B}	0.5 ± 0.2^{bB}	0.3 ± 0.1^{cB}	0.4 ± 0.0^{cB}
120	13.1 ± 2.3^{cA}	0.5 ± 0.0^{C}	5.1 ± 1.8^{aB}	0.3 ± 0.0^{cC}	N/A

[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 ($p > 0.05$).
N/A Not analyzed.
[f] Mean ± standard deviation of three replicates. Droplets were dispersed in SDS solution prior to analysis.
[g] Coconut milk emulsion prepared without addition of surface-active stabilizer.

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 (Tables 2 and 3). The flocculated coconut milk (i.e., heated at 90 °C) creamed less than the non-flocculated samples (30 °C or 70 °C, Fig. 1a) 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 (Fig. 1a), 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.

3.1.2. Coconut milk with added protein

The coconut milk emulsions prepared with 1 wt% protein (sodium caseinate or WPI) had smaller droplets (0.4 μm primary d_{43} compared to 6 μm in the absence of added protein, Table 3) and were stable to chilling at 5 °C. The caseinate sample was stable to freeze–thaw, either at −10 °C or −20 °C, while the WPI emulsion showed some loss in stability (Table 3 and Fig. 1).

The bulk properties and microstructure of coconut milk emulsions homogenized with sodium caseinate were not affected by heating for 1 h at 70 °C, 90 °C, or 120 °C (Table 2 and Fig. 1b). 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 canning process (Genato & Gonzalez, 1985).

There was no change in the droplet size and the appearance after heating WPI-stabilized coconut milk emulsion at 70 °C while a slight decrease in the stability of the emulsion was observed after being treated at 90 °C (Table 2 and Fig. 1c). Severe heating (120 °C, 1 h) of the coconut milk emulsified with WPI, however, resulted in a massive change in extensive droplet coalescence and separation of emulsion into free oil layer on top and a coagulated layer with large white aggregates dispersed in transparent fluid at the bottom.

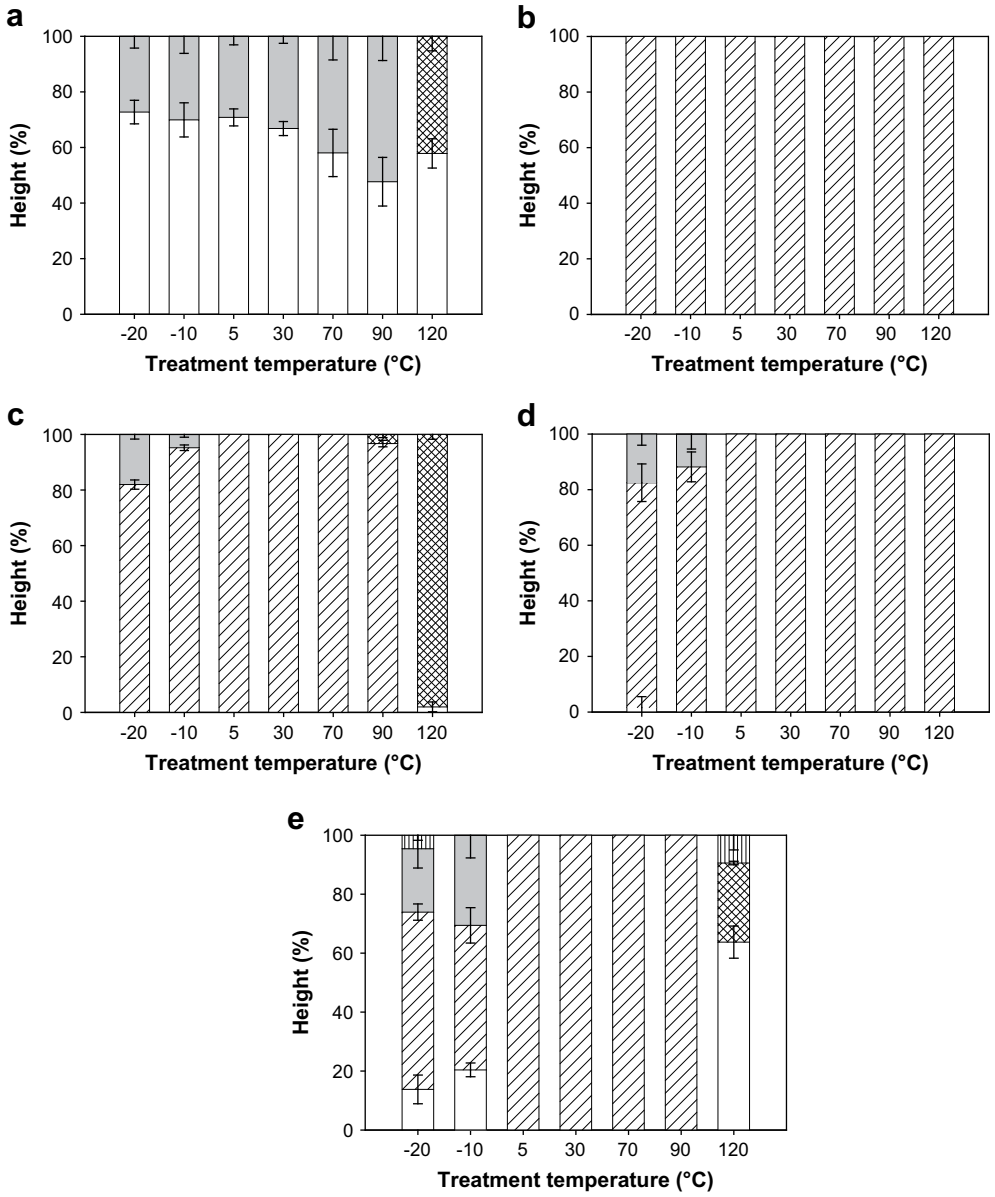

Fig. 1. Visual appearance after thermal treatments of homogenized coconut milk emulsions prepared with no additive (a), 1 wt% sodium caseinate (b), WPI (c), SDS (d), and Tween 20 (e). Open bars represent serum layer, filled bars represent cream layer, diagonal striped bars represent emulsion layer, diagonal crisscrossed bars represent coagulated layer, and vertical striped bars represent free oil layer.

3.1.3. Coconut milk with added surfactant

Coconut milk emulsions prepared with small-molecule surfactant (SDS or Tween 20) had smaller and non-flocculated droplets (about 0.4 μm primary d_{43} compared to 6 μm in the absence of surfactant, Table 3) and did not show any phase separation (Fig. 1d,e). They were stable to chilling but suffered significant destabilization following freeze–thaw (Table 2 and Fig. 1d,e). 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 samples 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, et al., 1993) and thicker layers have been associated to better freeze–thaw stability (Thanasukarn et al., 2004). It is likely that the negative charge on surface of SDS-stabilized droplets provided substantial electrostatic repulsion that was more effective than the non-ionic Tween 20 in preventing droplets from getting to close to each other, and thus less coalesced after freeze–thaw.

Coconut milk emulsified with SDS was stable to all heating treatments (i.e., no change in droplet size and no phase separation, Table 2 and Fig. 1d). The Tween 20 stabilized emulsions were stable after being heated for 1 h at temperatures up to 90 °C, despite the fact that 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 2 and Fig. 1e, note that because a free oil layer was observed the particle size could not be usefully measured).

Polysorbates, e.g., 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 broke down perhaps because the autoclave treatment used in our study (120 °C, 1 h) is more severe than that 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 correspond to the lower heat stability of coconut milk emulsions prepared with Tween 20 compared to those prepared with SDS.

The changes in stability for the processed coconut milk samples are summarized in Table 3 and in Section 3.2 we use calorimetry to investigate the thermal transitions underlying the differences seen.

3.2. Thermal properties and discussion

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, end point 27 °C) (Fig. 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) (Fig. 3). In the samples cooled to −40 °C (Fig. 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 a melting peak at 0 °C is seen in Fig. 3b and is absent in samples cooled to −15 °C (Fig. 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 on composition of the fat crystals. This suggests that whatever polymorphic transitions may occur in coconut oil during cooling 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 and only one in bulk could be 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. Alternatively the polymorphic transitions in emulsified lipids are known to be affected by the size of the droplets (Gulseren & Coupland, 2007) and the multiple peaks seen here may simply be due to changing polymorphic composition. The fat crystallization peaks were independent of phase transitions in the aqueous phase (i.e., cooling to −10 °C or −20 °C, Fig. 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 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,

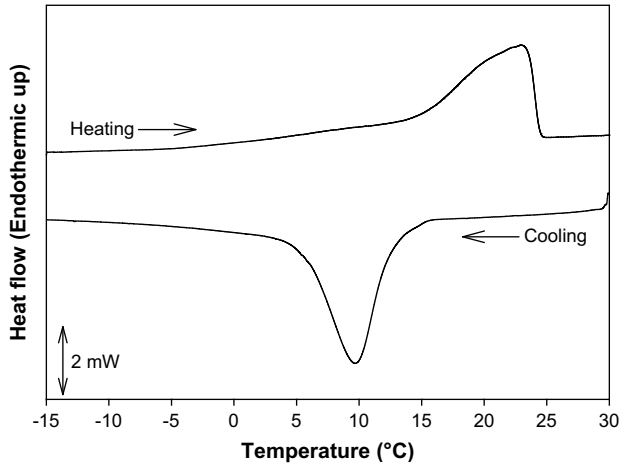

Fig. 2. Typical thermograms of coconut oil. Samples were cooled from 40 °C to −40 °C at 1.5 °C min^{-1} following by reheating at the same rate.

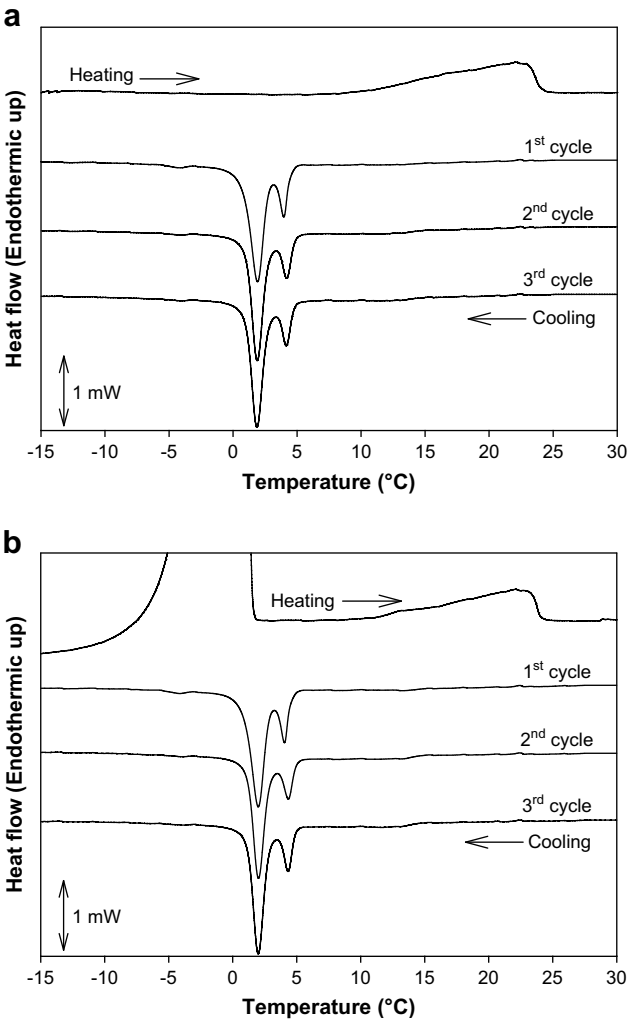

Fig. 3. Successive cooling thermograms and a representative heating thermogram of homogenized coconut milk repeatedly cycled between 30 °C and −15 °C (a), and −40 °C (b) at 1.5 °C min^{-1}.

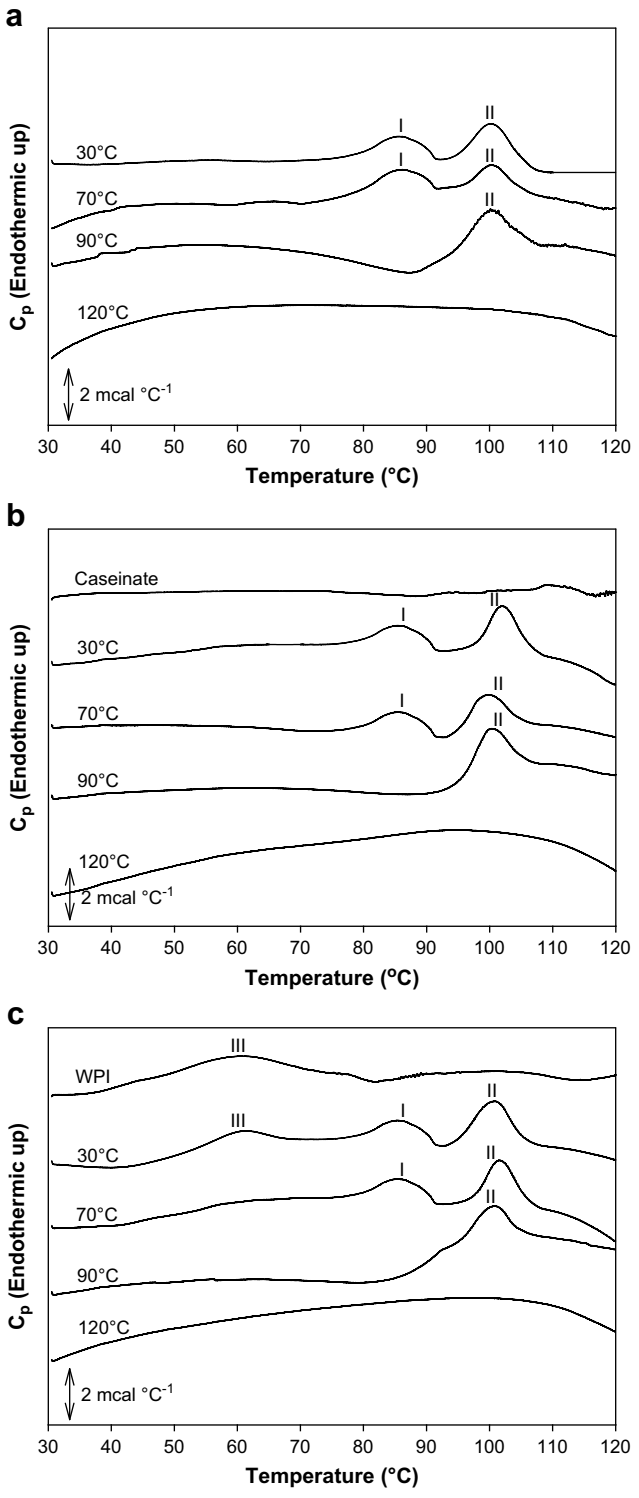

Fig. 4. Differential scanning microcalorimetric thermograms of coconut milk emulsions prepared with no additive (a), 1 wt% sodium caseinate (b), and WPI (c) after heated at different temperatures for 1 h. I and II indicate endothermic peaks due to thermal denaturation of coconut proteins whereas III indicates those of WPI.

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

Table 4
Summary of stability after thermal treatments of 10 wt% coconut milk emulsions prepared with 1 wt% stabilizer.

Treatment temperature (°C)	Stability[a]				
	Control[b]	Caseinate	WPI	SDS	Tween 20
−20	●●	○	●	●	●●
−10	●●	○	○	●	●●
5	●	○	○	○	○
30	●	○	○	○	○
70	●	○	○	○	○
90	●●	○	○	○	○
120	●●	○	●●	○	●●

[a] ○ Stable, ● some destabilization, ●● extensive destabilization.
[b] Coconut milk emulsion prepared without addition of surface-active stabilizer.

temperatures than this and similar isothermal studies revealed that none of the emulsified coconut oil crystallized under the chilled conditions described in Table 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 2 and Fig. 1). Longer storage might allow the droplets to eventually crystallize but even if they did, we suspect the emulsion

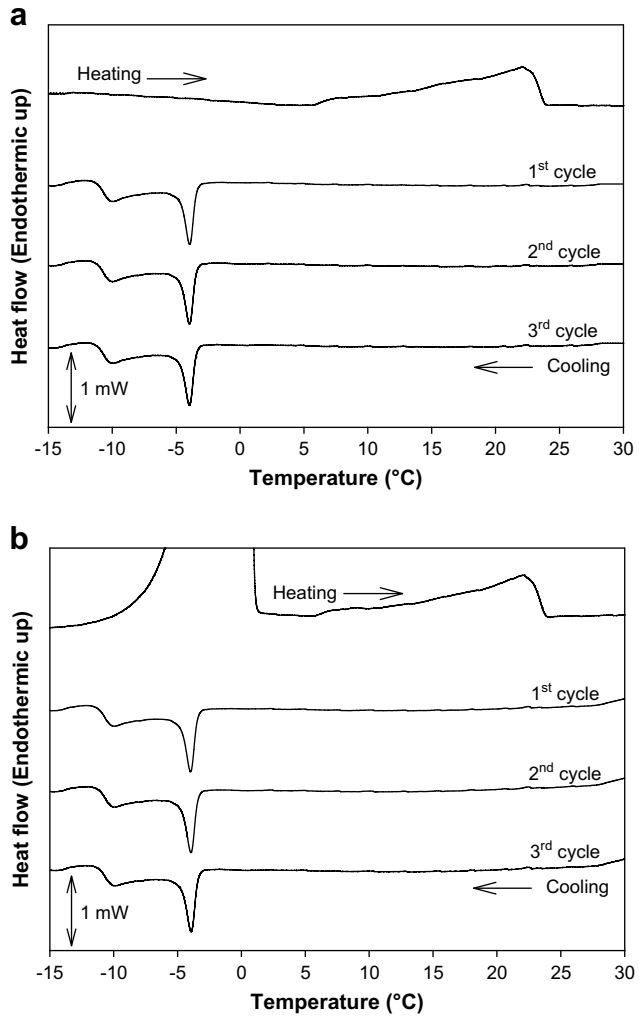

Fig. 5. Successive cooling thermograms and a representative heating thermogram of coconut milk emulsions prepared with 1 wt% sodium caseinate repeatedly cycled between 30 °C and −15 °C (a), and −40 °C (b) at 1.5 °C min^{-1}.

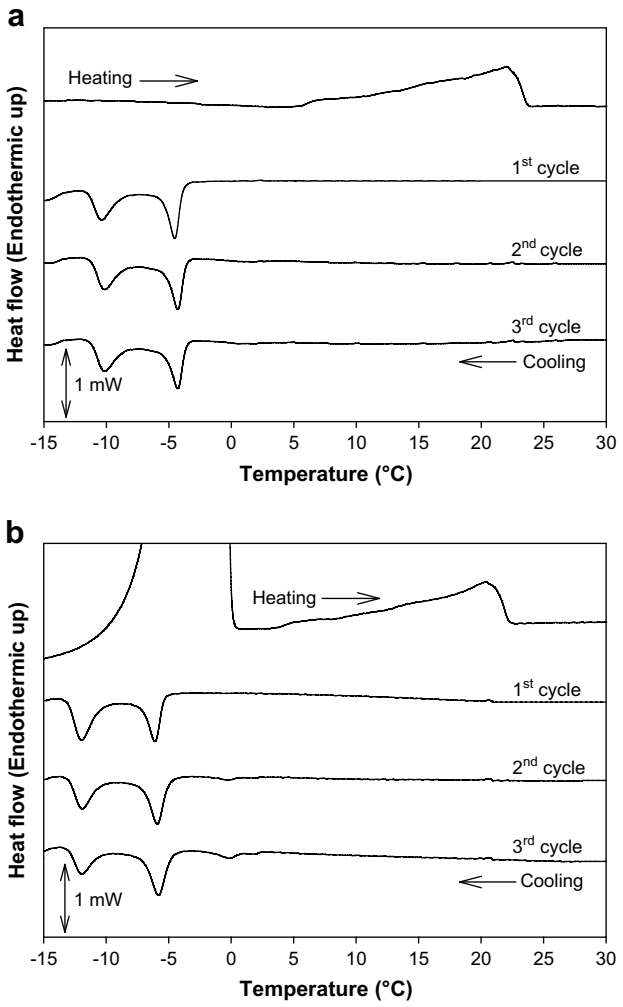

Fig. 6. Successive cooling thermograms and a representative heating thermogram of coconut milk emulsions prepared with 1 wt% WPI repeatedly cycled between 30 °C and −15 °C (a), and −40 °C (b) at 1.5 °C min^{-1}.

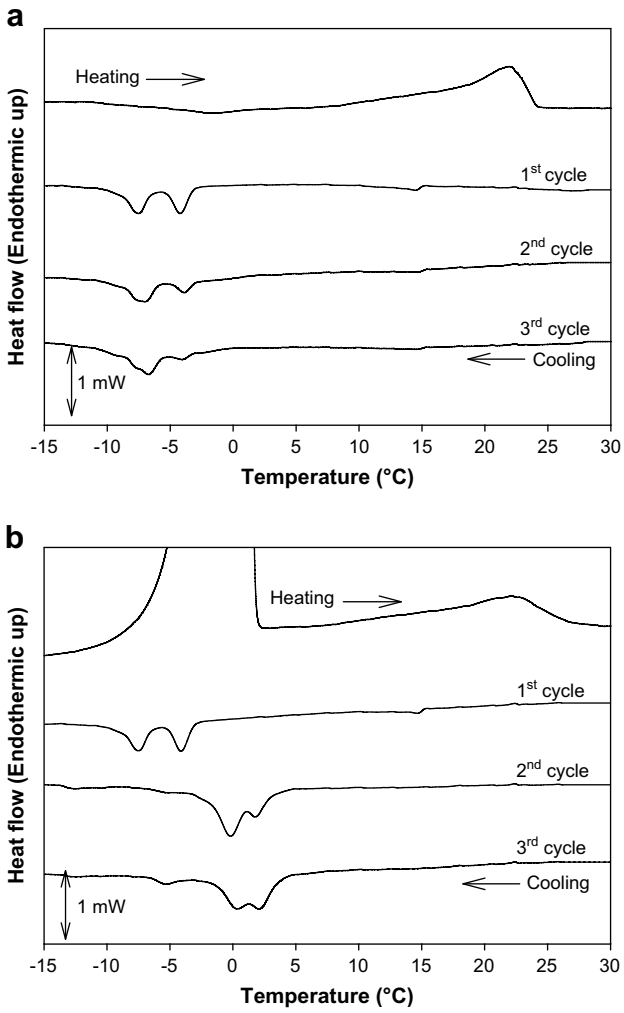

Fig. 7. Successive cooling thermogram and a representative heating thermogram of coconut milk emulsions prepared with 1 wt% SDS repeatedly cycled between 30 °C and −15 °C (a), and −40 °C (b) at 1.5 °C min^{-1}.

would still remain stable, as the emulsions were also stable to the much more severe freezing cycle.

Evidence of thermal denaturation of proteins was gained from microcalorimetry. Thermograms of coconut milk emulsions previously heated at 30 °C (unheated), 70 °C, 90 °C and 120 °C are shown in Fig. 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 soymilk (Kwok & Niranjan, 1995). Soy protein is mainly (~70%) composed of 7S and 11S globulins (Utsumi, Matsumura, & Mori, 1997), similar to the coconut proteins and it has been reported that the two endothermic peaks of soymilk (70 °C and 90 °C) correspond to the denaturation of the two soy protein fractions (Zhang, Takenaka, & Isobe, 2004).

The DSC heating curve of homogenized coconut milk was unchanged by prior heating at 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 4). 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 after heating at 90 °C and extensive destabilization following heating at 120 °C (Table 4).

3.2.2. Coconut milk with added protein

There were two crystallization peaks in the cooling thermogram of both emulsions prepared with added protein; a larger one at −5 °C and a smaller peak at about −12 °C (Figs. 5 and 6), while the unmodified coconut milk 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 (Fig. 6b) for the WPI samples only. Changes in crystallization pattern of the dispersed phase correspond to the changes in particle size seen resulting from freeze–thaw destabilization (Table 2).

No transition was observed in the thermograms of a 1 wt% sodium caseinate solution (data not shown) as the protein tends to be stable to heating at temperatures up to 140 °C (Singh, 1995). The

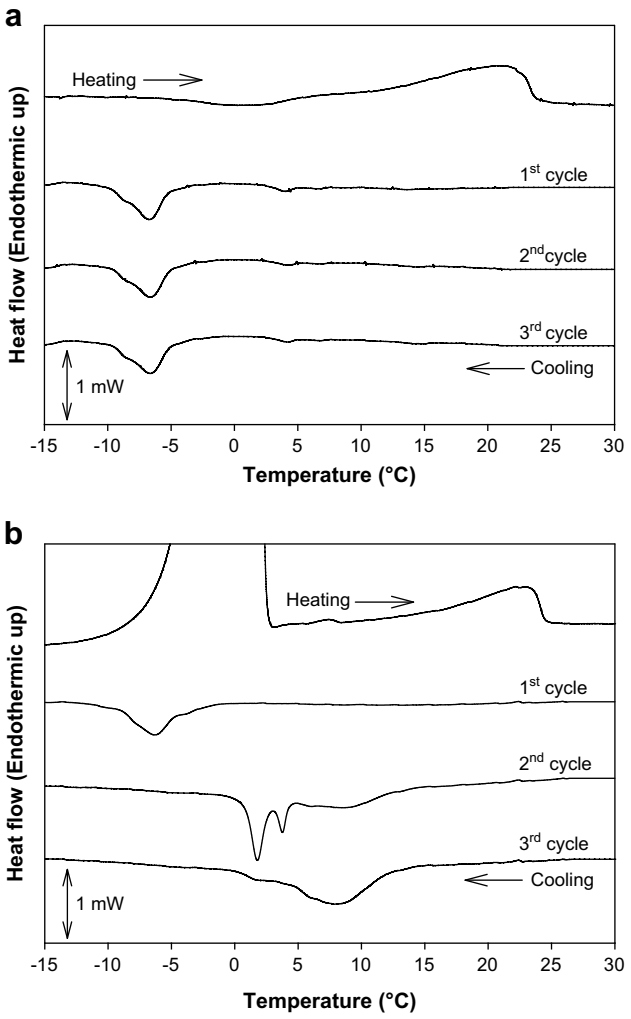

Fig. 8. Successive cooling thermograms and a representative heating thermogram of coconut milk emulsions prepared with 1 wt% Tween 20 repeatedly cycled between 30 °C and −15 °C (a), and −40 °C (b) at 1.5 °C min^{-1}.

thermograms of the heated, caseinate-stabilized coconut milk were similar to those of the coconut milk itself (Fig. 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 emulsion exhibited three exothermic peaks upon heating in microcalorimeter (Fig. 4c): peak I and peak II (85 °C and 100 °C) correspond with 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. Although WPI-stabilized emulsions have been reported to be stable against heating at pH values other than the isoelectric point of the protein (Demetriades et al., 1997; Hunt & Dalgleish, 1995; Kim et al., 2002; Kim, Decker, & McClements, 2005; Sliwinski et al., 2003), coconut milk emulsions made with WPI showed some degree of destabilization after being treated at 90 °C and 120 °C.

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, Fig. 7) while samples with added Tween 20 crystallized with a single peak (−6 °C, Fig. 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 that the composition of the interface can affect the crystallization properties of an emulsified lipid (Gülseren & Coupland, 2007; McClements, Dickinson, et al., 1993; McClements, Dungan, German, Simoneau, & Kinsella, 1993; Palanuwech & Coupland, 2003).

There was no change in the thermograms upon repeated cooling (to −15 °C, no ice formation) which is not surprising as the emulsions were very stable. However, upon freeze–thaw cycling (to −40 °C, ice formation), a dramatic change in cooling thermograms was observed in the coconut milks emulsified with SDS and Tween 20 (Figs. 7b and 8b), compared to those homogenized with proteins (Figs. 5b and 6b). Indeed cooling thermograms of the latter cycles, 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 it allows the formation of very fine droplets, it provides less protection than proteins against freeze–thaw destabilization (Palanuwech & Coupland, 2003; Thanasukarn et al., 2004; Vanapalli et al., 2002).

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 4. 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 layer which was 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.

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