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Structural and interfacial characteristics of oil bodies in coconuts (*Cocos nucifera* L.)

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ABSTRACT

This study investigated the structural characteristics of oil bodies from mature coconut (*Cocos nucifera* L.) fruit. The ultrastructure and the distribution of oil bodies in coconut endosperm were investigated using cryo-scanning electron microscopy. The interfacial characteristics of the oil bodies in suspensions isolated using two different protocols were studied using confocal laser scanning microscopy (CLSM), and the oleosins stabilizing the oil bodies were characterized using sodium dodecyl sulfate polyacrylamide electrophoresis. The oil bodies were found to be preferentially accumulated in endosperm tissues away from the inner endosperm and had a polydisperse size distribution, both intracellularly and in suspensions. The CLSM of oil bodies revealed uniform distribution of proteins and phospholipids at the interface along with glycolipids. Six different proteins were found to be associated with oil bodies some of which were disulfide-linked. This work provides new insights into the structure of coconut oil bodies and mechanisms for their stabilization.

1. Introduction

Recently, oil bodies, or oleosomes, from plants have received considerable attention because of their potential applications in the delivery of bioactive compounds (Acevedo et al., 2014; Boucher, Cengelli, Trumbic, & Marison, 2008; Nikiforidis, Matsakidou, & Kiosseoglou, 2014). They are present as discrete organelles within plant cells and their primary function is the storage of triacylglycerols (TAGs) (Tzen, Cao, Laurent, Ratnayake, & Huang, 1993; Tzen & Huang, 1992). Their diameter is about 0.6–2.0 μm in most oil seeds and up to 10–20 μm in some fruits (Ross, Sanchez, Millan, & Murphy, 1993; Tangsuphoom & Coupland, 2005). The structure of oil bodies consists of a core of up to 95% (w/w) TAGs that is stabilized by a 2–4 nm thick interfacial layer (Napier, Stobart, & Shewry, 1996). This layer is made up of phospholipids (up to 2% w/w) and proteins that are called oleosins (up to 8%, total proteins) (Huang, 1996). The detailed structure of oil bodies and the role of oleosins in the stabilization of oil bodies have been the subjects of several reviews (Huang, 1994; Murphy, 1990; Napier, Beaudoin, Tatham, Alexander, & Shewry, 2001; Napier et al., 1996; Tzen et al., 1993; Tzen & Huang, 1992).

Coconut (*Cocos nucifera* L.) is a fruit from a perennial plant that is grown in tropical countries, mainly for the production of coconut oil. In their natural state, the oil bodies containing the coconut TAGs remain embedded in the cells of solid endosperm, which is also referred to as coconut 'kernel' or 'meat' (Heathcock & Chapman, 1983). The oil bodies appear to be uniformly distributed throughout the kernel matrix but their size distribution within the cells is unknown. The aqueous oil body extract that is prepared by pressing grated coconut has a pH 6.2 and is known as 'coconut milk' (Seow & Gwee, 1997). The size of the oil bodies in freshly extracted coconut milk ranges from 2 to 25 μm and the oil bodies show minimal flocculation (Rodelas et al., 2008; Tangsuphoom & Coupland, 2008, 2009). At the natural pH of extraction, the oil body suspension in coconut milk has a net charge of −16 mV (Tangsuphoom & Coupland, 2009), which is due to the high number of glutamic acid residues in coconut proteins (Kwon, Park, & Rhee, 1996; Samson, Khaund, Cater, & Mattil, 1971).

The TAGs in coconut oil bodies are rich in saturated fatty acids; unsaturated fatty acids (mainly oleic $C_{18:1}$ and linolenic $C_{18:2}$) constitute < 5% (w/w) of the total fat (Appaiah, Sunil, Prasanth Kumar, & Gopala Krishna, 2014). Short- and medium-chain fatty acids form the bulk of the total fatty acids in coconut oil, with lauric (C_{12}) and myristic (C_{14}) acids being the most abundant (approximately 70% w/w of the total fatty acids) (Appaiah, Sunil, Kumar, & Krishna, 2015; Appaiah et al., 2014). The numerous health benefits that are associated with

Abbreviations: CLSM, confocal laser scanning microscopy; Con A, concanavalin A; POB, purified oil bodies; Rd-DHPE, rhodamine B triethylammonium salt; FG-FCF, fast green-FCF; SDS-PAGE, sodium dodecyl sulfate polyacrylamide electrophoresis; SEM, scanning electron microscopy; TAG, triacylglycerol; WC, washed cream; WGA, wheat germ agglutinin

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coconut oil are attributed to its high short- and medium-chain fatty acid content (Fernando et al., 2015; Garcia-Fuentes, Gil-Villarino, Zafra, & Garcia-Peregrin, 2002; Gil-Villarino, Garcia-Fuentes, Zafra, & Garcia-Peregrin, 1999). A small proportion of the coconut lipids (5%, w/w of the total fat) has been reported to be glycosylated with galactose (Krishnamurthy & Chandrasekhara, 1983). The polar lipids in coconut oil (1.5–2.0% w/w of the total fat) consist primarily of phospholipids, in particular phosphotidyl choline, phosphotidyl ethanolamine, and phosphotidyl inositol (Krishnamurthy & Chandrasekhara, 1983). Although the phospholipids are probably present in the interfacial layer that stabilizes the oil bodies, their distribution on the oil body surface is poorly understood.

The composition of the oleosins in coconut oil bodies was first investigated by Rodelas et al. (2008) who found that the coconut oleosins consist of two polypeptides with approximate molecular weights of 14.4 and 11 kDa. In comparison, Tangsuphoom and Coupland (2009) reported five different proteins to be associated with coconut oil bodies. The isoelectric point of coconut oil body oleosins was reported to vary from pH 4 to pH 10 (Rodelas et al., 2008). This is counter to the narrower isolectric range (pH 5–6.5) reported for oelosins in seed oil bodies, including maize and rapeseed (Tzen et al., 1993). This discrepancy in the number of oleosins and their isoelectric point reported previously may result from the different methods used for the separation and purification of oil bodies. Nevertheless, the distribution of the oleosins on the surface of coconut oil bodies and their characteristics have not yet been investigated.

In this study, we report the detailed microstructure of the coconut kernel matrix and the distribution of the oil bodies therein. The oil bodies were washed extensively and were investigated for distribution of oleosins and phospholipids on their surface. The effect of the washing steps on the distribution of the components of the interfacial layer is discussed. The physicochemical characteristics of coconut oil bodies are revisited and discussed in relation to the composition of the interfacial material and the underlying mechanisms that are responsible for the stability of the oil bodies. This is first such study investigating the interfacial characteristics of oil bodies from fruits and the aspects of coconut oil bodies reported in this work have not yet been explored.

2. Materials and methods

Unless otherwise stated, all chemicals were purchased from Sigma Aldrich Ltd. (St. Louis, MO) and the reagents were made up in Milli Q water. All experiments were repeated at least twice and the averages of the results from two or more individual experiments are reported.

2.1. Microstructure of coconut kernel by scanning electron microscopy (SEM)

Mature coconuts (product of Tonga), purchased from a local grocery store, were de-shelled and used for SEM imaging. SEM images of coconut kernel were recorded using a scanning electron microscope (Philips XL 30S FEG, Eindhoven, The Netherlands) equipped with an energy-dispersive detector and a cryo-unit (Gatan Alto 2500, Gatan, Abingdon, UK). Kernel sections of approximately 1 mm × 1 mm in size, either from the outer endosperm region under the testa or from the inner endosperm lining the water-filled cavity, were placed in two different orientations in the sample holder. This allowed the imaging of cells from two different dimensions, representing their side view and their cross section. The samples were allowed to freeze under liquid nitrogen slush and then transferred to the cryo-prep chamber under vacuum. The temperature of the sample during etching was −90 °C followed by sputter coating with gold for 240 s at 12 mA at a temperature < −120 °C. Images were recorded at 5 kV and a sample temperature below −180 °C.

2.2. Preparation of coconut milk and isolation of oil bodies

The testa of the coconut kernel was removed manually using a kitchen grater. The de-shelled and pared coconut kernel was then passed through a mincer to reduce its size. The minced coconut kernel contained: moisture 48.2% (w/w, vacuum drying, AOAC methods 990.19 and 990.20), total fat 38.2% (w/w, Mojonnier method, AOAC method 954.02), and protein 4.1% (w/w, Dumas method, AOAC method 968.06). The material that was removed during the paring contained fractions of kernel adhering to the testa layer; its composition was: total solids 41.6% (w/w), fat 35.2% (w/w), and protein 4.8% (w/w).

Potable soft water (20 °C) was added to the minced kernel at a ratio of 4:1 (minced coconut kernel:water) and mixed thoroughly. The mixture was then pressed in a pneumatic press under a force of 3.5 kg/cm^2 for 10 min. The resulting aqueous solution containing oil bodies was collected and preserved using sodium azide (0.02% w/w, in the final suspension). This oil body extract henceforth would be referred to as “coconut milk” in the manuscript. It was stored for a maximum of 4 days at 20 °C and was used for analysis without any further processing.

The oil bodies in the coconut milk were concentrated using two different methods. Firstly, a 20 mL sample of coconut milk was centrifuged at 10,000*g* for 20 min at 20 °C. The resulting oil bodies in the cream phase were washed by suspending them in phosphate buffer (10 mM, pH 7.5) followed by re-centrifugation. The washing step was repeated and the cream obtained after the second buffer wash was re-suspended in buffer and mixed with hexane (1:1) to remove any free oil that may have been released during the repeated centrifugation and wash steps. The suspension was re-centrifuged at 10,000*g* for 20 min at 20 °C and the resulting cream was suspended in 5 mL of phosphate buffer (10 mM, pH 7.5) and used for analyses. In further discussion, oil bodies that were concentrated and washed using this procedure are referred to as washed cream (WC), while the aqueous phase obtained after removal of the cream layer is referred to as the serum.

Secondly, the oil bodies were isolated using the method described by Tzen, Peng, Cheng, Chen, and Chiu (1997). In brief, 20 mL of coconut milk was mixed with 20 mL of 0.4 M sucrose and centrifuged at 10,000*g* for 20 min at 20 °C. The oil bodies separated in a cream layer, which was collected and suspended in phosphate buffer (5 mM, pH 7.5) containing 0.1% (w/w) Tween 20 and 0.2 M sucrose. The suspension was then diluted 1:1 using phosphate buffer (10 mM, pH 7.5) and centrifuged at 10,000*g* for 20 min at 20 °C. The cream layer obtained from this second centrifugation step was suspended in an aqueous solution of 9 M urea and allowed to mix thoroughly for 20 min. The urea suspension was diluted using phosphate buffer (10 mM, pH 7.5) at a ratio of 2:1 (suspension:buffer) and re-centrifuged at 10,000*g* for 20 min at 20 °C. The separated cream layer from this step was suspended in 20 mL of phosphate buffer (10 mM, pH 7.5) containing 0.6 M sucrose. Separate experiments to investigate the effect of individual washing steps were also carried out as above in which the oil bodies were washed either by aqueous solutions containing Tween 20 or 9 M urea. The suspension was then washed with hexane as described above and the resulting cream layer was re-suspended in 5 mL of phosphate buffer (10 mM, pH 7.5). The purified oil bodies that were concentrated and washed using this procedure are referred to as purified oil bodies (POB) in further discussion.

2.3. Structural characterization of oil bodies

2.3.1. Confocal laser scanning microscopy (CLSM)

The microstructure of oil body suspensions was investigated using CLSM and the method described by Gallier, Gordon, and Singh (2012). Oil body suspensions were diluted fivefold with phosphate buffer (pH 6.1); they were mixed with Nile Red (1 mg/mL in acetone, 1:50 v/v) to stain neutral lipids and with Fast Green FCF (1 mg/mL in Milli-Q water,

3:50 v/v) to stain proteins. The distribution of phospholipids on the oil body surface was investigated by staining the samples with the fluorescent head-group-labelled phospholipid analogue Lissamine™ rhodamine B, triethylammonium salt (Rd-DHPE, λ_{ex}: 560 nm, 1 mg/mL in chloroform, 1:20 v/v), lectin Alexa Fluor® 488 conjugate of concanavalin A (Con A, λ_{ex}: 488 nm, 1 mg/mL in 0.1 M sodium bicarbonate buffer, pH 8.3, 1:20 v/v), and lectin Alexa Fluor® 488 conjugate of wheat germ agglutinin (WGA, λ_{ex}: 488 nm, 1 mg/mL in phosphate buffer saline, pH 7.4, 1:20 v/v). All samples were observed using the 63 mm oil immersion objective lens of the confocal laser scanning microscope (Leica DM 6000B, Heidelberg, Germany) and the images were processed using the software ImageJ.

2.3.2. Sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE) to determine the oleosins associated with oil bodies

The proteins present on the oil body surface were extracted using the method described by Tzen and Huang (1992). A sample (1 mL) of coconut milk, WC, or POB suspension was mixed with 1 mL of diethylether and vortexed for 30 s. The tubes were then centrifuged at 13,600*g* for 10 min at 20 °C. The top ether layer was removed carefully; the bottom layer was washed again using 1 mL of diethylether followed by re-centrifugation and removal of the diethylether layer. Any residual ether was evaporated from the suspension by placing the tubes in a waterbath maintained at 37 °C for 10 min.

The samples were mixed with 1 mL of a solvent mixture consisting of chloroform and methanol (2:1) and vortexed for 30 s. The suspension was then centrifuged at 13,600*g* for 10 min at 20 °C and the intermediate layer was collected and suspended in 250 μL of water. An aliquot (750 μL) of the solvent mixture was added and the samples were centrifuged at 13,600*g* for 10 min at 20 °C. The interfacial layer was collected and suspended in 0.5 mL of water.

The extracts were mixed with SDS-PAGE sample buffer at ratios of 1:20 for coconut milk and 1:1 for WC and POB. For reducing conditions, the PAGE buffer contained 200 mM dithiothreitol and the samples were reduced by heating at 56 °C for 10 min. After preparation, the samples were centrifuged at 16,000*g* for 20 min and the subnatant phase was stored at 4 °C until analysis.

The samples were analyzed by the PAGE protocol described by Manderson, Hardman, and Creamer (1998), which was based on a method described previously (Laemmli, 1970). Aliquots (10–15 μL) of the samples were loaded on to Mini-Protean gels prepared in-house and run at a constant voltage of 180 V. The gels were stained using 0.3% (w/v) Coomassie brilliant blue R and the final destained gels were scanned using a molecular imager Gel Doc XR system (Bio-Rad Laboratories, CA) and the images were analyzed using ImageLab software.

2.4. Physicochemical characterization of oil bodies

2.4.1. Droplet size of oil bodies in coconut milk

The average size of the oil bodies in coconut milk was characterized using a Malvern MasterSizer 2000 Hydro MU (Malvern Instruments Ltd., Malvern, UK). A freshly prepared coconut milk sample and washed oil body suspensions were diluted 1:50, in either water or 1% (w/v) SDS to determine the extent of flocculation (Tangsuphoom & Coupland, 2008). The refractive indices of coconut oil and water used in the measurement were 1.456 and 1.33 respectively. Preliminary experiments revealed that dilution of the samples either in water or in buffer (10 mM, phosphate buffer, pH 6.1) did not affect the particle size (results not shown). In addition, there was no change in the droplet size at different dilutions (Fig. S1. See Supporting Information). The volume-mean diameter ($d_{4,3}$) and the Sauter-average diameter ($d_{3,2}$) were recorded at a laser obscuration of between 10 and 15%. Mean particle diameters were calculated from triplicate measurements from three independent coconut milk extracts.

2.4.2. Charge on oil bodies in coconut milk

The ζ-potentials of the oil bodies and serum proteins were measured using a Malvern Zetasizer Nano ZS instrument (Malvern Instruments Ltd., Malvern, UK) equipped with a 4 mW He/Ne laser with an output wavelength of 633 nm. The samples were diluted 1:25 (v/v) using 10 mM phosphate buffer (pH 6.1). They were filtered using 0.45 μm syringe filters, placed in the electrophoresis cell (DTS 1060C, Malvern Instruments Ltd., Malvern, UK), and allowed to equilibrate at 25 °C for 2 min. The refractive indices of coconut oil and water were 1.456 and 1.33, respectively, and the ζ-potential was computed using the Smoluchowski approximation according to Henry's law. An average ζ-potential value was calculated from 10 runs of duplicate measurements on three independent coconut milk extracts.

2.5. Characterization of lipids in coconut oil bodies

The TAGs in coconut oil bodies were extracted using the method described by Bligh and Dyer (1959). The phospholipids in the extract were separated from the neutral lipids by solid phase extraction using the method described by Avalli and Contarini (2005). A 10 mg/mL extract from the Bligh and Dyer fat extract in chloroform was loaded on to a HybridSPE® ultra cartridge (bed weight 30 mg, Sigma Aldrich Ltd, St. Louis, MO) pre-equlibrated with chloroform. The neutral lipids were washed-off the cartridge with 6 mL hexane, 3 mL hexane diethyl ether (8:2, v/v) mixture and finally again with 3 mL hexane diethyl mixture (1:1, v/v). The phospholipids retained on the cartridge were eluted by 4 mL methanol followed by 2 mL methanol plus 2 mL mixture consisting of chloroform methanol and water (3:5:2, v/v). The solvent fractions containing phospholipids from several runs were pooled before analysis. The solvents from both the neutral lipid as well as phospholipid fractions were evaporated and the dried samples were used for total fatty acid analysis by GC. The concentration of phospholipids in the Bligh and Dyer fat extract was 10 ± 0.2 mg/g of fat extract.

The fatty acid composition (free and ester forms) in coconut oil bodies was determined using the method described by Zhu, Ye, Verrier, and Singh (2013). A sample (10–20 mg) of the coconut fat from the Bligh and Dyer extraction was used for the analysis with methyl nonadecanoate (1 mg/mL) as an internal standard. The fatty acid composition of mono-, di-, and triglycerides in unfractionated fat was analyzed separately by methylation and extraction of these fatty acids. The conditions for the methylation of the fatty acids and their esters, their extraction, and their analysis by capillary gas chromatography and calculations of free fatty acids were as described by Zhu et al. (2013). The sterol composition in the coconut kernel was analyzed at Assure Quality Ltd. (Auckland, New Zealand) using the method of Laakso (2005).

3. Results

3.1. Microstructure of coconut kernel by Cryo-SEM

The distribution of oil bodies in the coconut kernel matrix was investigated by cryo-SEM imaging in two different regions: up to 1 mm depth from the testa and the inner endosperm surface lining the water-filled cavity. The testa layer of the coconut (T, Fig. 1A) was approximately 50 μm thick and had the typical appearance of lignified tissue. The cells in the kernel below the testa layer had a rigid cell wall that imparted a symmetrical shape to the cells, which were aligned in the direction perpendicular to the plane of the testa. No oil bodies could be identified either in the testa layer or in the tissues immediately beneath the testa layer.

Cells rich in oil bodies could be seen distinctly in the tissue approximately 150–200 μm below the surface (white arrows, Fig. 1A and 1B). These cells were approximately 50–100 μm in length and 50 μm in diameter (Fig. 1C and D) and appeared to overlap with adjacent cells. The oil bodies appeared to be largely spherical with diameters varying

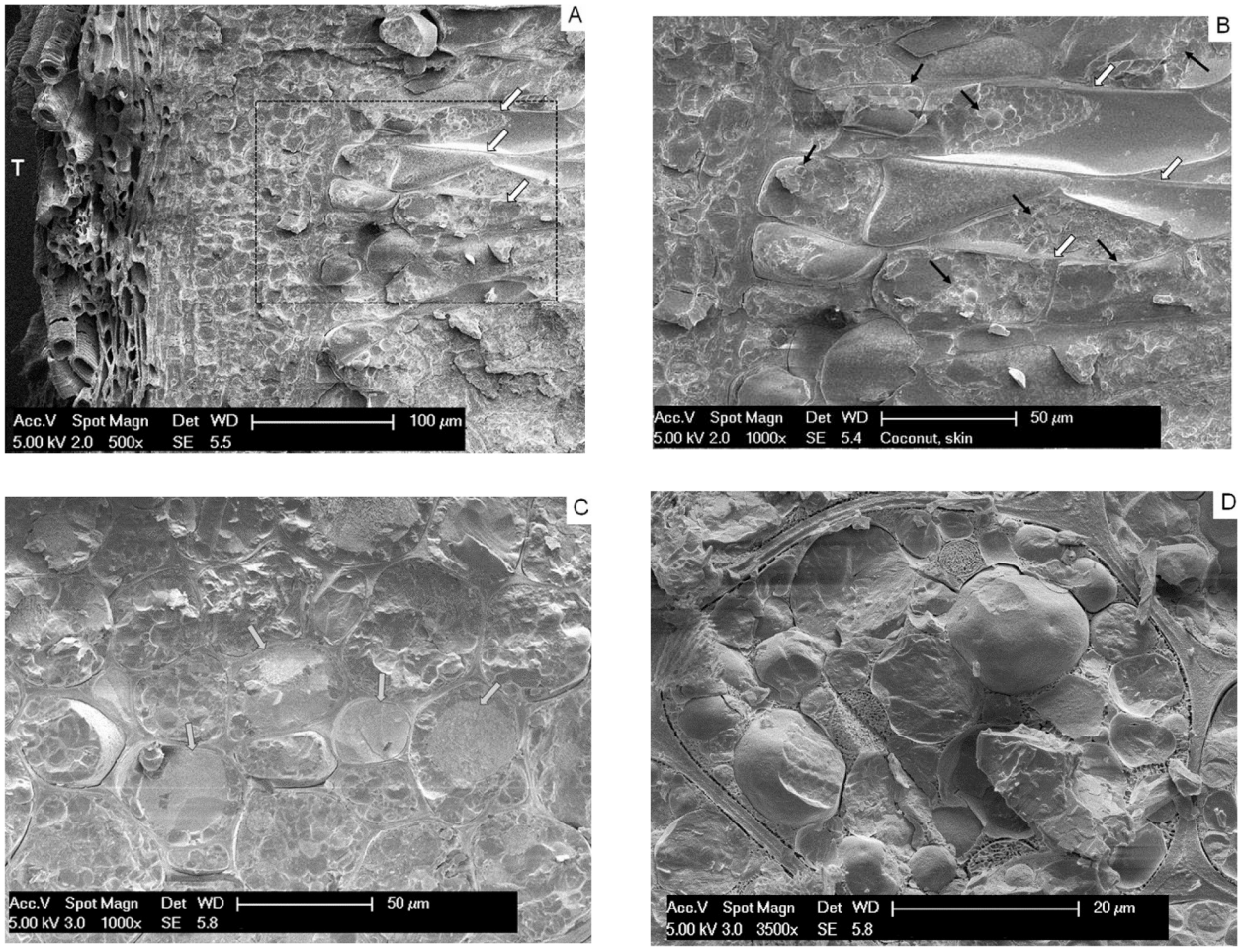

Fig. 1. Cryo-SEM images of the outer endosperm tissues adjacent to the testa. The plane of imaging is parallel (A and B) and perpendicular (C and D) to the testa layer. The region highlighted in the rectangular area in A is imaged at a higher magnification in B. For description of the arrows in A, B, and C, see the text.

from 1 to 20 μm (black arrows in Fig. 1B and Fig. 1D) with minor asymmetries that were probably caused by the oil bodies being pressed against each other and the cellular components. In addition, the cross section of the cells also revealed an asymmetric intracellular distribution of the oil bodies, with some cells devoid of any oil bodies in the plane of imaging (grey arrows, Fig. 1C and Fig. S2, see Supporting Information).

The relative abundance of the oil bodies was observed using three-dimensional CLSM imaging of a relatively thicker (1 mm) section of the kernel from the same region; this section was stained with a liphophilic dye, Nile Red. However, the free oil and oil bodies released from the kernel during the preparation of the sections and during imaging interfered with the imaging. Nevertheless, the oil bodies showed a polydisperse size distribution in the cells and appeared to be irregular (Supporting Information, Fig. S3), probably because they were pressed against cellular organelles and other surrounding oil bodies, consistent with the SEM images.

The microstructure of the tissue of the inner endosperm layer (Fig. 2) that lined the water-filled cavity of the coconut appeared to be significantly different from that of the tissue in the outer endosperm region near the testa. The cells of the inner endosperm lining the cavity (L, Fig. 2A) appeared to be flexible and amorphous without any defined structure. They were 50–100 μm in length with various diameters (Fig. 2B and C) and their intracellular ultrastructure was consistent with that reported for an aqueous matrix that is rich in non-lipid solutes, such as sugars (Gerschenson, Rojas, & Marangoni, 2001). The proportion of oil bodies in the cells was significantly lower and the oil bodies, when present, had a narrow size distribution with their approximate size being < 5 μm (white arrows, Fig. 2D).

3.2. Physicochemical characterization of coconut milk

Under the conditions of extraction, the recovery of fat and protein were approximately 50% and 60% w/w respectively. The composition of coconut milk is shown in Table S2 (see Supplementary information). The particle size distribution of the oil bodies in freshly extracted coconut milk is shown in Fig. 3. The oil bodies in coconut milk had a polydisperse size distribution and showed very little flocculation. The $d_{4,3}$ and the $d_{3,2}$ for the oil bodies were found to be 18.9 ± 1.7 and 9.3 ± 0.5 μm, respectively. These values did not change in the presence of 1% SDS, with $d_{4,3}$ and $d_{3,2}$ being 18.02 ± 1.6 and 8.2 ± 1.1 μm, respectively, indicating that the oil bodies in freshly prepared coconut milk were largely free of flocculation. In comparison, a slight increase in the oil body size ($d_{4,3}$) were observed in WC (36 ± 1.1 μm in water; 21.7 ± 0.1 μm 1% w/v SDS) and POB (31.7 ± 2.6 μm in water; 22.1 ± 0.2 μm in 1% w/v SDS).

The ζ-potential of oil bodies in coconut milk at its extraction pH (6.1) was −13 ± 1 mV. In comparison, the ζ-potential of oil bodies in WC and POB were −32.6 ± 1.8 and −33.8 ± 0.4 respectively at pH 7.5. Freshly prepared coconut milk showed spontaneous phase separation within a few minutes of extraction, partitioning into a small layer of sediments at the bottom, a middle clear layer consisting mainly of proteins, and a milky-white oil-body-rich top layer.

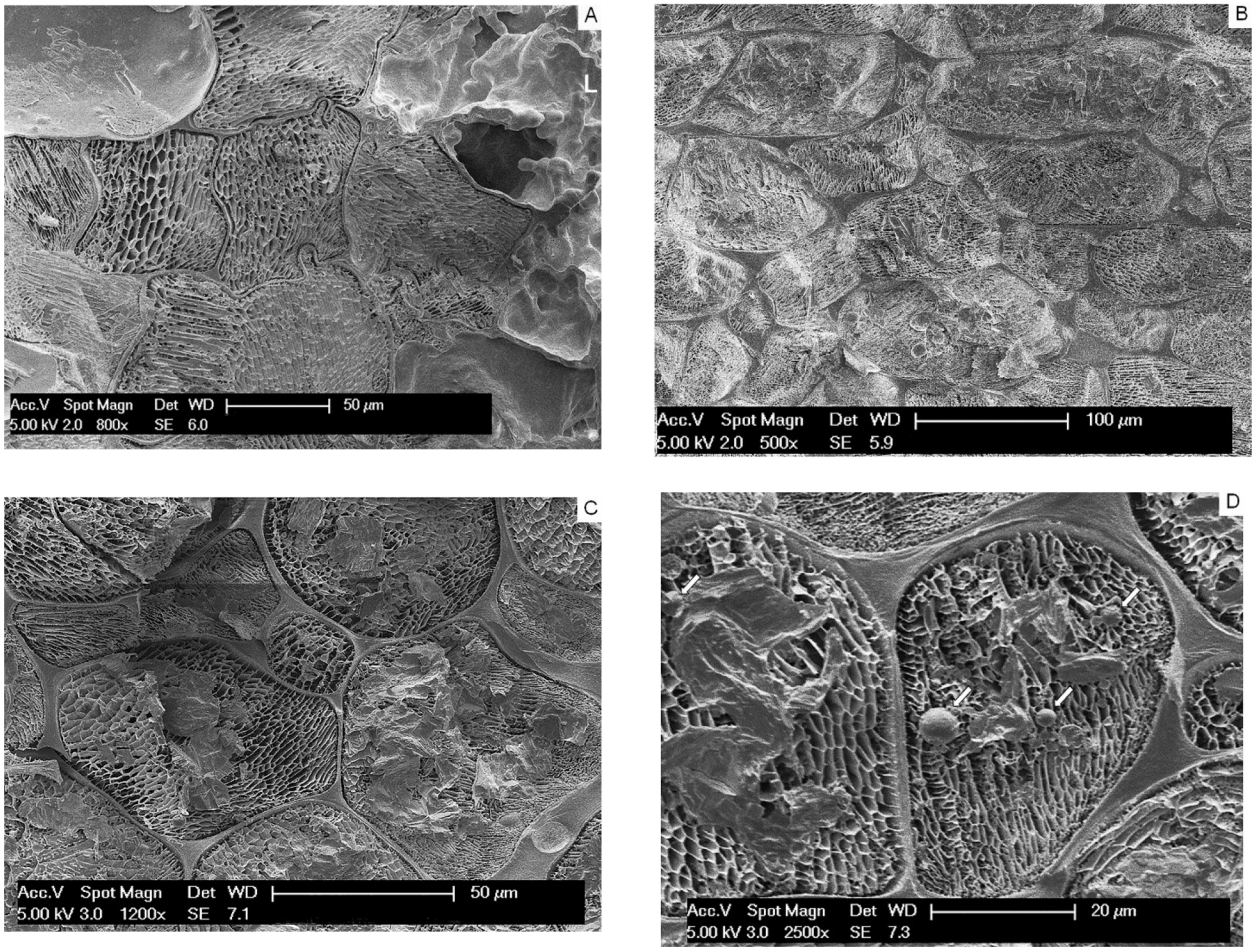

Fig. 2. Cryo-SEM images of tissues in the inner endosperm region lining the water-filled cavity of coconut. The plane of imaging is perpendicular (A and B) and parallel (C and D) to the lumen layer.

3.3. Structural characterization of coconut oil bodies

3.3.1. Microstructure of coconut oil bodies by CLSM

The structural characteristics of the oil bodies were investigated using CLSM. The diameter of the oil bodies ranged from 1 to 30 μm, indicating a polydisperse size distribution, in agreement with the light scattering data. The dye Nile Red exhibits intense red fluorescence in an organic phase that is rich in neutral lipids and hence acts as a hydrophobic probe (Greenspan, Mayer, & Fowler, 1985). The oil bodies showed a large core region exhibiting red fluorescence, indicating that this region was rich in neutral lipids or TAGs.

The distribution of proteins on the oil body surface was investigated by staining using Fast Green FCF (FG-FCF). The dye FG-FCF binds to proteins electrostatically, resulting in a fluorescent green complex. The oil bodies showed a uniform layer of proteins on the surface (Fig. 4B, D, and F). In the aqueous phase, some protein flocs appeared to loosely adhere to the oil bodies, but their proportion was relatively small. The oil bodies in WC and POB showed similar staining with Nile Red and FG-FCF (Fig. 4C–F), indicating that the repeated washing steps had minimum effect on the integrity and the surface proteins of the oil bodies.

The lateral distribution of phospholipids on the oil body surface was investigated by staining the oil bodies with the phospholipid analogue Rd-DHPE, that binds to phospholipids resulting in an intense red fluorescence. The oil bodies, irrespective of their size, showed uniform Rd-DHPE fluorescence intensities (Fig. 5A, C, and E), suggesting a uniform distribution of the phospholipids on the oil body surface. The Rd-DHPE fluorescence intensities of washed samples (WC and POB) were unaffected (Fig. 5C and E), indicating that the relatively severe conditions (POB) of washing with 0.1% (w/w) Tween 20 and 9 M urea solutions did not affect the distribution of phospholipids on the surface of the oil bodies.

The lectin conjugates Con A and WGA were used to investigate details of the surface characteristics of the oil bodies. Con A selectively binds to the α-manopyranosyl and α-glucopyranosyl residues on proteins/lipids and results in a fluorescent green complex, while WGA has the affinity to bind to *N*-acetyl-glucosamine and sialic acid residues in glycoproteins. When stained with Con A, the oil bodies in coconut milk showed green fluorescence on their surface (Fig. 5B). In comparison, when stained with WGA, they showed the absence of any staining.

The fluorescence intensities of Con A in WC and POB were similar to those in coconut milk (Fig. 5D and F), implying that the glycosyl groups in the interfacial layer were largely unaffected by the washing of the oil bodies. However, no fluorescence was observed when the oil bodies in WC and POB were stained using WGA, indicating that glycoproteins with *N*-acetyl-glucosamine and sialic acid residues were absent at the interface (results not shown) in line with results of unwashed oil bodies in coconut milk.

3.3.2. Composition of lipids by gas chromatography

The total fatty acid composition of the TAGs and phospholipids extracted from the oil bodies, as determined by gas chromatography, is shown in Table S2 (see, Supplementary Information). The neutral TAGs in oil bodies contained a high proportion of medium-chain fatty acids (C_6–C_{12}, approximately 68%) and saturated fatty acids (94% w/w of total fatty acids) with lauric and myristic acids accounting for nearly

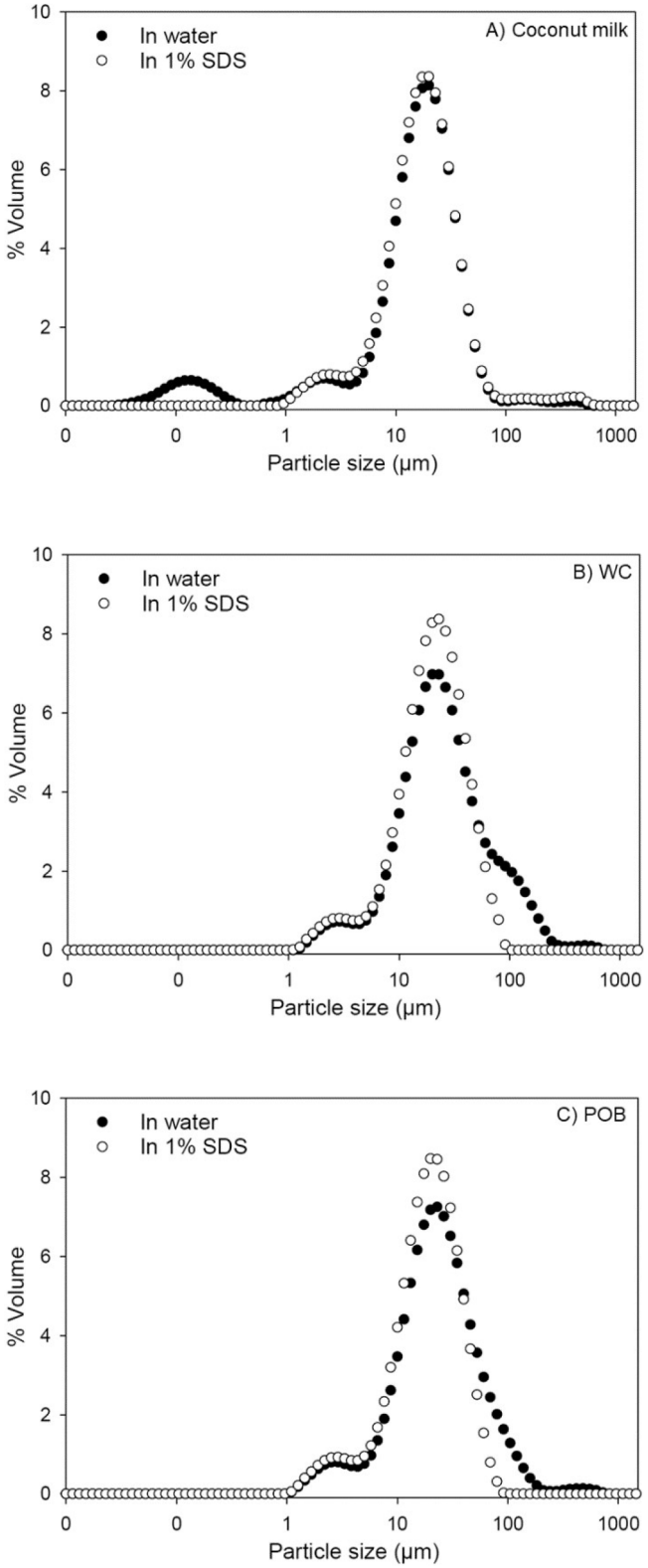

Fig. 3. Particle size distribution in oil body suspensions. (A) Coconut milk, (B) WC and (C) POB. Samples were diluted 1:50 in either water or 1% SDS. Data are representative of particle size distributions from two separate extractions.

70% of the total fatty acids. The main unsaturated fatty acids in the neutral TAGs were oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids, although their proportion was approximately 7.5% (w/w, total fatty acids). The fatty acid composition of phospholipids followed a similar trend but had higher levels of palmitic (C16:0, 13.6% w/w, total fatty acids) and oleic acid (14%, w/w total fatty acids). Overall, the total fatty acid composition of coconut oil bodies agrees with previous studies (Appaiah et al., 2014). The free fatty acid content in coconut milk was very low (Fig. S4, see Supporting Information), indicating a high stability of coconut fat.

The sterol profile of pared coconut kernel before pressing revealed β-sitosterol (15 mg/100 g) to be the major sterol in coconuts. The levels of other sterols (cholesterol, brassicasterol, ergosterol, campesterol, and stigmasterol), if present, were probably below the detection limit of the method (10 mg/100 g).

3.3.3. Oleosins associated with oil bodies: SDS-PAGE

To identify the oleosins in coconut oil bodies, samples of coconut milk and oil-body-rich fractions derived from coconut milk upon centrifugation were analyzed by SDS-PAGE (Fig. 6). A fresh coconut milk sample showed > 15 bands with molecular weights between 10 and > 250 kDa (Fig. 6A and B, lane M). Several of these polypeptide bands (regions G1, G2, and G3; Fig. 6A, lane M) were disulfide-linked (regions H1, H2, and H3, Fig. 6B) indicating that, in the fresh unprocessed coconut milk, the extracted proteins showed extensive disulfide bonding.

Upon centrifugation, most of the polypeptide bands noted in the PAGE of coconut milk were retained in the subnatant fraction. This fraction showed the absence of oil bodies when checked by light microscopy (results not shown). In their native state (pH 6.1), the proteins in the subnatant remained largely un-aggregated and had a net charge of −10 mV. The most prominent bands in the subnatant fraction were those corresponding to approximate molecular weights of 50, 25, and 15 kDa. In contrast, the WC showed distinct peptide bands in regions G1 and G2 (Fig. 6A), which were absent in the subnatant fraction, indicating that the polypeptides in these bands may have been associated with oil bodies. Under reducing conditions, WC showed 10 polypeptide bands between 10 and 75 kDa (Fig. 6B), with the most prominent bands at approximately 50 kDa, two bands between 25 and 37 kDa, and one band between 10 and 15 kDa. Further, most of the peptide bands in regions H1 and H2 showed disulfide bonding.

A more extensive washing procedure resulted in the loss of some of the polypeptides from the POB sample. Distinct differences in the protein bands of POB and WC could be identified in regions G2 and H2 (Fig. 6A and B, respectively). Under non-reducing conditions, at least three bands identified in WC were absent in POB (region G2, Fig. 6A); under reducing conditions, around five bands that were present in WC were absent in POB (region H2, Fig. 6B). This suggests that some of the proteins (> 15 kDa) that were associated with the oil bodies in WC were removed during the extensive steps of washing with 0.1% (w/w) Tween 20 and 9 M urea solution, indicating that these proteins were loosely bound to the surface of the oil bodies. Moreover, at least one protein band in region H1 and two protein bands in region H2 (Fig. 6B) in the POB sample showed disulfide bonding, indicating that these proteins were linked by disulfide linkages in native oil bodies.

The most prominent band amongst the protein bands of POB was that corresponding to a molecular weight of approximately 14 kDa. The intensity and the position of this band were consistent for both WC and POB, irrespective of the analysis conditions (reducing versus non-reducing). Interestingly, this band was difficult to identify in the coconut milk sample. Its absence may be explained by the low overall concentration of these proteins in coconut milk.

4. Discussion

This study has elucidated, for the first time, the distribution of oil bodies in the matrix of coconut kernel. The oil bodies appeared to be accumulated in cells of the outer endosperm whereas the inner endosperm tissue lining the central cavity was devoid of any oil bodies. This is contrary to the findings of Heathcock and Chapman (1983), who, using birefringence measurements, reported the coconut lipids to be uniformly distributed in tissues throughout the kernel matrix (from

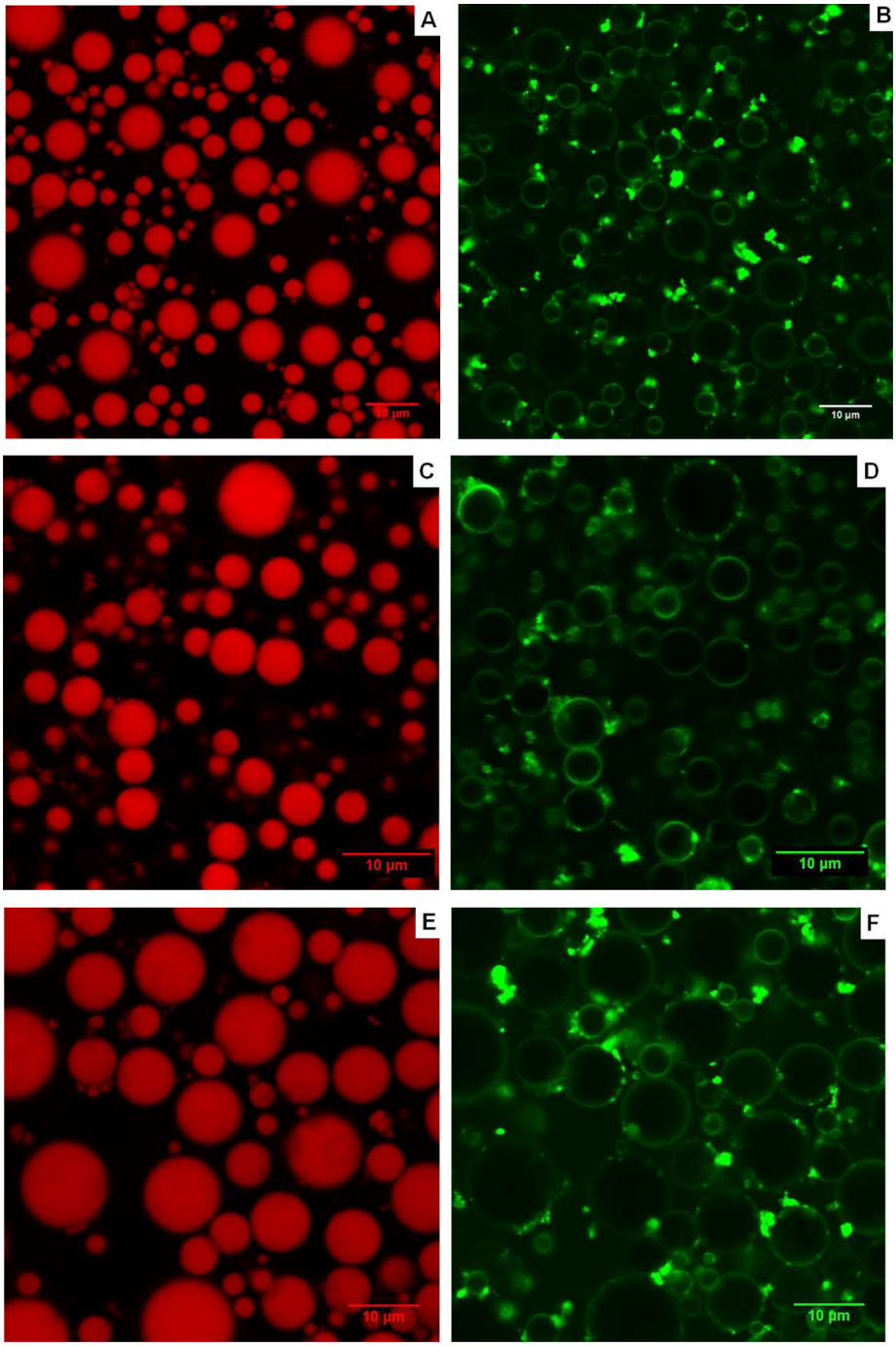

Fig. 4. CLSM images of oil bodies in coconut milk (A and B), WC (C and D), and POB (E and F). Oil bodies are stained red by Nile Red (A, C, and E) and proteins are stained green by Fast Green FCF (B, D, and F). The images are representative of coconut milk from three individual extractions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the testa to the inner endosperm lining the cavity). It is possible that slicing thin sections for birefringence measurements, in the previous study, may have released the oil bodies from the cells, which resulted in a continuous birefringence signal along the kernel matrix. It is also possible that the non-lipid material in the cells also contributed to the birefringence noted by these authors. Freezing of the samples for cryo-SEM imaging in our study allowed fixation of the oil bodies and the tissue components, minimizing any interference from expressed oil bodies or cellular components.

The ultrastructure of coconut kernel showed that the oil bodies appeared to be preferentially accumulated in specific regions of the cells (Fig. 1A and B and grey arrows in Fig. 1C). This suggests that the distribution of the oil body regions was asymmetric along the long axis of the cell. It is likely that the intracellular region that was devoid of any oil bodies consisted of other cell components and that this region overlapped with those that were rich in oil bodies.

Appaiah et al. (2014) compared the proximate compositions of coconut testa and coconut kernel and noted that they were remarkably similar. Indeed, in our study, the compositions of these fractions, when separated during the manufacture of coconut milk, were found to be similar, despite the testa layer not appearing to contain any oil bodies (Fig. 1A). This is likely to be because the cells that were rich in oil bodies in the kernel were located very close (< 200 nm) to the testa layer. It is possible that the intermediate layers below the testa, rich in

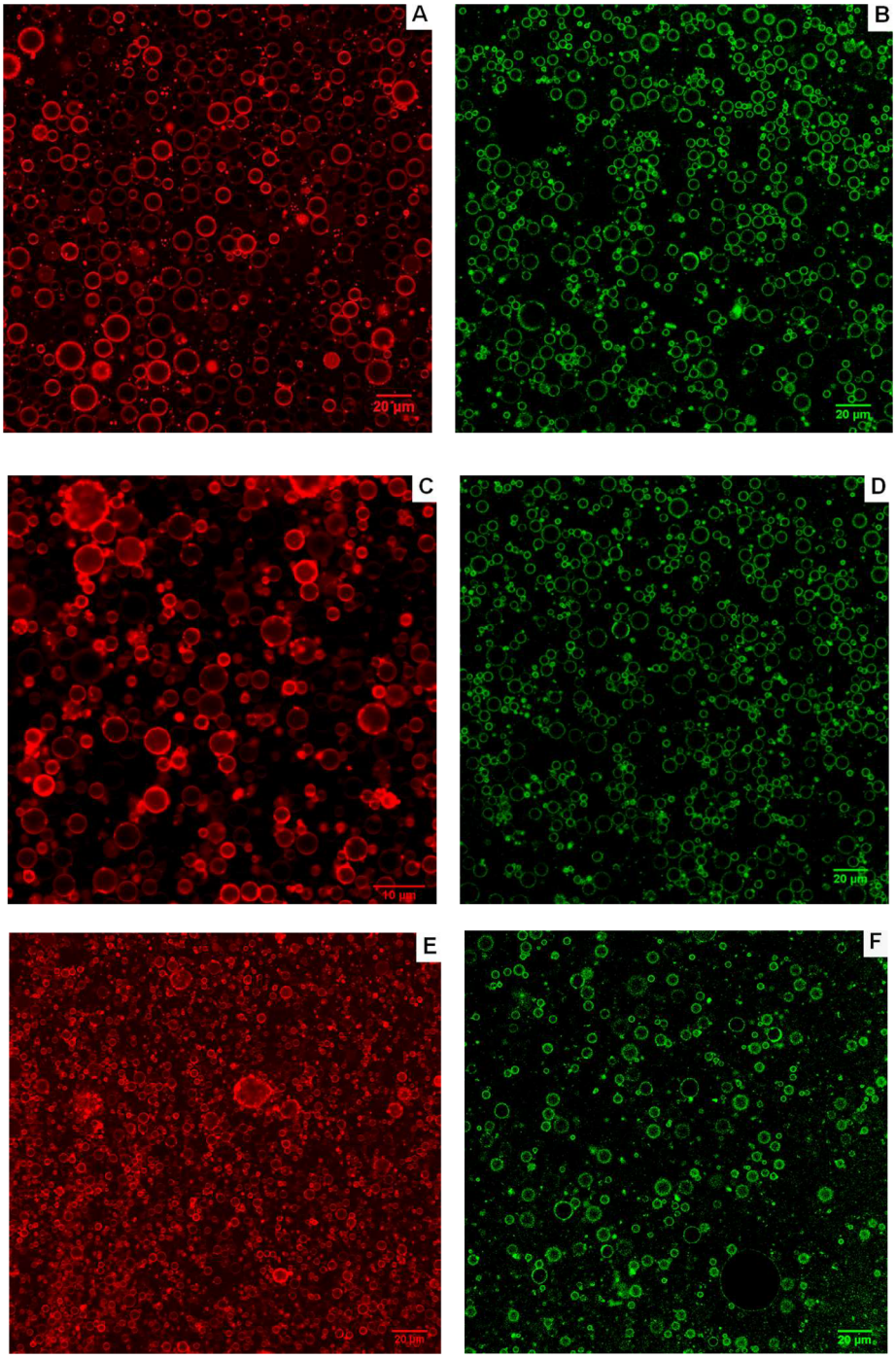

Fig. 5. CLSM images showing the interfacial distribution of phospholipids and glycosyl groups on the interface of oil bodies in coconut milk (A and B), WC (C and D), and POB (E and F). Phospholipids are stained red by Rd-DHPE (A, C, and E) and the distribution of glycosyl groups is stained red by binding to Con A (B, D, and F). The images are representative of coconut milk from three individual extractions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

oil bodies, were removed concurrently during paring, which contributed to the higher fat content in the testa.

The physicochemical characteristics of the oil bodies, including the composition of the TAGs, were largely similar to those reported in previous studies (Appaiah et al., 2014; Tangsuphoom & Coupland, 2005, 2008), in this study. The proportion of palmitic ($C_{16:0}$) and oleic acids ($C_{18:1}$) in the total fatty acid composition of phospholipids was twice as that in neutral TAGs. The sterol content in oil bodies was slightly lower than those previously reported, which may be explained by the difference in the variety of the coconut used.

The oil bodies in the coconut milk had a polydisperse size distribution and showed little flocculation. However, repeated centrifugation during washing resulted in slight coalescence of the oil bodies in WC and POB (up to 20% increase in $d_{4,3}$) probably due to the compaction of oil bodies by centrifugal force during multiple wash steps.

The CLSM images of the oil body extracts revealed previously unexplored aspects of oil bodies. The interfacial layer covering the oil bodies consisted of both oleosins and phospholipids. The surface covering of phospholipids was uniform without any liquid-ordered domains, as reported for the fat globules of bovine milk (Gallier, Gragson, Jiménez-Flores,

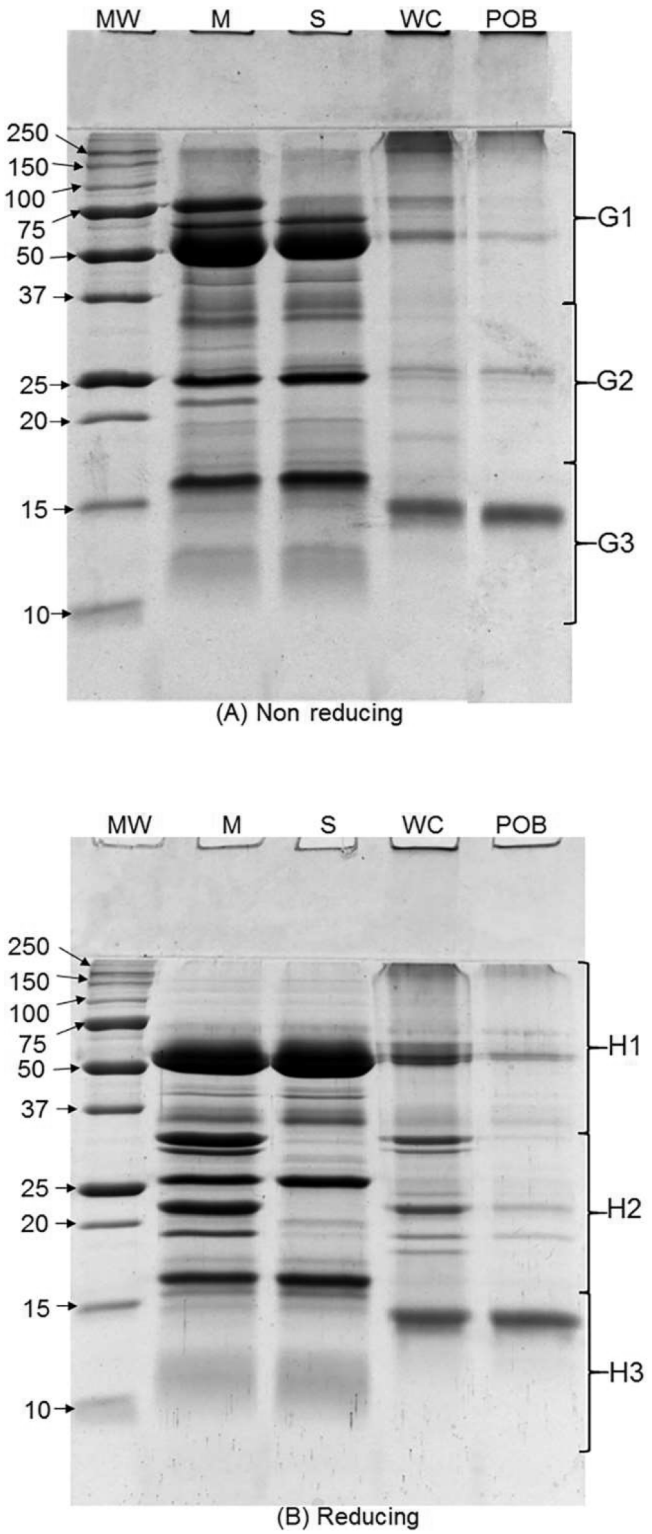

Fig. 6. SDS-PAGE profile of coconut milk and its fractions obtained by centrifugation. The samples were analyzed under either (A) non-reducing or (B) reducing conditions. MW, molecular weight marker, weights in kDa; M, coconut milk; S, serum obtained after removing the cream layer upon centrifugation; WC, washed cream; POB, purified oil bodies.

& Everett, 2010). The absence of these domains may be explained by the absence of cholesterol and sphingomyelin (Gallier et al., 2012) in coconuts. The surface of the oil bodies revealed the presence of α-manopyranosyl and α-glucopyranosyl groups. Galactosylated glycolipids have been reported previously in coconut oil bodies (Krishnamurthy & Chandrasekhara, 1983) and it is likely that these groups originated from glycolipids.

The oil bodies showed a uniform layer of proteins that were free of any *N*-acetyl-glucosamine and sialic acid residues on the oil body surface. The relative thickness of the protein stained layer appeared to be thinner than that reported for almond oil bodies in our previous study (Gallier et al., 2012). Indeed, the SDS-PAGE analysis of the oil bodies revealed that multiple proteins were present (WC and POB). These proteins that were associated with the oil bodies could be grouped into two categories based on how strongly they were bound to the oil bodies. At least six different proteins (WC) appeared to be loosely bound by non-covalent interactions and these proteins could be dislodged from the surface by washing with Tween 20 and urea solutions.

The displacement of surface proteins by Tween 80 (0.5–2% w/v, after 24 h) was observed by Nikiforidis and Kiosseoglou (2011) in maize seed oil bodies. However, in our study, the effect of Tween 20 wash alone on desorption of proteins from the oil body surface was minimum (Fig. S5, See Supporting Information). This could be explained by the slower rate of displacement of proteins by Tween (Nikiforidis & Kiosseoglou, 2011) and it is likely that the low concentration of Tween 20 (0.1% w/v) and the short time scales (30 min) of washing used during the preparation of POB, were insufficient for any significant desorption of proteins form the oil body surface.

Nikiforidis and Kiosseoglou (2011) attributed the interfacial proteins displaced by Tween 80 to be caleosins and steroleosins. These proteins have shorter patches of hydrophobic sequences that can interact with hydrophobic lipid core (Lin & Tzen, 2004; Shimada & Hara-Nishimura, 2010) implying that the hydrophobic interactions keeping these proteins adsorbed on to the oil bodies are relatively weak. It is uncertain whether the proteins dislodged during the washing steps in the preparation of POB in our study belonged to these groups of proteins. Their desorption by urea suggests that these proteins were bound to the oil body surface by non-covalent interactions mainly hydrophobic interactions and hydrogen bonding.

The oil bodies showed a second group of proteins that were unaffected by the harsh washing steps of purification of the oil bodies. From the SDS-PAGE results, the oil bodies showed a preferential accumulation of a protein band corresponding to an approximate molecular weight of 14 kDa in oil-body-rich samples (Fig. 6, WC and POB). The molecular weight of this band corresponded to the broader range of oleosins (15–26 kDa) found in a diverse group of oil seeds (Huang, 1996; Tzen et al., 1993) and was similar to that reported for coconut oleosin (Rodelas et al., 2008). It is possible that the polypeptides in this band had large patches of hydrophobic sequences, which interacted with the hydrophobic core and contributed to the anchor regions of oleosins noted in the classic structure of oil bodies (Huang, 1994). Preliminary studies (results not shown) on characterization of sequences in this band using in-gel digestion by trypsin followed by tandem mass spectrometry analysis of resulting peptides yielded very few peptides suggesting that the polypeptides in this band may contain sequences devoid of any charged residues (Lysines and Arginines), a classic feature of oleosins.

It can also be concluded from Fig. 6 that the coconut oleosins consisted of at least two distinct protein groups, that could be distinguished based on disulfide bonding. This is in contrast to the results of Rodelas et al. (2008), who reported only two protein bands with approximate molecular weights of 14.4 and 11 kDa in the SDS-PAGE of coconut oleosins. This anomaly can be explained based on differences in the method of oil body purification and the variety of coconut used in the two studies. In our study, the oil bodies were washed extensively using Tween 20 and urea solutions, which is expected to remove any proteins that are loosely bound to the oil body surface by non-covalent interactions without affecting their integrity (see Fig. 4D and F). Nevertheless, the difference may also result from variability in the oleosin composition within the same species.

An interesting question that arises here is whether the oil bodies

with different sizes differed in their composition of interfacial materials (distribution as well as type). Napier et al. (2001) proposed that the limiting factor contributing to the size of oil bodies is the oleosin content that is available for stabilization. Although this finding correlates well for oil bodies from different species of oil seeds that vary greatly in size, it is not known whether this is applicable to oil bodies from the same species. However, the CLSM results showed a uniform distribution of phospholipids and proteins on the surface of oil bodies of different sizes, suggesting that the interfacial compositions of the oil bodies were similar, irrespective of their size. Nevertheless, further experiments are necessary to test this hypothesis.

From the close packing of the oil bodies seen in the SEM images (Fig. 1B–D), it may be tempting to conclude that the larger oil bodies are formed by the coalescence of smaller oil bodies. However, the co-existence of distinct oil bodies of various sizes in the SEM images (Figs. 1 and 2) and the uniform staining of interfacial components in aqueous oil body solutions (Figs. 4 and 5) indicate that the oil bodies may be relatively stable in mature coconut fruit and that any coalescence after their expression may be minimal. The biogenesis of TAGs occurs in the endoplasmic reticulum and the interfacial materials (oleosins and phospholipids) are incorporated into the structure in the phospholipid bilayer of the endoplasmic reticulum under reducing conditions (Napier et al., 1996). Once expressed, the oil bodies in cytoplasm may be stabilized against coalescence by steric hindrance by the oleosins and a high net negative charge arising from the interfacial components (Tzen et al., 1993). It is possible that oil bodies of different sizes are produced at different stages of maturity of the fruit. A systematic study investigating the oil bodies in coconut fruit at different stages of maturity will give more insight into the reasons for the polydispersity in their size.

5. Conclusions

The structural features of coconut oil bodies elucidated in this study indicate that the oil bodies originating from in the fruit endosperm may be essentially possess similar structural features to those originating in seeds (Tzen et al., 1993). A more detailed study investigating the molecular distribution of the interfacial components on the oil body surface is currently underway. Nevertheless, the identification of biomaterials on coconut oil body interface i.e. proteins, phospholipids, disulfide linkages and glycosyl groups opens new avenues of research into their interactions for improving the functional properties of oil bodies.

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Conflicts of interest

Authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2018.09.125.

References

Acevedo, F., Rubilar, M., Jofré, I., Villarroel, M., Navarrete, P., Esparza, M., ... Shene, C. (2014). Oil bodies as a potential microencapsulation carrier for astaxanthin stabilisation and safe delivery. *Journal of Microencapsulation, 31*(5), 488–500.

Appaiah, P., Sunil, L., Kumar, P., & Krishna, A. (2015). Physico-chemical characteristics and stability aspects of coconut water and kernel at different stages of maturity. *Journal of Food Science and Technology (New Delhi, India), 52*(8), 5196–5203.

Appaiah, P., Sunil, L., Prasanth Kumar, P. K., & Gopala Krishna, A. G. (2014). Composition of coconut testa, coconut kernel and its oil. *Journal of the American Oil Chemists' Society, 91*(6), 917–924.

Avalli, A., & Contarini, G. (2005). Determination of phospholipids in dairy products by SPE/HPLC/ELSD. *Journal of Chromatography A, 1071*(1–2), 185–190.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*(8), 911–917.

Boucher, J., Cengelli, F., Trumbic, D., & Marison, I. W. (2008). Sorption of hydrophobic organic compounds (HOC) in rapeseed oil bodies. *Chemosphere, 70*(8), 1452–1458.

Fernando, W. M. A. D. B., Martins, I. J., Goozee, K. G., Brennan, C. S., Jayasena, V., & Martins, R. N. (2015). The role of dietary coconut for the prevention and treatment of Alzheimer's disease: Potential mechanisms of action. *British Journal of Nutrition, 114*(1), 1–14.

Gallier, S., Gordon, K. C., & Singh, H. (2012). Chemical and structural characterisation of almond oil bodies and bovine milk fat globules. *Food Chemistry, 132*(4), 1996–2006.

Gallier, S., Gragson, D., Jiménez-Flores, R., & Everett, D. (2010). Using confocal laser scanning microscopy to probe the milk fat globule membrane and associated proteins. *Journal of Agricultural and Food Chemistry, 58*(7), 4250–4257.

Garcia-Fuentes, E., Gil-Villarino, A., Zafra, M. F., & Garcia-Peregrin, E. (2002). Changes in plasma lipid composition induced by coconut oil. Effects of dipyridamole. *Journal of Physiology and Biochemistry, 58*(1), 33–41.

Gerschenson, L. N., Rojas, A. M., & Marangoni, A. G. (2001). Effects of processing on kiwi fruit dynamic rheological behaviour and tissue structure. *Food Research International, 34*(1), 1–6.

Gil-Villarino, A., Garcia-Fuentes, E., Zafra, M. F., & Garcia-Peregrin, E. (1999). Coconut oil induces short-term changes in lipid composition and enzyme activity of chick hepatic mitochondria. *Journal of Nutritional Biochemistry, 10*(6), 325–330.

Greenspan, P., Mayer, E. P., & Fowler, S. D. (1985). Nile red – A selective fluorescent stain for intracellular lipid droplets. *Journal of Cell Biology, 100*(3), 965–973.

Heathcock, J. F., & Chapman, J. A. (1983). The structure of fresh and desiccated coconut. *Food Microstructure, 2*(1), 81–90.

Huang, A. H. C. (1994). Structure of plant seed oil bodies. *Current Opinion in Structural Biology, 4*(4), 493–498.

Huang, A. H. C. (1996). Oleosins and oil bodies in seeds and other organs. *Plant Physiology, 110*(4), 1055–1061.

Krishnamurthy, M. N., & Chandrasekhara, N. (1983). Polar lipids of coconut. *Journal of Food Science and Technology (New Delhi, India), 20*(5), 206–209.

Kwon, K., Park, K. H., & Rhee, K. C. (1996). Fractionation and characterization of proteins from coconut (*Cocos nucifera* L.). *Journal of Agricultural and Food Chemistry, 44*(7), 1741–1745.

Laakso, P. (2005). Analysis of sterols from various food matrices. *European Journal of Lipid Science and Technology, 107*(6), 402–410.

Laemmli, U. K. (1970). Cleavage of structural proteins during assembly of head of bacteriophage-T4. *Nature, 227*(5259), 680.

Lin, L. J., & Tzen, J. T. C. (2004). Two distinct steroleosins are present in seed oil bodies. *Plant Physiology and Biochemistry, 42*(7–8), 601–608.

Manderson, G. A., Hardman, M. J., & Creamer, L. K. (1998). Effect of heat treatment on the conformation and aggregation of β-lactoglobulin A, B, and C. *Journal of Agricultural and Food Chemistry, 46*(12), 5052–5061.

Murphy, D. J. (1990). Storage lipid bodies in plants and other organisms. *Progress in Lipid Research, 29*(4), 299–324.

Napier, J. A., Beaudoin, F., Tatham, A. S., Alexander, L. G., & Shewry, P. R. (2001). The seed oleosins: Structure, properties and biological role. In J. A. Callow (Ed.), Advances in Botanical Research, Vol 35: Incorporation Advances in Plant Pathology, vol. 35 (pp. 111–138).

Napier, J. A., Stobart, A. K., & Shewry, P. R. (1996). The structure and biogenesis of plant oil bodies: The role of the ER membrane and the oleosin class of proteins. *Plant Molecular Biology, 31*(5), 945–956.

Nikiforidis, C. V., & Kiosseoglou, V. (2011). Competitive displacement of oil body surface proteins by Tween 80-Effect on physical stability. *Food Hydrocolloids, 25*(5), 1063–1068.

Nikiforidis, C. V., Matsakidou, A., & Kiosseoglou, V. (2014). Composition, properties and potential food applications of natural emulsions and cream materials based on oil bodies. *RSC Advances, 4*(48), 25067–25078.

Rodelas, A. J. D., Regalado, E. S., Bela-ong, D. B., Garcia, R. N., Laurena, A. C., & Mendoza, E. M. T. (2008). Isolation and characterization of the oil bodies and oleosin of coconut (*Cocos nucifera* L.). *Philippine Agricultural Scientist, 91*(4), 389–394.

Ross, J. H. E., Sanchez, J., Millan, F., & Murphy, D. J. (1993). Differential presence of oleosins in oleogenic seed and mesocarp tissues in olive (*Olea europaea*) and avocado (*Persea americana*). *Plant Science, 93*(1–2), 203–210.

Samson, A. S., Khaund, R. N., Cater, C. M., & Mattil, K. F. (1971). Extractability of coconut proteins. *Journal of Food Science, 36*(5), 725–728.

Seow, C. C., & Gwee, C. N. (1997). Coconut milk: Chemistry and technology. *International Journal of Food Science & Technology, 32*(3), 189–201.

Shimada, T. L., & Hara-Nishimura, I. (2010). Oil-body-membrane proteins and their physiological functions in plants. *Biological and Pharmaceutical Bulletin, 33*(3), 360–363.

Tangsuphoom, N., & Coupland, J. N. (2005). Effect of heating and homogenization on the stability of coconut milk emulsions. *Journal of Food Science, 70*(8), E466–E470.
Tangsuphoom, N., & Coupland, J. N. (2008). Effect of pH and ionic strength on the physicochemical properties of coconut milk emulsions. *Journal of Food Science, 73*(6), E274–E280.
Tangsuphoom, N., & Coupland, J. N. (2009). Effect of surface-active stabilizers on the surface properties of coconut milk emulsions. *Food Hydrocolloids, 23*(7), 1801–1809.
Tzen, J. T. C., Cao, Y. Z., Laurent, P., Ratnayake, C., & Huang, A. H. C. (1993). Lipids, proteins, and structure of seed oil bodies from diverse species. *Plant Physiology, 101*(1), 267–276.
Tzen, J. T. C., & Huang, A. H. C. (1992). Surface structure and properties of plant seed oil bodies. *Journal of Cell Biology, 117*(2), 327–335.
Tzen, J. T. C., Peng, C. C., Cheng, D. J., Chen, E. C. F., & Chiu, J. M. H. (1997). A new method for seed oil body purification and examination of oil body integrity following germination. *Journal of Biochemistry, 121*(4), 762–768.
Zhu, X. Q., Ye, A. Q., Verrier, T., & Singh, H. (2013). Free fatty acid profiles of emulsified lipids during in vitro digestion with pancreatic lipase. *Food Chemistry, 139*(1–4), 398–404.