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Influence of ultra-sonication on stability and physicochemical properties of coconut milk

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Abstract
The impact of ultra-sonication (20 KHz) on stability, rheological and physicochemical properties of coconut milk was studied. The coconut milk (10 % fat) was sonicated at 20 KHz for 5, 10 and 15 min. The emulsion stability index (ESI) of coconut milk was increased due to sonication treatment with respect to treatment time and the maximum value of ESI was 36.15 min at 15 min treatment time. It was observed that the sonication treatment had synergetic effect on physicochemical properties such TSS, protein, FFA and viscosity. All the samples exhibited pseudoplastic behaviour with an average flow behaviour index (n) and consistency coefficient (K) of 0.193 and 2.682 Pa s^n respectively. Among the power law and Bingham model, Bingham model showed higher correlation coefficient ($R^2 = 0.983$). The microstructure analysis revealed that longer exposure of sonication time improved the particle size reduction due to cavitation effect.

Keywords: Ultra sonication, coconut milk, Emulsion stability index, viscosity, physicochemical properties

1. Introduction

Coconut milk is the natural oil-in-water emulsion extracted from endosperm of matured coconut (*Cocoas nucifera* L) with or without adding water (Seow and Gwee, 1997) [1]. It is used as an important ingredient for traditional foods in South East Asia. Coconut milk contains 50% moisture, 40% fat, 3% protein, 1.5% ash and 5.5% carbohydrates (Woodroof, 1970)[2]. Coconut milk emulsion is naturally stabilized by coconut protein (globulins and albumins) but separate into white cream and aqueous layer during processing and storage. This is stabilized by proper emulsifiers or stabilizers depending on quantity and quantity of emulsifiers (Timmins and Kramer, 1978) [3]. Traditionally, coconut milk is processed by thermal methods like pasteurization, sterilization and UHT to increase the shelf life. But the thermal processes are associated with disadvantages like protein denaturation, decrease in nutrient content and change in product quality parameters (Chiewchan *et al.* (2006) [4]; Tangsuphoom and Coupland (2005) [5]; Khuenpet *et al.* (2016) [6]). To overcome these difficulties, traditional thermal processing technologies are being replaced by non-thermal technologies.

Ultra sonication technique is one of the fast, versatile and non-toxic technique among the other non-thermal techniques such as gamma radiation, pulse electric field, pulsed light etc. (Kentish and Ashokkumar, 2011)[7]. This can be applied for cleaning, homogenising, emulsification, extraction, microbial disinfection and enzyme inactivation ((Mcclements, 2015) [8]; Ordoñez *et al.* (1987)[9]; O'donnell *et al.* (2010)[10]). Sonication is the act of applying sound energy to agitate particles in a sample within the range of 20 KHz to 100 KHz frequency. Small vacuum or cavity bubbles are formed during sonication process because of successive compression and rarefaction of sound waves. These bubbles collapse at certain point which will generate very high temperature (3000 K to more than 10,000 K) and pressure (greater than 100 MPa) with in fraction of second. This phenomenon is called cavitation effect which is responsible for changes in sample during process (Povey and Mason, 1998) [11].

When the ultra-sonication (20 KHz) was applied to milk for 5 min and 10 min, creaming index of the milk was increased with increase in treatment time. This change was due to cavitation phenomenon, shear stress and turbulence during treatment (Ertugay *et al.*, 2004) [12]. Some of the authors reported that the ultra-sonication had synergetic effect on milk fat homogenization by cavitation effect (Shanmugam *et al.* (2012) [13]; Bosiljkov *et al.* (2011) [14]). Jena and Das (2006)[15] reported the effect of sonication time (0.5 to 3 minutes) on particle size of coconut milk and also modelled an equation which gave the number of particles below 2 μm after particular sonication time. Wu *et al.* (2000)[16] studied the effect of ultra-sonication (20 KHz) on milk (150 ml) homogenization, which was achieved at high power (450 W) after 10 min of sonication due to cavitation effect. Lad and Murthy (2012) [17] investigated the effect of ultra-sonication (20 KHz – 120 and 250 W power for 12 min) on the stability of sunflower oil in water emulsion made by coconut milk protein (CMP-1.2 %). The result showed that the mean diameter of emulsion droplet size was highly affected by sonication with coconut milk protein. It was concluded that, sonication process had the capability to produce emulsion with smaller droplet size.

The main objective of this study was to investigate the effect of ultra-sonication on emulsion stability, rheological properties and physicochemical properties of the coconut milk.

2. Materials and methods

2.1 Preparation of coconut milk

Fresh coconuts were purchased from the local market of Coimbatore, India. The shell was broken into two halves and endosperm was removed using the knife. Upper brown layer of endosperm was removed and the endosperm was cut into small pieces. White coconut pieces were blended by the addition of distilled water at a ratio of 3:1, respectively. Then the coconut milk was squeezed manually by using double layer cheese cloth. The fat content of coconut milk was determined using the Mojonnier ether extraction method (AOAC, 2002) [18] and used for further analysis.

2.2 Ultra sonication system

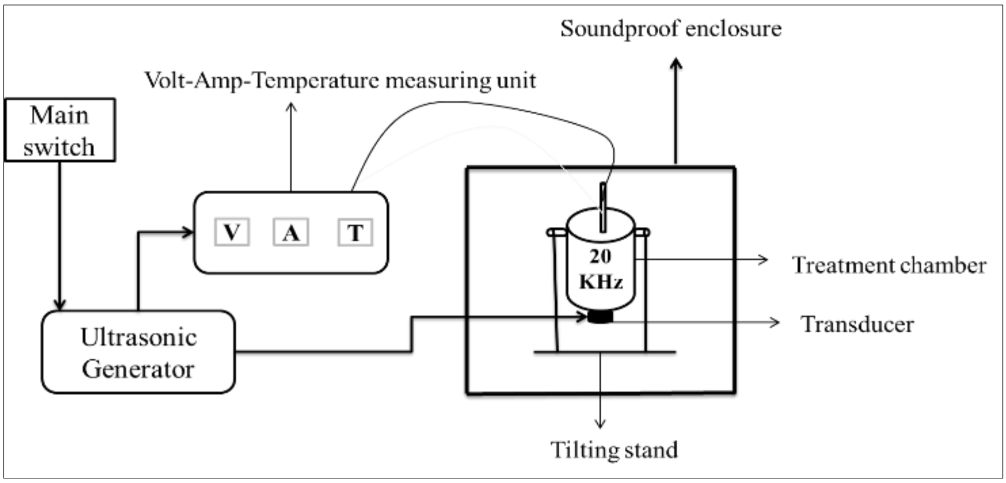

Fig 1: Schematic diagram of ultra-sonication system

The schematic diagram of ultra-sonication system (20 KHz frequency) showed in Fig. 1, consisted of ultrasonic generator, Volt-Amp-Temperature measuring unit, soundproof enclosure, ultrasonic transducer and treatment chamber. The ultrasonic generator converts the AC current into DC and amplifies the electrical current. The Volt-Amp-Temperature measuring unit consisted of Volt meter, Amps meter and temperature sensor. The input voltage and current to ultrasonic transducer was measured by volt and amps meter respectively. The ultrasonic transducer made of piezo electric material, converts the electrical current into vibrational sound energy. The transducer was fixed at the bottom of cylindrical treatment chamber (Diameter = 80 mm and Height = 80 mm) and the treatment chamber was fixed to tilting stand. During sonication, the sound waves were propagated from bottom to surface of the sample

100 ml of coconut milk (10% fat) was sonicated in treatment chamber for 5, 10 and 15 min. The actual power delivered to the sample was determined to be 0.0523 W/ml by calorimetric method (Povey and Mason, 1998) [11]. All the samples were treated at ambient temperature and each treatment was triplicated.

2.3 Emulsion stability index (ESI)

Emulsion stability index (ESI) of the coconut milk was estimated by using turbidimetric method (Rodsamran and Sothornvit, 2018) [19]. The mixture of 6 ml of sample and 2 ml of coconut oil was homogenized at 10,000 rpm for 1 min. From the homogenized sample 50 µl was pipetted out at 0 and 10 min duration into test tube containing 5 ml of 0.1% sodium dodecyl sulfate (SDS). The absorbance was recorded as A_0 and A_{10} at 500 nm using UV spectrophotometer with respect to the blank solution containing 50 µl of distilled water and 5 ml of 0.1% SDS. ESI of the sample was expressed as minutes calculated using the formula (2.1).

$$\text{ESI (min)} = \frac{A_0 \times 10}{A_0 - A_{10}} \qquad (2.1)$$

Where, $A_0$is absorbance at 0 min and A_{10} is absorbance at 10 min

2.4 Creaming index

15 ml of coconut milk was transferred into test tube (15 X 150 mm), covered with aluminium foil and stored for 24 hours at 4ºC. After 24 hours the sample was formed into two layers, an opaque cream layer at the top and a transparent layer at the bottom. The creaming index was calculated by using formula (2.2) (Jiang *et al.*, 2016) [20].

$$\text{Creaming index (\%)} = \frac{\text{Height of the aqueous layer } H_A}{\text{Total height of emulsion } H_E} \times 100 \qquad (2.2)$$

2.5 Determination of total soluble solids (ºBrix), total protein and free fatty acid

Total soluble solids (TSS) content of coconut milk was determined by using a digital hand Refractometer (ATAGO, Tokyo) and expressed in terms of ºBrix.

Total protein content of coconut milk was determined by Lowry's method (Horwitz, 1975) [21].

The free fatty acid (FFA) content of coconut milk samples was determined by AOAC (2002) [18] method. The FFA was calculated as percentage of lauric acid per 100 ml of coconut milk, considering lauric acid to be higher fatty acid present in coconut milk (Waisundara *et al.*, 2007) [22].

2.6 Rheological Properties

Anton-paar rheometer (MCR52 model) was used to perform the rheological measurements (viscosity, shear stress and shear rate). The temperature was maintained using automatic temperature control peltier system. Viscosity of different samples was measured by parallel plate geometry (diameter = 50 mm; gap = 1 mm) in the shear rate range of 0 to 500 s^{-1}. Two millilitre of sample was loaded on plate and left for 4 - 5 min to allow temperature equilibrium.

2.7 Microscopic study

Samples of coconut milk (15 µl) were placed on a microscope slide, gently covered with a cover slip and observed under 450X magnification using optical microscope (Nikon, SMZ 745T) equipped with digital camera. Photographs were taken from different fields on each slide and represented in micrographs.

3. Results and Discussion

3.1 Effect of ultra-sonication on emulsion stability index (ESI)

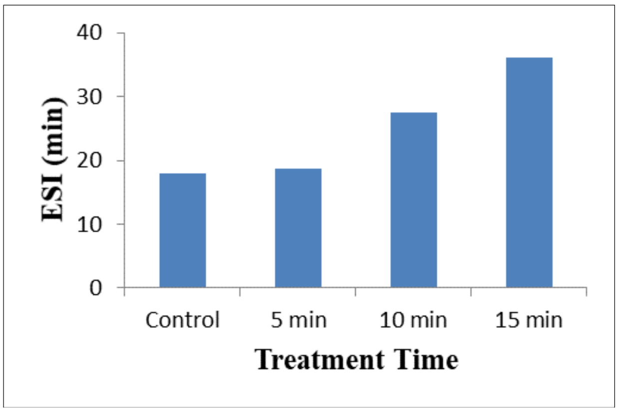

Fig 2: Emulsion stability index of sonicated (0, 5, 10 and 10 min) coconut milk

The Emulsion stability index (ESI) indicates the ability of emulsion to resist the change in its structure over defined time period (Rodsamran and Sothornvit, 2018) [19]. As shown in Fig. 2 the ESI of coconut milk increased with increase in sonication time. The ESI mainly depends upon protein solubility and surface hydrophobicity. During sonication the denaturation of protein might have increased the surface activity and adsorption of oil and water interface, which might have increased the ESI of coconut milk (Soria and Villamiel, 2010) [23]. Sui *et al.* (2017)[24] reported the emulsion stability index of sonicated (150 W) soya bean protein isolate was increased from 42.06 min to 221.03 min with increase in treatment time from 12 min to 24 min.

3.2 Effect of ultra-sonication on creaming index

Creaming index is an important parameter to evaluate stability of coconut milk. If the creaming index percentage is less, the stability of coconut milk is more. It was observed from the Fig. 3 the creaming index of coconut milk decreased with increase in treatment time. This may be due to reduction of fat or protein globule size and prevention of flocculation by the cavitation effect. Lad and Murthy (2012)[17] reported that the sonication (20 KHz - 250 W) of coconut milk protein and oil emulsion had significant effect on creaming index due to reduction in oil droplet size.

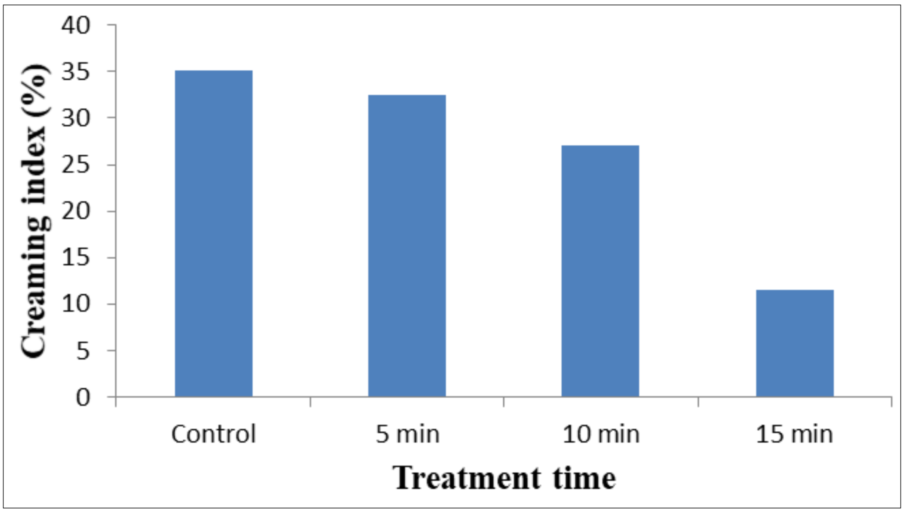

Fig 4: Creaming index of sonicated (0, 5, 10 and 15 min) coconut milk

3.3 Effect of ultra-sonication on physicochemical properties of coconut milk

The changes in TSS, protein, FFA and viscosity of coconut milk due to ultra-sonication are presented in Table 1. The total soluble solid content of coconut milk was enhanced with sonication treatment. From the Table 1, maximum brix (9.5 ºBrix) was observed at 15 min sonication of coconut milk compared to control (8.8 ºBrix). Increase in TSS might be due to mass transfer effect generated during the cavitation process. During cavitation process, shear and shock waves may damage the cell walls of protein tissues which might enhance the rate of diffusion. This would ultimately result in the solubilisation of more solids (Zou and Hou, 2017) [25]. Nadeem *et al.* (2018)[26] reported that the TSS of sonicated (20 KHz for 6 min) carrot grape blend juice increased from 12.5 ºBrix to 13 ºBrix due to cavitation effect.

In this study the protein content of sonicated coconut milk increased from 5.18 to 6.51 g/100ml. This might be due to denaturation of lipo protein during cavitation process. Villamiel and De Jong (2000)[27] stated that the ultra-sonication treatment (20 KHz) was denatures the whey protein in whole and skim milk.

The free fatty acid content decreased from 1.72 % to 1.11 % of lauric acid. The decrease in FFA may due to inactivation of lipase activity during sonication process. The Lipase enzyme activity was responsible for the production of lauric acid (Waisundara *et al.*, 2007) [22].

Table 1: Physicochemical properties of sonicated coconut milk

	Control	5 min	10 min	15 min
TSS (˚Brix)	8.80	9.00	9.20	9.50
Protein (g/100ml)	5.18	5.33	6.23	6.51
FFA (% of lauric acid)	1.72	1.69	1.13	1.11
Viscosity (Pa s)	0.018	0.020	0.022	0.025

The linear regression model equation of coconut milk is given below.

For TSS (˚Brix): y = 0.23x + 8.55 (R^2 = 0.988) (3.1)

For protein (g/100ml): y = 0.489x + 4.59 (R^2 = 0.924) (3.2)

For FFA (% of lauric acid): y = -0.239x + 2.01 (R^2 = 0.833) (3.3)

For viscosity (Pa s): y = 0.0022x + 0.0158 (R^2 = 0.996) (3.4)

3.4 Effect of ultra-sonication on rheological properties

The samples were analysed by using power law model (3.5) and Bingham model (3.6). All the treatments were carried out with three replications and average is reported.

Power law model: $\sigma = K(\gamma)^n$ (3.5)

Bingham model: $\sigma = \sigma_0 + \eta\gamma$ (3.6) Where σ is shear stress (Pa), K is the consistency coefficient (Pa s^n), γ is shear rate (s^{-1}), *n* is the flow behavior index (dimensionless), σ_0 is Yield stress (Pa) and η is viscosity (Pa s)

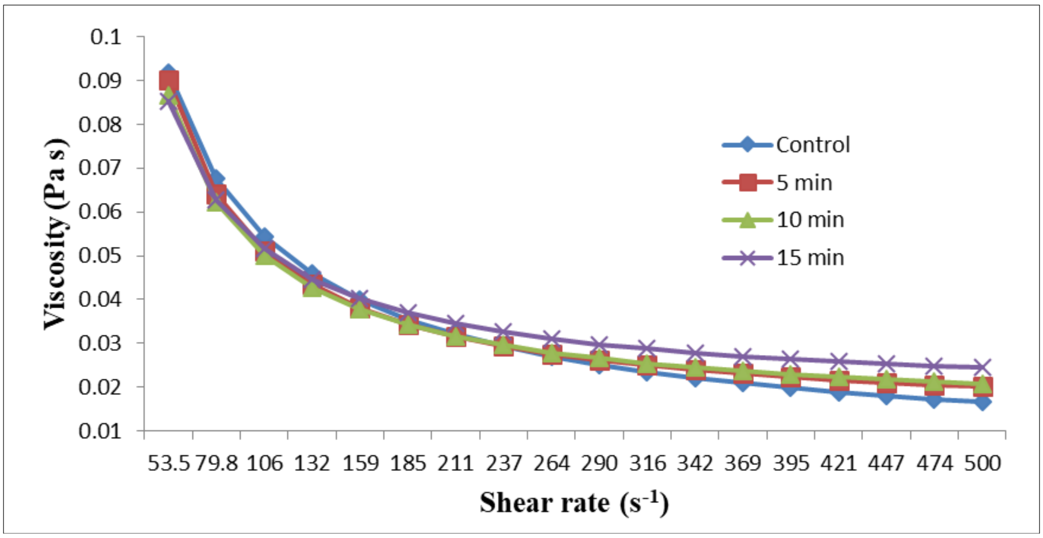

Fig 5: Effect of sonication on viscosity of coconut milk

Fig. 5 shows that the viscosity decreased with increase in shear rate for all coconut milk samples. When the shear force was applied on coconut milk, larger particles might be broken into smaller and rearranged themselves into parallel direction with shear force. The interaction between these particles increased, as a result of decrease in viscosity (Simuang *et al.*, 2004) [28]. The viscosity of the samples increased with the treatment time due to increase in TSS.

Table 2: Parameters of the power law and Bingham models for the flow behaviour of coconut milk

Treatments	Power law model				Bingham model		
	n	K (Pa s^n)	Viscosity (Pa s)	R^2	Yield stress (Pa)	Viscosity (Pa s)	R^2
Control	0.159	3.150	0.0071	0.782	4.28	0.0068	0.975
5 min	0.163	3.000	0.0076	0.756	4.19	0.0075	0.997
10 min	0.183	2.735	0.0089	0.754	3.94	0.0087	0.987
15 min	0.233	2.313	0.019	0.867	3.64	0.015	0.964

Table: 2 shows that the flow behaviour parameters of coconut milk from power law model (flow behaviour index, consistency coefficient and viscosity) and Bingham model (yield stress and viscosity). According to power law model, the average flow behaviour index (*n*), consistency coefficient (K) and viscosity were 0.193, 2.683 Pa s^n and 0.011 Pa s, respectively. The flow behaviour index (n) of all the samples was less than one, which stated that the coconut milk exhibited pseudoplastic behaviour. Simuang *et al.* (2004) [28] reported that the flow behaviour of coconut milk at different temperatures (70 - 90 ºC) exhibited pseudoplastic behaviour. According to Bingham model, the yield stress of the samples decreased from 4.28 to 3.64 Pa with respect to treatment time. Decrease in yield stress might be due to increase in homogeneity of the sample by reduction in size of fat globules.

3.5 Microstructure of coconut milk emulsion
The microstructure of sonicated coconut milk was examined using an optical microscope. Fig.6 shows the micrographs of treated coconut milk samples for 5, 10 and 15 min. The size of the fat globule was larger and non-uniformly dispersed in untreated coconut milk. In 5 min sonication treatment, the size of fat globules was not much affected but when the treatment time was increased to 10 and 15 min the size of fat globules were decreased. These changes might be due to the cavitation phenomenon, which breaks the fat globules into small size. Wu *et al.* (2000) [16] reported that the sonication (20 KHz for 1, 6 and 10 min) of milk had a significant effect on size of fat globules with longer exposure time.

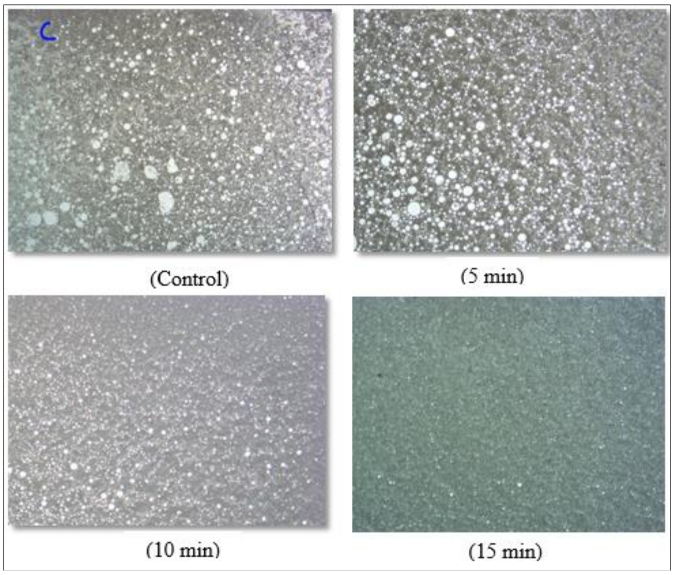

Fig 6: Micrographs (450X magnification) of 0, 5, 10 and 15 min sonicated coconut milk

4. Conclusion
Ultra-sonication had positive influence on the stability of coconut milk by incresing the ESI and reducing the creaming index. The TSS and protein conent of coconut milk was significantly incresed by increasing the sonication time. All coconut milk samples exhibited pseudoplastic behaviour (n<1). The flow behaviour of coconut milk was well fitted to Bingham model (R^2 = 0.983). From the micrographs, it was inferred that, longer exposure time resulted better fat globule size reduction.

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