PAPER

A COMPARATIVE STUDY OF THE PHYSICOCHEMICAL PROPERTIES AND EMULSION STABILITY OF COCONUT MILK AT DIFFERENT MATURITY STAGES

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ABSTRACT

Based on chemical analysis, mature coconut (MC) milk had the highest moisture content ($p<0.05$), followed by immature coconut (IMC) and overlay mature coconut (OMC) milk, respectively. OMC milk had the highest lipid content while IMC milk showed the lowest lipid content ($p<0.05$). The lowest protein and carbohydrate contents were found in MC milk ($p<0.05$). Cocosin with MW of 55 kDa was observed as the major protein in all coconut milks; however, the band intensity slightly decreased with increasing maturity stages. Increase in oil droplet size was observed with increasing maturity stages. Therefore, maturity stages have an influence on the chemical compositions, properties and emulsion stability of coconut milk.

Keywords: coconut milk, droplet size, emulsion stability, physicochemical properties, protein pattern

1. INTRODUCTION

Coconuts (*Cocos nucifera)* are extensively used in many traditional foods of the Asian and Pacific regions (ONSAARD *et al.*, 2005). Coconut milk is commonly used in several cuisines such as curries and desserts (TANSAKUL and CHAISAWANG, 2006). It contains high amounts of medium chain saturated fatty acids (MCFAs), especially lauric acid (RAGHAVENDRA and RAGHAVARAO, 2010). Lauric acid is converted into a very valuable compound known as monolaurin, which has antiviral and antibacterial properties. The consumption of coconut milk may help to protect the body from infections (DEBMANDAL and MANDAL, 2011).

Coconut milk is a milky white oil-in-water emulsion extracted from grated coconut meat with or without the addition of water. The emulsion in coconut milk is naturally stabilised by coconut proteins (globulins and albumins), as well as phospholipids (RAGHAVENDRA and RAGHAVARAO, 2011). The major protein (~65%) in coconut endosperm is an 11S globulin known as cocosin with a molecular weight (MW) of 55 kDa (GARCIA *et al.*, 2005), and is believed to play a significant role in stabilising the coconut milk emulsion (TANGSUPHOOM and COUPLAND, 2008). Generally, both intrinsic factors (e.g. protein compositions, etc.) and environmental conditions (e.g. pH, temperatures, etc.) can affect the stability of the coconut milk emulsion (RAGHAVENDRA and RAGHAVARAO, 2010).

On the other hand, the instability of the coconut milk emulsion is required for the production of virgin coconut oil (VCO). In recent years, coconut milk is immensely used for the extraction of VCO. Moreover, VCO has gained much popularity in the scientific community due to the presence of MCFAs, its high degree of saturation and good stability. It can be obtained by breaking the emulsion of coconut milk using different extraction methods (RAGHAVENDRA and RAGHAVARAO, 2010). Thus, to maximise the yield of VCO, coconut milk emulsion must be destabilised to a high degree, so that oil can be released and separated effectively

The quality and stability of coconut milk emulsion could be governed by intrinsic factors, especially at different maturity stages. However, no information exists regarding the influence of maturity stages on the characteristics and emulsion stability of coconut milk. A better understanding of the physicochemical properties and emulsion stability of coconut milk at different maturity stages could be beneficial in the manufacturing of VCO with prime quality and high yield. Therefore, this comparative study was carried out to evaluate the physicochemical properties and emulsion stability of milk obtained from coconut at three different maturity stages.

2. MATERIALS AND METHODS

2.1. Chemicals

Sodium hydroxide, boric acid and Nile blue A were purchased from Sigma (St. Louis. MO, USA). Sodium dodecyl sulphate and isooctane were obtained from Merck (Darmstadt, Germany). Methanol, ethanol, acetic acid, chloroform, petroleum ether, hydrochloric acid, sulphuric acid, n-hexane and cyclohexane were procured from Lab-Scan (Bangkok, Thailand). Chemicals for electrophoresis were obtained from Biorad (Richmond, VA, USA) and protein molecular weight marker was procured from GE healthcare (Buckinghamshire, UK).

2.2. Preparation of coconut meat and coconut milk

Coconuts at three different maturity stages including immature coconut (IMC) (9-10 months old from pollination), mature coconut (MC) (11-12 months old from pollination) and overlay mature coconut (OMC) (14-15 months old from pollination) were purchased from a plantation site in Yaring District, Pattani Province, Thailand and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Songkhla. Coconuts were subjected to deshelling, paring and removal of water. Coconut kernel was collected manually and grated using a rotary wedge cutter machine. To prepare coconut milk, the grated coconut meat was pressed using a hydraulic press machine (Model stainless steel hydraulic press A2, Sakaya, Bangkok, Thailand) with a maximum pressure of 10.35 MPa for 2 min. Thereafter, coconut milk was collected and analysed.

2.3. Proximate analysis of coconut meat and coconut milk

Coconut meat and coconut milk at three different maturity stages were analysed for moisture, ash, lipid and protein contents according to the method of AOAC (AOAC, 2000). The protein content was calculated using 6.25 (as the factor) and the carbohydrate content was calculated as the difference from the sum total of the aforementioned proximate analysis components. The values were expressed as g/100 g (wet weight basis).

2.4. Colour determination

Coconut milk colour was measured using a colourimeter (HunterLab, Model colourFlex, VA, USA). The colour was reported as L^*, a^*, b^* values, indicating lightness, redness/greenness and yellowness/blueness, respectively. Total difference in colour (ΔE^*) and the difference in chroma (ΔC^*) were also calculated using the following equations:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^*, Δa^* and Δb^* are the differences between the corresponding colour parameter of the sample and the white standard ($L^* = 93.55$, $a^* = 0.84$, $b^* = 0.37$).

$$\Delta C^* = C^*_{sample} - C^*_{Standard}$$

where $C^* = \sqrt{(a^*)^2 + (b^*)^2}$

2.5. pH measurement

A digital pH meter (Eutech, pH700 Thermo Scientific, USA) was used to measure the pH values of coconut milk.

2.6. SDS- polyacrylamide gel electrophoresis (SDS-PAGE)

The protein patterns of coconut milk were determined by SDS-PAGE, according to the method of LAEMMLI (1970), using 4% staking gel and 12% separating gel. The coconut milk samples (10 mL) were homogenised with 10 mL of 50 g/L SDS at a speed of 12,000 rpm for 1 min. The homogenate was heated at 95°C for 1 h, followed by centrifugation at 7000 × g for 10 min at 25°C using a centrifuge (Beckman coulter, Allegra™ centrifuge, CA,

USA). The protein concentration of the supernatant was determined by the Biuret method (ROBINSON and HOGDEN, 1940), using bovine serum albumin (BSA) as a standard. The prepared samples were mixed with sample buffer containing 2% SDS, 10% glycerol and 0.05% bromophenol blue in 0.5 M Tris-HCl, and the resulting solution had a pH of 6.8. Under reducing condition, β-mercaptoethanol was added to the sample buffer in order to obtain a final concentration of 5% and the mixtures were heated at 95°C for 3 min prior to loading. The prepared mixtures (20 μg protein) were loaded onto the gel. Electrophoresis was performed using a vertical gel electrophoresis unit (Mini-protein II; Bio-Rad Laboratories, Richmond, VA, USA) at a constant voltage of 20 mA/gel. After electrophoresis, the gels were stained with 0.5 g/L Coomassie Brilliant Blue R-250 in 500 mL/L methanol and 75 mL/L acetic acid for 30 min. Finally, they were destained with a mixture of 500 mL/L methanol and 75 mL/L acetic acid for 30 min and destained again with a mixture of 50 mL/L methanol and 75 mL/L acetic acid for 1 h. The relative mobility (R_f) of proteins was calculated and their molecular weight was estimated from the plot between R_f and log (MW) of standards.

2.7. Microstructure determination of oil droplets

2.7.1 Confocal laser scanning microscopy (CLSM)

The microstructures of coconut milk samples were examined with a confocal laser scanning microscope (CLSM) (Model FV300; Olympus, Tokyo, Japan.). The samples were dissolved in Nile blue A solution (1:10) and manually stirred until uniformity was obtained. Fifty microlitres of sample solutions were smeared on the microscope slide. The CLMS was operated in the fluorescence mode at excitation and emission wavelengths of 533 and 630 nm, respectively; using a Helium Neon Red laser (HeNe-R) for lipid analysis. A magnification of 400x was used.

2.7.2 Phase contrast microscopy

Oil droplets in coconut milk were observed under a phase contrast microscope (Model IX50; Olympus, Tokyo, Japan) equipped with camera. Samples were placed on a glass slide, covered with cover slip and observed at 400x magnification.

2.7.3 Determination of particle size

The particle size distribution of coconut milk emulsion was determined using a laser particle size analyser (LPSA) (Model LS 230, Beckman Coulter®, Fullerton, CA, USA) as per the method of CASTELLANI *et al.* (2006). Prior to analysis, the sample (5 mL) was diluted with 1 mL sodium dodecyl sulphate (SDS) in order to dissociate flocculated droplets. The surface-weighted mean particle diameter (d_{32}) and the volume-weighted mean particle diameter (d_{43}) of the emulsion droplets were measured.

2.7.4 Determination of coalescence and flocculation

Coconut milk samples were diluted with distilled water in the presence and absence of SDS. The coalescence index (C_i) and flocculation factor (F_f) were calculated using the following equations (INTARASIRISAWAT *et al.*, 2014):

$$F_f = \frac{d_{43}\text{-SDS}}{d_{43}+\text{SDS}}$$

$$C_i = \frac{(d_{43}+SDS,t - d_{43}+SDS,in)}{d_{43}+SDS,in} \times 100$$

where d_{43}+SDS and d_{43}-SDS are the volume-weighted mean particle diameter of the emulsion droplets in the presence and absence of SDS, respectively; d_{43}+SDS, in and d_{43}+SDS, t are the volume-weighted mean particle diameter of the emulsion droplets in the presence of SDS at time 0 and the designated storage time (24 h), respectively. Determination was conducted at room temperature (28-30°C).

2.7.5 Statistical analysis

Experiments were carried out in triplicate using three different lots of samples. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple range test. For paired comparison, T-test was used (STEEL and TORRIE, 1980). Statistical analysis was performed using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1. Proximate compositions of coconut meat and milk

The proximate compositions of coconut meat and milk at three different maturity stages are shown in Table 1. MC meat had the highest moisture content (61.07 g/100 g), followed by IMC (53.94 g/100 g) and OMC (39.50g/100 g), respectively. A similar trend was observed in coconut milk, in which MC had the highest moisture content (61.55 g/100 g), followed by IMC (55.36 g/100 g) and OMC (36.59 g/100 g), respectively. The high moisture content of MC meat and milk was more likely due to the absorption of coconut water inside the endosperm, since the beginning of germination. Water uptake is an essential step towards germination (BEWLEY and BLACK, 1994). On the other hand, low moisture content was observed in OMC meat and milk. The result suggested that the absorbed water in the endosperm was utilised during embryo development (BEWLEY and BLACK, 1994). It was found that there was a general increase of lipid content in coconut meat and milk with increasing maturity. OMC meat and milk were found to have the highest lipid content ($p<0.05$). The lipid content of coconut increased with maturity stage due to the accumulation of lipids in the endosperm (LÓPEZ-VILLALOBOS *et al.*, 2001). Lower protein content was observed in both MC meat and milk as compared with those of IMC and OMC ($p<0.05$). Cocosin is a reserve protein found in coconut endosperm and serves as nitrogen source during germination (BALASUNDARESAN *et al.*, 2002). The result suggested that proteins could be degraded and utilised at the beginning of germination. Since water was utilised during the germination of OMC, the proportion of proteins was slightly increased. The ash content of both meat and milk decreased with maturity. The ash contents are indices of the mineral content (OBASI *et al.*, 2012). It has been reported that coconut water contains sugars, vitamins, minerals, amino acids and phytohormones(YONG *et al.*, 2009). The decrease in ash content in OMC meat and milk suggested that minerals are more likely used up during the germination process. A significantly lower carbohydrate content was observed in MC meat and milk ($p<0.05$). The obtained results are in accordance with that of JEGANATHAN (1970) who found that coconut milk at the mature stage, had a carbohydrate content of 5.5%. WHITE *et al*. (1989)

found that coconut milk at the mature stage is composed predominantly of galactose and arabinose with a small amount of mannose and glucose. BALASUBRAMANIAM (1976) reported that galactomannans and cellulose are present in the kernel of maturing and matured coconuts, whereas mannans are almost absent from very immature kernel and increased with maturation. Endosperm rich nutrients appear to function as a food reservoir for embryo development (BALASUNDARESAN *et al.*, 2002). Thus, reserved materials, particularly carbohydrates, were degraded and utilised during maturity. The decrease in carbohydrate plausibly led to the increased proportion of lipid in MC, as compared with the IMC sample. The results revealed that different maturity stages had marked impact on the chemical composition of coconut meat and milk.

Table 1. Proximate composition of coconut meat and milk at three different stages of maturity.

Content (g/100 g)	Coconut Meat			Coconut Milk		
	IMC	MC	OMC	IMC	MC	OMC
Moisture	53.94±0.60b	61.07±1.02a	39.50±0.82c	55.36±0.15b	61.55±0.13a	36.59±1.05c
Lipid	18.59±0.89b	20.86±0.95b	32.45±0.35a	17.28±1.46c	30.34±0.96b	44.20±0.85a
Protein	4.79±0.16a	3.95±0.09b	4.45±0.56a	3.35±0.29a	2.90±0.06b	3.34±0.49a
Ash	1.15±0.02a	1.14±0.04a	1.04±0.03b	1.03±0.05a	1.00±0.04a	0.80±0.03b
Carbohydrate	21.53±0.98a	13.05±0.95b	22.34±0.85a	22.98±1.21a	4.21±0.93c	15.07±1.63b

IMC: Immature Coconut, MC: Mature Coconut and OMC: Overlay Mature Coconut.
Values are presented as Mean±SD (n=3).
Different lowercase letters in the same row, within the same commodity, indicate significant difference ($p<0.05$).

3.2. Colour of coconut milk

L^*, a^* and b^* values of coconut milks at three different stages of maturity are shown in Table 2. The coconut milks were milky white in colour as evidenced by high L^*-value (lightness). In general, coconut milk is an oil-in-water emulsion, where oil droplets are dispersed in the water phase. Light scattering of oil droplets is mostly associated with the white colour of coconut milk. All samples had low a^* and b^*- values, suggesting that deterioration did not occur in all samples. It was observed that ΔE^* and ΔC^* decreased with increasing maturity stages, where IMC had the highest values, followed by MC and OMC samples, respectively. The highest L^*- value was found in the MC sample ($p<0.05$). The turbidity, cloudiness, or opaque appearance of emulsion is dependent on light scattering which is mediated by the dispersed oil droplets (MCCLEMENTS, 2002).

Table 2. Colour and pH of coconut milk at three different stages of maturity.

Samples	L^*	a^*	b^*	ΔE^*	ΔC^*	pH
IMC	92.90±0.24a	0.10±0.05c	5.09±0.06a	4.83±0.05a	4.09±0.06a	7.00±0.01a
MC	94.86±1.83c	-0.28±0.04b	4.48±0.12a	4.48±0.13b	3.84±0.12b	6.39±0.02b
OMC	93.09±0.18b	-0.35±0.03a	4.22±0.08b	3.88±0.08c	3.23±0.08c	5.58±0.13c

IMC: Immature Coconut, MC: Mature Coconut and OMC: Overlay Mature Coconut.
Values are presented as Mean±SD (n=3).
Different lowercase letters in the same column indicate a significant difference ($p<0.05$).

Lightness is not only determined by lipids or oils, but also by the size of oil droplets, which is another prime factor governing the colour, particularly the lightness of coconut milk. Some differences in indigenous pigments present in coconut milk of different maturity stages were also presumed.

3.3. pH of coconut milk

The pHs of freshly prepared coconut milks at three different stages of maturity are shown in Table 2. IMC milk was found to have a pH of 7.0 while MC milk was slightly acidic in pH (pH 6.39). The lowest pH (5.58) was obtained in OMC milk ($p<0.05$). Reserved food materials such as proteins, carbohydrates and lipids provide nourishment to growing embryo (SAMSON *et al*., 1971). The breakdown of these stored food materials by some enzymes, possibly occurred for embryo development. Acidic metabolites or degradation products such as acidic amino acids may contribute to the lowered pH. The pH of 5.58 found in OMC milk is close to the isoelectric point of coconut proteins (pI = 4-5) (SAMSON *et al.*, 1971; MONERA and DEL ROSARIO, 1982; Kwon *et al.*, 1996). Therefore, pH may affect the emulsifying properties of proteins in coconut milk, especially those stabilising oil droplets in the aqueous phase.

3.4. Electrophoretic patterns of coconut milk proteins

The protein patterns of coconut milks at different stages of maturity under reducing and non-reducing conditions are shown in Fig. 1. Under non-reducing condition, there were six protein bands with MW of 55, 46, 33, 25, 18 and 16 kDa. The major protein in coconut endosperm is 11S globulin, which is referred to as cocosin, with MW of 55 kDa (GARCIA *et al.*, 2005). A hexamer (55 kDa) consists of acidic (32-34 kDa) and basic (22-24 kDa) subunits, which are linked by a disulphide bridge (GARCIA *et al.*, 2005; TANGSUPHOOM and COUPLAND, 2008). Some proteins present in the aqueous phase of coconut milk could act as emulsifier to stabilise fat globules (PEAMPRASART and CHIEWCHAN, 2006). Cocosin plays a prominent role in regulating the stability of coconut milk (TANGSUPHOOM and COUPLAND, 2008). In the present study, smaller bands of proteins with MW higher than 55 kDa were found. Bands with higher intensity were found in IMC as compared with others. In the OMC sample, protein bands with MW of 70 and 46 kDa almost disappeared, indicating the degradation of proteins during germination. Under the reducing condition, several major protein bands with MW of 55, 33, 31, 25, 21, 20, 18 and 16 kDa were observed, which are in agreement with previous reports of GARCIA *et al.* (2005) and DEMASON and SEKHAR (1990). Under the reducing condition, cocosin dissociated into acidic and basic polypeptides with the coincidental appearance of proteins with MW of 32 and 22 kDa. Other proteins with MW greater than 55 kDa were not found. The occurrence of protein with MW of 20 kDa was also observed under reducing conditions. Protein with MW of 36 kDa was found only in the MC sample. In general, the OMC sample showed the lower band intensity of most proteins as compared with others. The result confirmed that the protein composition of coconut milk changed with maturity stages.

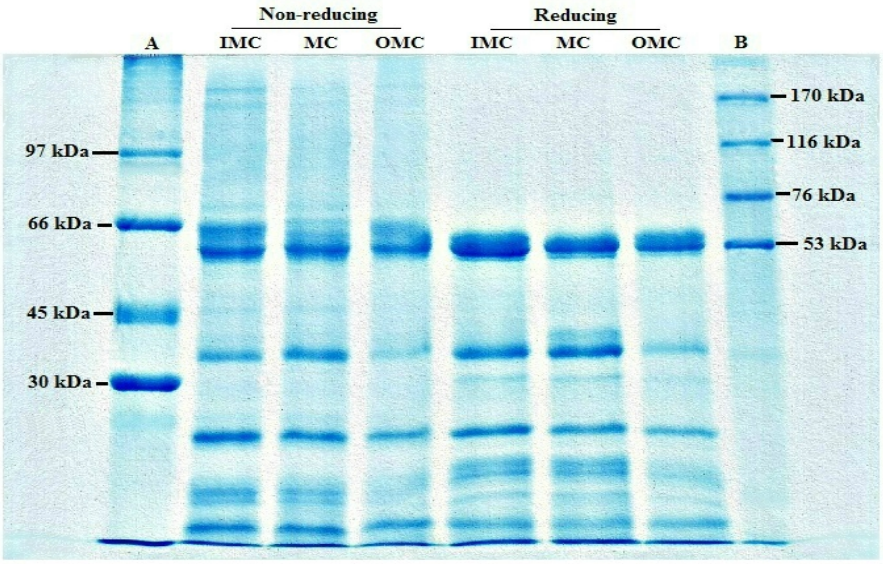

Figure 1. SDS-PAGE patterns of coconut milk proteins with three different maturity stages. A: low molecular weight standards; B: high molecular weight standards; IMC: Immature Coconut, MC: Mature Coconut and OMC: Overlay Mature Coconut.

3.5. Microstructure of oil droplets in coconut milk

The microscopic structures of coconut milk emulsions at three different stages of maturity were visualised by confocal laser scanning microscopy (CLSM) and phase contrast microscopy (Fig. 2). In the same coconut milk sample, similar results were observed when both CLSM and phase contrast microscopies were used. CLSM generally provides higher clarity and better resolution images of the emulsion microstructure than convention optical microscopy. The observation of the microstructure of the emulsion was facilitated using a fluorescence dye such as Nile blue A, in order to label the lipid. However, phase contrast microscopy provides excellent contrast, and a halo is formed even around a small oil droplet. For IMC (Fig. 2a), smaller oil droplets with uniform shape and size were distributed uniformly in the aqueous phase. An emulsion with the same size of oil droplets is referred to as a monodisperse emulsion, whereas that containing a range of droplet sizes is referred to as a polydisperse emulsion (MCCLEMENTS, 2004). Coconut milk is an oil-in-water emulsion naturally stabilised by coconut proteins (BIROSEL *et al.*, 1963). In the present study, IMC had higher protein/lipid ratio as compared with the MC sample (Table 1). High protein content can lead to efficient localisation of protein films at the oil-water interphase. Thus, this could increase the emulsion stability of coconut milk. Moreover, proteins could stabilise the coconut milk emulsion by lowering the interfacial tension between two phases, in which oil droplets are dispersed uniformly throughout the water phase. However, polydisperse emulsion was observed in MC and OMC (Figs. 2b and c) with a wide range of oil droplet sizes. Large sizes of oil droplets were abundantly observed in the OMC. In OMC, coconut milk contained a high amount of lipid. Thus, the present proteins may not be sufficient to stabilise the emulsion. The low pH of OMC milk could be another factor enhancing the destabilisation of emulsion, by lowering the repulsion of protein film surrounding the oil droplets. In general, the emulsion was less stable as evidenced by the larger droplets with non-uniform distribution. The results clearly indicated that maturity stages affected oil droplet size.

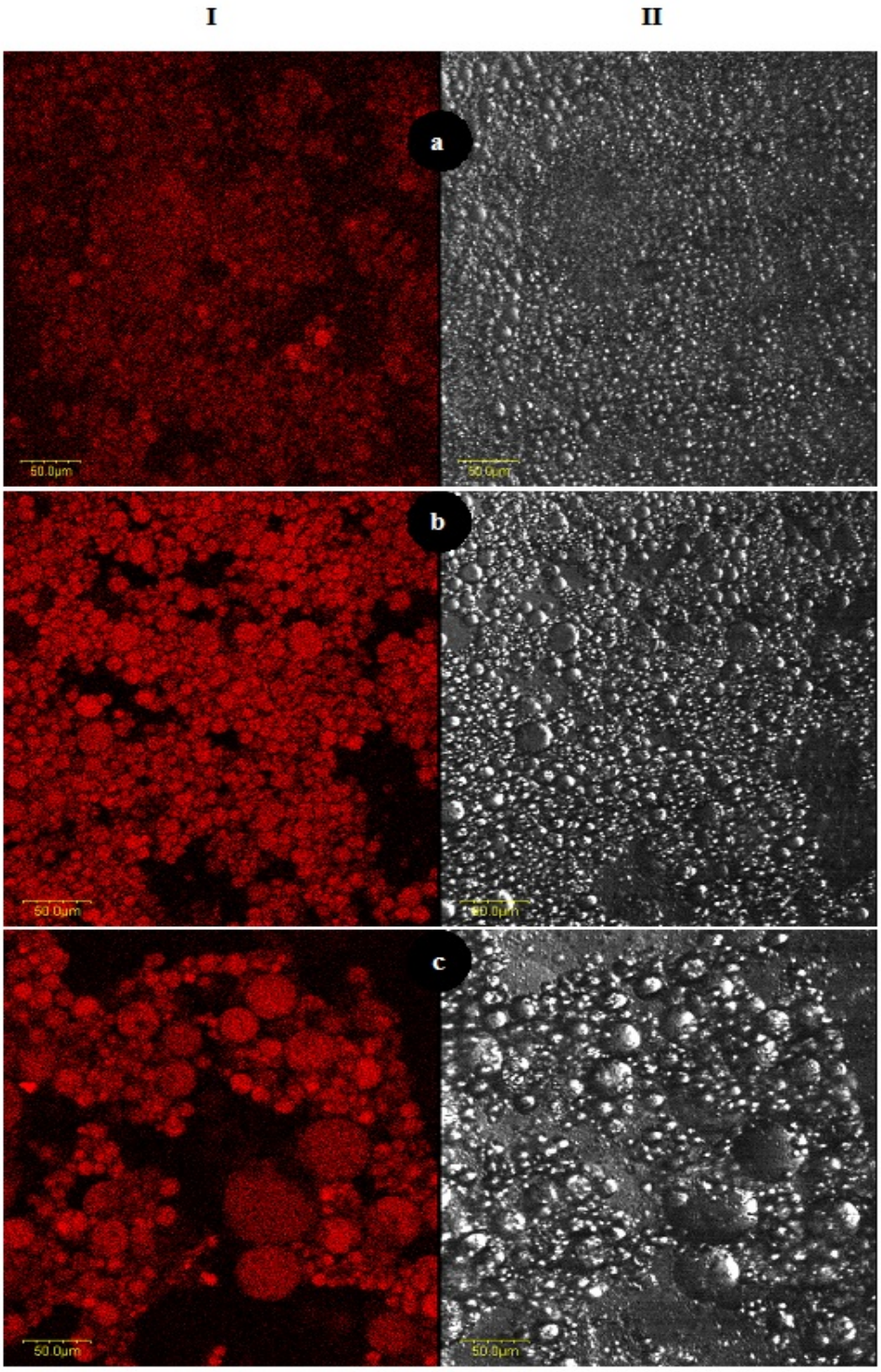

Figure 2. Confocal laser scanning micrographs (I) and phase contrast microscopy (II) of coconut milk at three different stages of maturity: (a) IMC: Immature Coconut; (b) MC: Mature Coconut; (c) OMC: Overlay Mature Coconut. Magnification: 400×. Scale bar = 50 μm.

3.6. Particle size distribution

Particle size distributions expressed as d_{32} and d_{43} of coconut milk emulsions at three different stages of maturity are shown in Table 3. The d_{32} increased from 3.38 μm (IMC) to 5.48 μm (OMC), while d_{43} increased from 5.29 μm (IMC) to 13.38 μm (OMC) with increasing maturity stages. Coconut milk from OMC contained the largest oil droplets (d_{43} and d_{32}), followed by those from MC and IMC. The d_{32} is related to the average surface area of droplet exposed to the continuous phase per unit volume of emulsion. The smaller d_{32} indicates higher specific surface area (INTARASIRISAWAT *et al.*, 2014). The d_{43} is the sum of the volume ratio of droplets in each size class multiplied by the mid-point diameter of the size class. The d_{43} can be used as the index of coalescence and flocculation (HEBISHY *et al.*, 2013). The proteins in coconut milk are known to function as emulsifier, which stabilises the oil droplets in coconut milk (DIONISIO, 1963; MONERA and DEL ROSARIO, 1982). The largest size of oil droplets in OMC can be attributed to the pH of the OMC milk, close to pI. As a result, there was a decrease in repulsion between protein films surrounding the oil droplets, thereby facilitating the coalescence. When the repulsive forces dominate, the droplets tend to remain as individual entities (MCCLEMENTS, 2004) and form a stable emulsion. After 24 h of storage, both d_{32} and d_{43} increased (Table 3), indicating the coalescence of oil droplets. Among all samples, a slight increase in d_{32} and d_{43} were observed in the OMC sample. The result suggested that the collapse of emulsion in the OMC milk was pronounced at the initial time and less coalescence occurred after 24 h. Conversely, the emulsion collapsed continuously in the MC and IMC samples.

Table 3. Droplet size and stability of coconut milk at three different stages of maturity.

Samples	Storage Time (h)	d_{32} (μm)	d_{43} (μm)	F_f	C_i
IMC	0	3.38±0.10C	5.29±0.20C	1.18±0.03A	-
	24	4.16±0.40b	8.10±0.10c	0.77±0.04b	53.22±0.59a
MC	0	5.07±0.25B	12.31±0.15B	0.90±0.90A	-
	24	5.55±0.60a	14.22±0.21a	0.78±0.03b	15.52±0.89b
OMC	0	5.48±0.13A	13.38±0.03A	1.26±0.04A	-
	24	5.66±0.26a	13.44±0.01b	1.26±0.01a	0.45±0.50c

F_f: Flocculation factor, C_i: Coalescence index.
IMC: Immature Coconut, MC: Mature Coconut and OMC: Overlay Mature Coconut.
Values are presented as Mean±SD (n=3).
Different uppercase letters in the same column at the initial storage time (0 h) indicate significant difference ($p<0.05$). Different lowercase letters in the same column after the designated storage time (24 h) indicate significant difference ($p<0.05$).

3.7. Coalescence and flocculation

The coalescence index (C_i) and flocculation factor (F_f) of the coconut milk emulsions were investigated to determine the instability of the emulsion as shown in Table 3. Emulsions are thermodynamically unstable due to the unfavourable contact between oil and water (FREDRICK *et al.*, 2010) and their physical structures are likely to change over time by various mechanisms including coalescence and flocculation. In IMC, higher *Ci* was observed after 24 h, as compared with the MC and OMC samples ($p<0.05$). On the other

hand, F_f decreased, suggesting that the individual oil droplets assembled to form larger oil droplets as evidenced by the increase in droplet size. The increase in d_{43} also reconfirmed the assembly of individual droplets into larger flocs (INTARASIRISAWAT *et al.*, 2014). The formation of larger oil droplets indicates poor emulsion stability (FREDRICK *et al.*, 2010). The interactions between oil droplets depend on the quality and quantity of proteins (DAMODARAN, 2005). The proteins in IMC were plausibly not effective in stabilising the coconut milk emulsion, especially after the extended storage. However, the lowest rate of C_i was observed for OMC. This coincided with the lowest rate of changes in d_{32} and d_{43} of OMC. For OMC, initial pH close to pI might not favour the solubility of proteins and as such, it was presumed to have poor emulsifying property. Additionally, the partial crystallization of lipid within the oil droplets could be another factor that favours the destabilisation of emulsion by coalescence (ROUSSEAU, 2000). Nevertheless, the emulsion initially found was slightly altered upon storage time. The results suggested that the oil droplet size and emulsion stability of coconut milk depended on the maturity stages.

4. CONCLUSIONS

Coconut milk and meat at three different maturity stages had varying proximate compositions. Cocosin with MW of 55kDa was predominantly observed in coconut milks, regardless of the maturity stage. Polydisperse emulsion was observed in coconut milk at the mature and overlay mature stages, whilst the monodisperse counterpart was found in coconut milk of the immature stage. The stability of coconut milk emulsion depends on intrinsic factors, mainly pH and protein content. Thus, the maturity stages had influence on the physicochemical properties and emulsion stability of coconut milk. The present study has provided a better understanding of the impact of maturity stage on the characteristics and emulsion stability of coconut milk, used as the starting material for VCO production. OMC was more appropriate for the production of VCO with higher yield as compared with other stages.

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