



Heating and megasonic interventions for improvement of aqueous-based oil extraction from fresh and cold stored coconut meat

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ABSTRACT

The efficiency of oil recovery is essential for the economics of the coconut oil extraction process. This study evaluated the application of increased heat and/or megasonic steps for enhancing aqueous-based oil separation from coconut meat. Combinations of heating temperature (60 or 70 °C) and time (30 or 50 min) were studied in freshly prepared, or in 20 and 44 h cold stored (5 °C) coconut-water mixtures (1 L). The megasonic effect after heating (60 °C, 30 min) was evaluated at 2 MHz frequency and energy densities of 44–349 kJ/kg. Oil extraction from fresh coconut meat was higher when increasing heating temperature (10–13%). Net ultrasound yield in cold stored coconut meat was improved from 1.1 to 3.2% with increased sonication time from 2.5 to 20 min. Megasonic effects on mixtures heated (60 °C, 30 min) were corroborated in fresh coconut meat at a larger scale, which demonstrates the potential for megasonic to enhance aqueous-based coconut oil extraction processes.

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1. Introduction

Coconut (*Cocos nucifera* L.) is an important international commodity. Mature coconuts contain a hard-thick flesh layer, or kernel, which is utilised as the raw material of many products such as copra or dried coconut, coconut oil and coconut milk. Refined and virgin coconut oil (VCO) are two goods easily commercialised as edible oils. Refined coconut oil (RCO) is obtained from copra and is also utilised for the production of soap or cleansing agents. VCO is produced from fresh coconut meat either from expeller or aqueous based processes without the need of further refining, or with the use of solvents for extraction (Liu, 2016; Pham, 2016; Siriphanich et al., 2011; Tan et al., 2014).

RCO occupies larger volumes of production and is mainly manufactured in the Philippines, Indonesia and India. Thirty seven percent of RCO is produced in the Philippines (1.1 million tonnes, in 2014) (FAOSTAT, 2017) and is exported to Europe and America (Pham, 2016; Villarino et al., 2007). Recently, demand for VCO has

increased due to its recognition as a healthy ingredient which provides a higher antioxidant content compared to RCO. Moreover, VCO contains lauric acid, a medium-chain fatty acid, and short-chain fatty acids such as capric, caproic and caprylic which have antiviral and antibacterial activities (DebMandal and Mandal, 2011; Marina et al., 2009; Pham, 2016; Siriphanich et al., 2011; Villarino et al., 2007).

Despite the potential health benefits of VCO, little has been reported concerning its industrial production. In 1973 the first pilot plant process proposed using aqueous extraction, where application of hot water at 60 °C was utilised to facilitate the release of oil followed by centrifugation for oil recovery (Hagenmaier et al., 1973). In modern processes, coconut crushed endosperm (approximately millimetric particle size) can be heated in water to obtain coconut milk or dried before it is fed through an oil expeller (Berma Procesys Corp, 2017; Liu, 2016). Alfa-Laval plants are capable of processing up to 600,000 coconuts per day to produce coconut milk (Seow and Gwee, 1997). Coconut milk is a natural oil-in-water emulsion that can contain from 5 to 29% fat. In commercial coconut preparations such as light, milk, cream or concentrated products (Liu, 2016), the globulins and albumins from coconut

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meat, are responsible for emulsion stability (Patil and Benjakul, 2017; Raghavendra and Raghavarao, 2010). For aqueous based extraction of VCO, emulsion breakage in coconut milk is achieved through a series of centrifugation stages that separate clean virgin oil and skim milk. However, up to 24% of the total oil content can be found in the solid coconut waste produced after the aqueous based coconut milk extraction process (Sulaiman et al., 2013).

A number of processing aids have been suggested to increase yields in coconut oil extraction processes, including chilling and thawing, fermentation or enzymatic treatments (Che Man, Suhardiyono, Asbi, Azudin, & Wei, 1996; Mansor et al., 2012; Raghavendra and Raghavarao, 2010). Novel technologies such as high frequency ultrasound treatment named currently megasonic treatment, consisting of sound irradiation of the oily biomass with high frequency standing waves (0.4–4 MHz). It has been successfully applied in aqueous-based oil extraction of olives (Juliano et al., 2017a,b), palm nuts (Juliano et al., 2013a,b), and in avocado fruits (Martínez-Padilla et al., 2018).

In the case of coconut oil extraction, preliminary results showed that treatment of a coconut-water mixtures with megasonic standing waves improved the process (Juliano et al., 2017a,b). Megasonic treatment caused a denser cream layer after centrifugation when compared to the non-treated control. Application of high frequency ultrasound, above 400 kHz, is milder treatment than the traditional high intensity low frequency ultrasound (18–24 kHz) and does not form imploding bubbles from unstable cavitation and hence does not cause cell breakage (Leong et al., 2015). The positive effects of megasonic treatment on oil separation are either due to standing wave particle-droplet separation or microstreaming effects caused by stable cavitating bubbles (Juliano et al., 2017a,b). Conversely, low frequency ultrasound has been applied as an emulsification step for production of nanoemulsions (Kentish et al., 2008; Ramisetty et al., 2015).

shelled and the separated endosperms were utilised as samples for the trials. Particle size was reduced by shredding the coconut endosperms (coarse particles near 1–3 mm) using a commercial blender (PB9800 Café Series Blender, Sunbeam, Australia) at speed 3 (~2500 rpm) for 2 min. Some of the separated coconut meat or shredded endosperm samples were stored at 5 °C for 20 or 44 h, as shown in the experimental design in Table 1.

2.2. Wet extraction

A heating step was implemented at lab-scale to study the aqueous coconut extraction process and the effect of coconut meat cold storage time simultaneously. A 1 L coconut-meat water mixture was prepared by mixing coconut meat with demineralised water (22 ± 2 °C) manually at 1:4 ratio. Samples of the mixture were heated in a water bath in a circular stainless-steel vessel (12.5 × 16 cm). Extraction variables were explored by conducting a factorial design in duplicate, with three levels of cold storage at 5 ± 1 °C (0, 20 and 44 h), two heating temperatures (60 and 70 ± 2 °C) and two heating times (30 and 50 min) (Table 1, Experiment 1).

A schematic diagram of the experimental process for aqueous based oil recovery is shown in Fig. 1. After heating the 1:4 coconut meat-water mixture, a visible and defined coconut cream layer was separated by gravity (approximately in 2 min). The gravity-separated cream layer was then removed with a spoon from the skim milk. All gravity-separated cream layers were centrifuged at 5020 g (4200 rpm) (Beckman Coulter J6-MI, rotor JS-4.2, USA) for 30 min and 40 °C to obtain a centrifuged-cream layer and a skim milk residue. Oil content in the centrifuged-cream layer was measured as described in Section 2.4. Each trial was carried out at least in triplicate. The oil yield and oil extractability were calculated as:

$$\text{Oil yield [\%]} = \frac{\text{Oil extracted from gravity - separated cream layer [g]}}{\text{Mass of coconut meat [g]}} \times 100 \quad (1)$$

$$\text{Oil extractability [\%]} = \frac{\text{Oil extracted from gravity - separated cream layer [g]}}{\text{Mass of oil in coconut meat [g]}} \times 100 \quad (2)$$

The purpose of this study was to firstly investigate the effect of cold storage of coconut meat and heating of coconut-water mixtures for improved VCO aqueous-based separation and secondly to investigate the effect of megasonic treatment time on oil extraction in both fresh and cold stored coconut meat. The megasonic VCO extraction was then scaled-up and evaluated at selected process conditions.

2. Materials and methods

2.1. Sample preparation

Coconuts were purchased from a local fruit market (Hoppers Crossing, Victoria, Australia). Coconuts were stored at 5 °C for less than 2 months. For each treatment, a batch of 8 coconuts was selected. Mature undamaged coconuts were dewatered and de-

2.3. Megasonic treatment

The high frequency ultrasound system, or megasonic reactor, consisted of a rectangular stainless-steel vessel of 18.2 × 22.5 × 6.2 cm containing the 2 MHz transducer plate (16 × 16 × 3.2 cm) (Sonosys, Germany) (Fig. 2a). The distance between transducer and vessel wall was 3 cm. Compressed air was circulated through the transducer plates to cool the transducer during sonication. Electrical power draw during sonication was measured with a power meter (Belkin, F7C005AU, China).

Megasonic trials were carried out with 1 L coconut meat-water mixture (200 g coconut meat: 800 g water) at 60 °C for 30 min, which is the temperature applied in traditional aqueous-based virgin coconut oil industrial processes (Berma Procesys Corp, 2017). Fresh meat samples (2 L) were divided into two portions, one treated using the traditional process and the other was used for

Table 1
Experimental design.

Experiment number/Batch number	Effect	Variables	Levels	Volume	Constant conditions	Ultra-sound variables	Design/ Statistical analysis
1/1	Wet extraction variables	Cold storage time x heating temperature x heating time	0, 20, 44 h 60, 70 °C 30, 50 min	1 L		0 MHz	Factorial 3 × 2 × 2 Multilevel factorial analysis, Analyse of variance (duplicate) 4 levels (3 replicates) One way- analyse of variance
2/2.1a 2/2.1b	Megasonic	Ultrasound treatment time	0, 5, 10, 15 min	1 L	Fresh sample Preheat at 60 °C for 30min	2 MHz 87-262 kJ/kg	5 levels (4 replicates) One way- analyse of variance
2/2.2	Megasonic	Ultrasound Treatment time	0, 2.5, 5, 10, 20 min	1 L	Cold stored sample Preheat at 60 °C for 30min	2 MHz 44-349 kJ/kg	Factorial 2 × 6 Multilevel factorial analysis, Analyse of variance (triplicate)
3/3	Megasonic and mass balance at larger scale	Ultrasound frequency x different layers	0, 2 MHz	2.5 L	Fresh sample Preheat at 60 °C for 30min	2 MHz 5 min 87 kJ/kg	

the megasonic treatment trials (Fig. 1).

The megasonic treatments were carried out at 2 MHz at maximum power draw (291 W) at four time intervals (0, 5, 10, 15 min) in triplicate using fresh coconut meat, and five time intervals (0, 2.5, 5, 10, 20 min) in four replicates using cold stored (20 h) coconut meat (Table 1, Experiment 2). Energy density was calculated by the time of applied treatment, measured electrical power draw and total mass of the treated sample. Corresponding minimum and maximum energy densities were 44 and 349 kJ/kg, respectively.

Ultrasound system was placed into a water bath to apply

previous heating treatment proposed (60 °C, 30 min). The temperature of coconut meat-water mixtures increased by a few degrees reaching 66, 69 and 70 °C at 5, 10 and 15 min of high frequency ultrasound treatment (megasonic), respectively. The control sample (non-ultrasound) run in parallel with the same initial coconut mixture was matched to this temperature for comparison.

A larger scale megasonic experiment was carried out with 2.5 L fresh coconut meat-water mixture to establish an oil mass balance across liquid and solid layers (Table 1, Experiment 3). Previous heating treatment was also applied (60 °C, 30 min) to the sample.

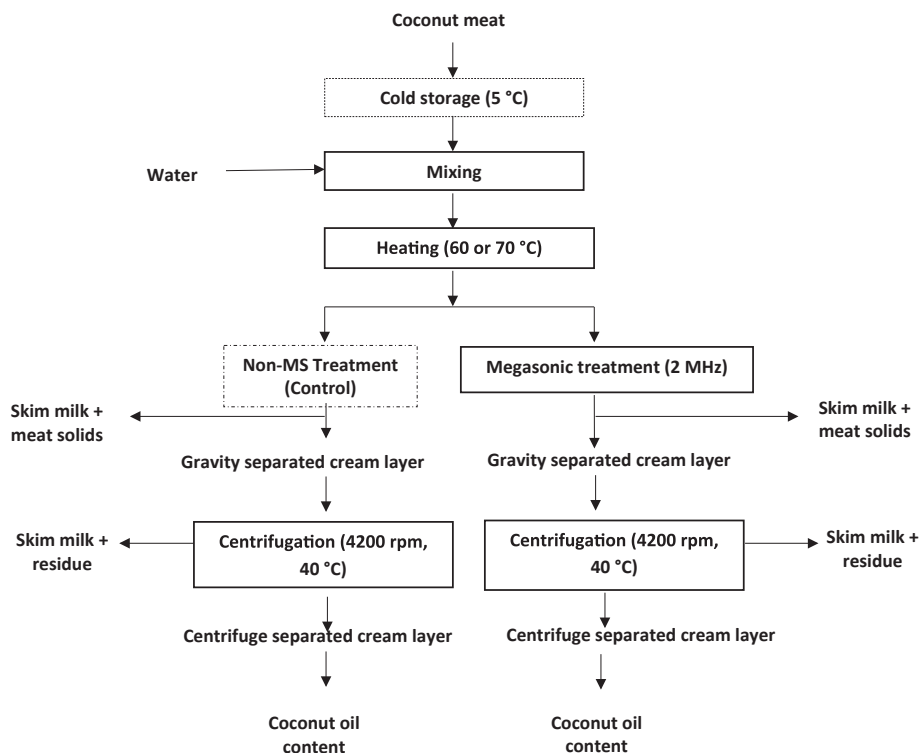


Fig. 1. Flowchart for the evaluation of 2 MHz megasonic treatment time effect on coconut oil separation.

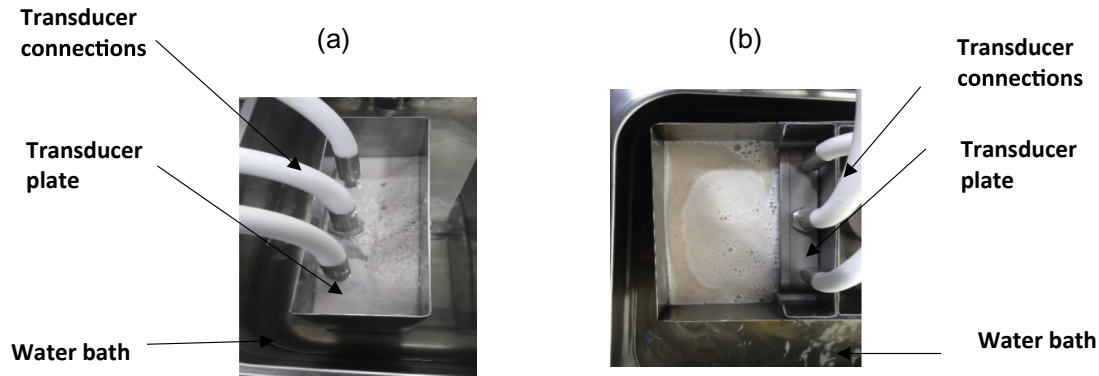


Fig. 2. Megasonic systems setup (a) 1L scale, (b) 2.5 L scale.

The ultrasonic vessel dimensions were $17 \times 20 \times 13$ cm and is shown in Fig. 2b. In this case, both gravity-separated (top) cream and bottom layers obtained after the heating treatment were centrifuged for both control and megasonic treatments. Test was conducted in triplicate. The centrifuged-cream, skim milk and solid residue were taken from either the top or bottom layers and analysed for oil content (see Section 2.4).

The net ultrasound yield (NUY) and the net ultrasound extractability (NUE) were obtained by the equations below: where the yield and extractability were calculated as per Eq. (1) and Eq. (2), respectively, after megasonic and control (non-megasonic) treatments.

$$NUY [\%] = Oil\ yield_{MS} - Oil\ yield_{control} \quad (3)$$

$$NUE [\%] = Oil\ extractability_{MS} - Oil\ extractability_{control} \quad (4)$$

2.4. Oil content

Oil content in coconut meat or in the centrifuged-cream layer was measured as hexane extractable oil, where 100 g of sample was placed in 500 mL centrifuge flask and filled with 100 mL of hexane (analytical grade, SupraSolv, Germany). The tube was shaken for 1 min and heated for 30 min in a 60 °C water bath. De-ionised water (100 mL) was then added in the flask and centrifuged (Centrifuge J6-MI with a rotor JS-4.2, Beckman Coulter, USA) at a force of 5020 g (4200 rpm) for 30 min at 40 °C. The hexane layer was separated by an air displacement pipette and an additional 50 mL of hexane were added to the residue, heated for 30 min in a 60 °C water bath, and centrifuged again at same conditions (5020 g, for 30 min at 40 °C). Total hexane layers from the cream and residue were kept for overnight drying in a SpeedVac concentrator with a refrigerated vapour trap (SC250EXP, RVT4104, Thermo Scientific, Australia). The obtained coconut oil was weighed and the oil content or hexane extractable oil was calculated as:

$$Oil\ content [\%] = \frac{Oil\ hexane\ extracted [g]}{Mass\ of\ sample [g]} \times 100 \quad (5)$$

2.5. Statistical analysis

An analysis of variance from the multilevel factorial design was performed for experiments 1 and 3. Significant differences between means in experiment 2 were determined using one-way ANOVA test. Differences between means were assessed by Dunnett multiple comparisons. A 95% confidence level was considered (Minitab 17, Minitab Inc., PA, USA).

3. Results and discussion

Individual oil contents of several batches are summarised in Table 2, and ranged from 18.9 to 25.6% which indicated wide variability among the purchased coconuts. This variability observed across batches may be attributed to differences in geographical origin and maturity stages among selected fruits (Pham, 2016; Siriphanich et al., 2011), and were below the oil content in coconut meat reported elsewhere (Raghavendra and Raghavarao, 2010; Seow and Gwee, 1997).

3.1. Aqueous-based extraction parameters

The effect of combined parameters, i.e. cold storage time of coconut meat, heating temperature, and heating time of coconut meat-water mixtures on oil yield is summarised in Table 3. The effect of temperature ($p \leq 0.05$) and storage time ($p \leq 0.05$) were statistically significant, while heating time ($p = 0.19$) was not. Moreover, the temperature-storage time interaction ($p \leq 0.05$) was

Table 2
Oil content in coconut meat used for various experiments ($n = 2$).

Batch number	% , g oil 100 g ⁻¹ coconut meat
1	21.2 ± 0.5
2.1a	21.8 ± 0.9
2.1b	25.6 ± 1.2
2.2	18.9 ± 0.5
3	23.5 ± 0.6

Table 3
Oil yield from different cold storage time and heating temperature-time treatments in aqueous extraction. Different letters (a, b, c) show statistical significant differences between samples with a 95% confidence level ($n = 2$, $p \leq 0.05$).

	Oil yield (% , g oil 100 g ⁻¹ coconut meat)			
	Heating temperature and time			
	60 °C, 30 min	60 °C, 50 min	70 °C, 30 min	70 °C, 50 min
0 h	10.3 ± 1.2 ^a	10.2 ± 0.3 ^a	13.4 ± 0.0 ^b	12.4 ± 0.6 ^b
20 h	12.7 ± 0.0 ^b	12.6 ± 0.8 ^b	12.9 ± 0.1 ^b	13.1 ± 0.4 ^c
44 h	13.9 ± 0.1 ^c	13.7 ± 0.2 ^c	14.0 ± 0.3 ^b	13.5 ± 0.1 ^c

also statistically significant. Same statistical differences were computed for oil extractability.

As seen in Table 3, extracted oil in cream increased with additional cold storage time after 44 h of coconut meat storage. This result could be explained as during cold storage, endogenous pectinases and cellulases may have disrupted the cell walls and enhanced oil body released, despite lower enzymatic activity expected at 5 °C (Goulao et al., 2010). It was reported that endogenous proteases can also hydrolyse peptide bonds in the interior of the polypeptide chain resulting in destabilising coconut milk emulsion and reaching higher oil separation (Raghavendra and Raghavarao, 2010). This idea can be supported as enzymes (added) were utilised to enhance coconut oil extraction and employed a similar conditioning step at 60 °C for 30 min (Che Man et al., 1996).

In fresh non-stored samples, higher heating temperature of coconut meat-water mixture at 70 °C increased oil yield by 2–3% in comparison to a 60 °C heating. In this case, high temperature could enhance the permeability of the tissue cell walls improving enzyme activity on polysaccharides and proteins, as it was reported by Goulao et al. (2010), therefore promoting aqueous oil extraction.

For cold stored coconut meat, heating temperature and time did not influence the oil yield in the cream layer indicating that the enzymatic effect is heating time independent and a similar effect can be achieved faster at an elevated treatment temperature of 70 °C. In this case, the activity of endogenous enzymes already took place during cold storage, and the heating effects became unnoticeable. Either extended storage or heating provide similar improvements to those seen in malaxation operations typically used for olive or avocado oil extraction (Clodoveo et al., 2013; Wong et al., 2014). In addition, an increase in malaxation temperatures in olive oil process, but at a lower temperature than 30 °C, have shown to increased oil yield (Clodoveo et al., 2013).

3.2. Megasonic treatment in a 1 L system

Preliminary studies have shown that megasonic treatments of coconut meat-water mixture enhanced oil separation as observed by a thicker cream layer after centrifugation (Juliano et al., 2017a,b). Fig. 3a and Fig. 3b show the mean oil yield of fresh (ultrasound treatments 5–15 min; 87–262 kJ/kg) and stored (ultrasound treatments 2.5–20 min; 44–349 kJ/kg) coconut meat after different treatment times at 2 MHz. Significant differences were only observed in cold stored samples ($p \leq 0.05$), mainly at high ultrasound time treatments. Dunnett multiple comparisons with a control (0 min) indicate that oil content after 10 and 20 min megasonic treatment (2 MHz) in stored coconut meat were significantly different (Fig. 3b).

The NUY values were calculated using the mean of oil yield only for cold stored samples (20 h). Oil in the fresh sample control varied widely; therefore, data was shown on an individual basis rather than averaged; i.e., for each treatment time a parallel control was conducted and 9 individual NUY values were calculated. For the 5 min megasonic treatment, megasonic effects were not pronounced and in certain cases the oil obtained in the control sample was higher than in the megasonic sample due to the mentioned variability, therefore giving a negative NUY value.

For cold stored coconut NUY values increased linearly ($R^2 = 0.91$) with sonication time, showing an additional oil yield of 1.1–3.2%. NUY values followed also a linear increase from 5.8 to 16.8% ($R^2 = 0.91$). These results corroborate previous reports showing enhanced oil separation in olive, palm nuts and avocado oil processes after increased megasonic treatment time (Juliano et al., 2013a,b; Juliano et al., 2017a,b; Martínez-Padilla et al., 2018).

Previous studies (Johansson et al., 2016; Juliano et al., 2013a,b; Torkamani et al., 2014) have characterised the megasonic reactor

used in this work with a luminol methods to measure free radical formation from cavitation. The frequency employed in this study (2 MHz) produced smaller bubbles that intimately produce microstreaming currents with limiting unstable cavitation effects and no luminol emission. In comparison, low frequency ultrasound systems have shown to provide strong unstable cavitation and bubble implosion capable of destroying cellular tissue (Vilkhu et al., 2008). Even though no previous studies have been reported on the use of low frequency ultrasound for enhancing coconut oil extraction, other studies have observed that extended low frequency application times (24 kHz, 1–5 min, 91–1857 kW/m³) reduced oil droplets and promote emulsification, however, the use of emulsifiers (Tween 80 and Span 80) was necessary to stabilise the coconut oil in water emulsions achieved (Ramisetty et al., 2015).

In these experiments, variations in fresh coconut meat treated samples may be due to intrinsic emulsification created by stable cavitation phenomena such as microstreaming, which improves the interaction between proteins (2–3% proteins in coconut milk; Liu, 2016) and oil droplets in the emulsified cream layer and which may create a more stable emulsion. Further emulsion destabilisation

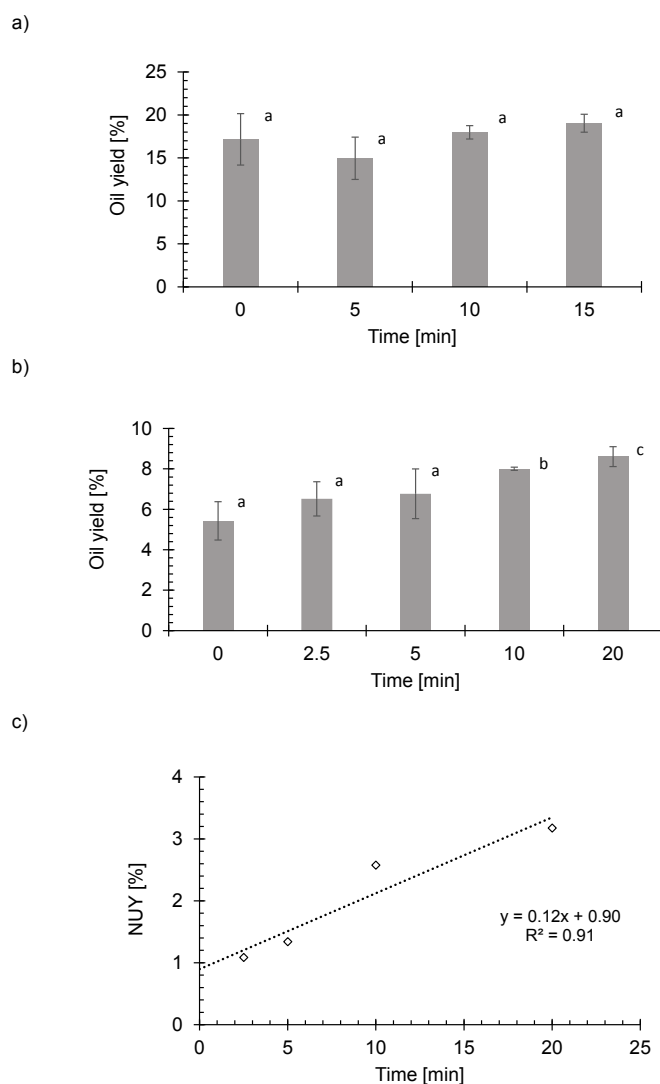


Fig. 3. Ultrasound treatment time effect on oil yield in fresh (a) and cold (b) stored coconut meat. Different letters (a, b, c) show statistical significant differences between samples with a 95% confidence level ($p \leq 0.05$). Net ultrasound yield (NUY) as a function of ultrasound treatment time for cold stored (5 °C, 20 h) coconut meats (c).

phenomena may have occurred during additional sonication up to 20 min due to additional aggregation between droplets or protein transfer into the aqueous phase. Other studies should evaluate the levels of protein transfer between the cream and aqueous phases after sonication on fresh and cold stored coconut.

3.3. Oil mass balance and megasonic scale up

The coconut oil yield distribution in the top and bottom gravity-separated layers obtained in a 2.5 L scale megasonic set-up is shown in Fig. 4a, with or without 5 min megasonic treatment at 2 MHz in fresh coconut. The highest oil yield was found in the top cream layer, with the megasonic treated sample showing an additional 1% net oil yield or 4.2% net oil extractability. Therefore, the megasonic effect demonstrated above in fresh coconut in the 1 L system was corroborated in the 2.5 L system. A reduction of oil in solids, indicates that possible oil was removed from the solids through microstreaming flow caused by large number of stable cavitating bubbles.

Fig. 4b shows that the sum of oil in top cream, top skim milk and top residues in top cream layer significantly increased oil content by 0.9% while oil content in the bottom layer was reduced by a similar amount although the number of samples needs to be increased to raise the level of significance. Table 4 shows the average NUY and NUE values in the top and bottom gravity-separated layers. The negative NUE values observed in the bottom layers confirmed that oil release was higher and migrated to the top gravity-cream layer, after megasonic treatment.

As reported previously, high frequency sonication in coconut meat-water mixtures promoted oil separation through two paths: differential positioning of oil droplets at the antinodes leading to oil droplet coalescence, and acoustic microstreaming flows and rubbing effects caused by non-collapsing or stable cavitation bubbles intimately located across the tissue structure, as discussed in studies of similar oily fruits (Juliano et al., 2013a,b; Juliano et al., 2017a,b; Martínez-Padilla et al., 2018).

In the future, design of large scale reactors requires considering sound attenuation effects, since a sharp decline of sound pressure

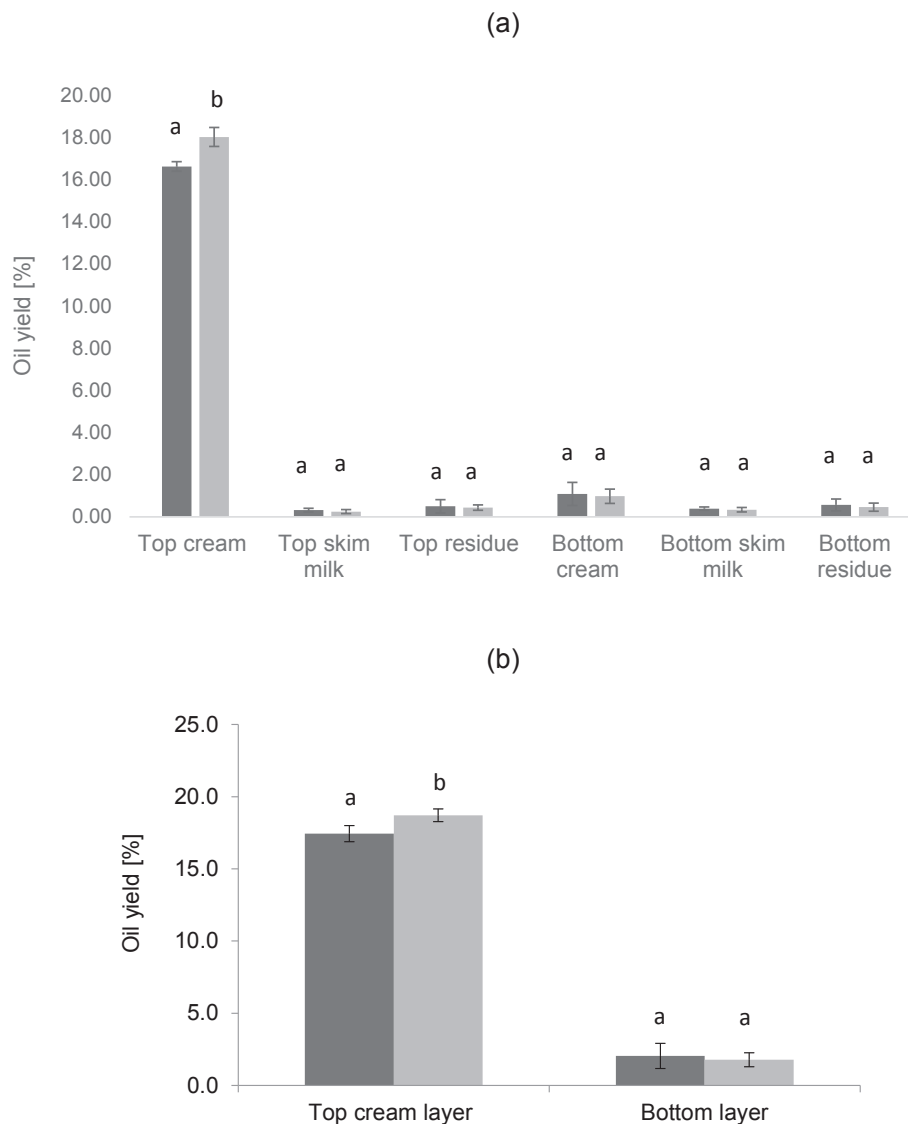


Fig. 4. Oil distribution in the top and bottom gravity-separated layers obtained in 2.5 L scale megasonic set-up (a). Sum of oil in top cream, top skim milk and top residues gravity-separated layers, and respective sum of the bottom layers (b). Control (dark grey), Megasonic treatment (pale grey). Different letters (a, b) show statistical significant differences between samples with a 95% confidence level ($p \leq 0.05$).

Table 4

Net ultrasound extractability (NUE) and net ultrasound yield (NUY) in the top and bottom gravity-separated layers obtained in a 2.5 L scale 2 MHz megasonic set-up (a).

Layer	NUE	Standard deviation
Top cream	4.2%	2.0
Top skim milk	−0.3%	0.1
Top residue	−0.3%	0.8
Bottom cream	−0.5%	1.0
Bottom skim milk	−0.2%	0.4
Bottom residue	−0.5%	1.0
Layer	NUY	
Top cream	0.99	0.45
Top skim milk	−0.070	0.04
Top residue	−0.067	0.18
Bottom cream	−0.123	0.23
Bottom skim milk	−0.060	0.10
Bottom residue	−0.110	0.23

has been observed in this type of system when sonicating beyond 100 mm in water (Leong et al., 2015).

4. Conclusions

Cold storage of coconut meat at 5 °C beyond 20 h provided enhanced aqueous based oil recovery. Heating of coconut meat-water mixtures at 60 and 70 °C was seen to be more beneficial in improving oil yield when using higher temperature. Megasonic treatments of coconut meat and coconut meat-water mixtures provided enhanced oil recovery effects in both fresh and cold stored samples after 5 min sonication at 2 MHz frequency. An increased oil recovery effect was observed with increased sonication treatment time only for the aqueous based process using cold stored coconut meat. The aqueous based separation of oil showed potential for scalability as the results from a 1L megasonic extraction vessel were corroborated at the 2.5 L scale. Sound waves generated from 2 MHz during 5 min (32.5 kJ/kg) were enough to obtain 2.7% of extra oil extractability, which is highly significant for industrial scale implementation of the megasonic technology since it will result in large cost savings.

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