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Resarch Article

Enhancing the Emulsion Stability of Coconut Milk by Ultrasonic Treatment

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ABSTRACT

This research aimed to enhance the emulsion stability of coconut milk by nonthermal ultrasonic process. The coconut milk was sonicated at different ultrasound frequency (20, 30 and 40 KHz), treatment time (5, 10 and 15 min) and fat content (8, 10 and 12 %) and analysed using Box Behnken statistical design under response surface methodology. The optimum emulsion stability was achieved with 20 Khz ultrasound frequency, 15 min treatment time and 10% fat content. It is found that the acoustic energy is responsible for fat globule reduction due to energy cavitation and high pressure shock waves. It was also observed that when increasing the ultra sound frequency the AED, Emulsion stability and creaming index are increased while particle size was reduced. The stable coconut milk gave the result as AED, ESI, creaming index and particle size as 4.14 KJ/100 ml, 32.54 min, 15.9 % and 106.53 nm, respectively.

Keywords: Ultra sonication, Non thermal, Coconut milk, AED, ESI, Creaming index and Particle size

INTRODUCTION

Coconut milk is the milky white 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 lauric acid, 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 (Gonzalez, 1986)[3]. The instability of coconut milk will occur because of creaming, flocculation and coalescence mechanism among protein and fat globules (McClements, 2015) [4]. To stabilize the coconut milk by addition of suitable emulsifiers followed by homogenization for reducing the fat globule size (Chiewchan et al., 2005) [5]. Particle size of fat globules plays predominant role in deciding the stability of emulsion. Hence, obtaining uniform small average emulsion droplet diameter becomes essential to achieve stability of coconut milk emulsion.

High pressure homogenizer and ultrasonic devices (sonicators) are frequently used for size reduction of emulsions (Smith and Dairiki 1974; Floury et al., 2000) [6][7]. The final particle size of emulsion in case of ultra-sonication depends on sonication parameters (ultrasound frequency, power, intensity etc.), treatment time, fat content of emulsion, emulsifier and quantity of emulsifier (Smith and Dairiki 1974)[6]. The ultrasound waves cause an interfacial instability at oil-water interfaces (Li and Fogler,1978)[8]. Ultra sound waves are sinusoidal and energy is propagated throughout the system. When the ultrasound travels through liquid medium the formation and bursting of small cavity bubbles will occur due to successive expansion (rarefaction) and compression cycles (Roberts, 1991)[9]. While collapsing of cavity bubble, each bubble will act as a hotspot and generates energy resulting in increasing temperature and pressure with in the medium up to 5000 K and 500 atm, respectively for a fraction of seconds (Suslick, 1989; Vollmer et al., 1998)[10][11]. This cavitation phenomenon will causes the physical and chemical change in liquid.

Ultra sonication is one of the promising methods in processing of coconut milk to maintain emulsion stability and also no work has been done so far on processing of coconut milk using ultra sound technology. The current study aims to optimize the

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ultrasonic frequency, treatment time and fat content based on emulsion stability of coconut milk.

MATERIALS AND METHODS

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)[12] and used for further analysis.

Ultra sonication treatment

The bath type ultra sonicator system (Vidhya 2018, TNAU)[13] used for treating the coconut milk. Three sonication chambers are made, each with different frequency (20, 30 and 40 KHz). The transducer was fixed at the bottom of cylindrical treatment chamber (Diameter = 80 mm and Height = 80 mm). Treatment chamber was fixed with thermocouple to measure the temperature change during treatment. 100 ml of coconut milk (8, 10 and 12% fat content) was sonicated in each treatment chamber for 5, 10 and 15 min. The actual power delivered to the sample was determined by calorimetric method (Povey and Mason, 1998)[14].

Acoustic energy density (AED)

The treated coconut milk temperature (T) was recorded as a function of time using a T-type thermocouple (Tiwari et al., 2009).

Mathematically,

$$\text{Ultrasonic power } (P_{cal}) = mC_p \frac{\Delta T}{\Delta t}$$

Where,

m – Mass of liquid (Kg)

C_p – Specific heat of coconut milk (J/Kg K)

ΔT – change in temperature (K) in sample

Δt – treatment duration (s)

Therefore, the acoustic energy density can be calculated by using following equation.

$$\text{Acoustic energy density (AED)} = \frac{\text{Power (KJ)}}{\text{Volume of the sample (ml)}}$$

Emulsion stability index (ESI)

Emulsion stability index (ESI) of the coconut milk was estimated by using turbidimetric method (Rodsamran and Sothornvit, 2018)[16]. 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 and 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).

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

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

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 4°C. 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 (Jiang et al., 2016)[17].

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

Particle size analysis

The mean particle size of the samples was measured using a laser diffraction particle analyzer (Nano particle size analyzer SZ-100, HORIBA Scientifics, Plate 3.5). The scattering pattern with relative refractive index 1.09 (ratio of the refractive index of coconut oil-1.45 and that of the dispersion medium-1.33) was used to calculate the mean particle size of the droplets by the internal software of the instrument. Coconut milk was diluted using distilled water to 0.1 dilution factor to avoid multiple scattering effects (Tangsuphoom and Coupland 2008)[18]

Statistical design

Box-Behnken method under response surface methodology was used to determine the interactions of three independent variables on three levels. Design Expert Version 6.0.8.1 software was used for analyzing the statistical significance. The complete design consisted of 13 experiments are in Table 1.

Table 1: Ultrasonic treatment combinations with reference to RSM

Treatments	Ultrasonic frequency (KHz) A	Treatment time (minutes) B	Coconut milk fat content (%) C
	20	10	12
	20	10	8
	20	5	10
	20	15	10
	30	10	10
	30	5	12
	30	15	12
	30	5	8

30	15	8
40	5	10
40	15	10
40	10	12
40	10	8

RESULTS AND DISCUSSION

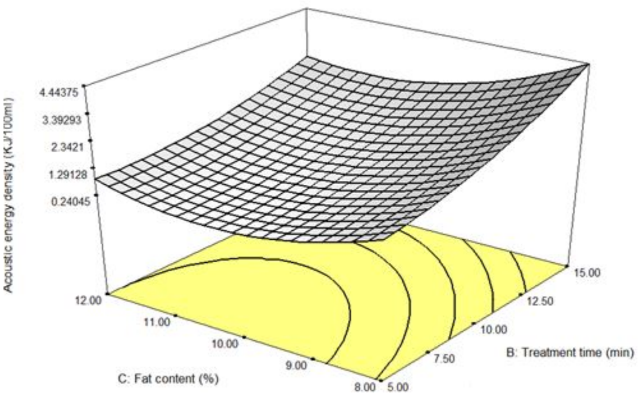
Acoustic energy density (AED)

AED of coconut milk at different ultra sound treatment time and fat content is presented in figure 1. Maximum AED (4.39 KJ/100 ml) was attained for 40 KHz frequency, 10 min treatment time and 8 % fat content. Minimum AED (0.94 KJ/100 ml) was attained for 30 KHz frequency, 10 min treatment time and 10 % fat content. From the figure.1, it was observed that the AED of coconut milk increased significantly ($p < 0.01$) with increase in treatment time and decreased significantly ($p < 0.05$) with increase in fat content.

The best fit second order quadratic equation (R2 value = 0.916) for AED of ultra sonicated coconut milk is as follows.

$$\text{Acoustic energy density (KJ/100 ml)} = + 0.94 + 0.051 *A + 1.11 *B - 0.67 *C + 1.31 *A^2 + 0.45 *B^2 + 0.97 *C^2 - 0.29 *A *B + 0.24 *A *C - 0.31 *B * C$$

Figure 1: Acoustic energy density of coconut milk at different ultrasound treatment time (5, 10 and 15 min) and fat content (8, 10 and 12 %)



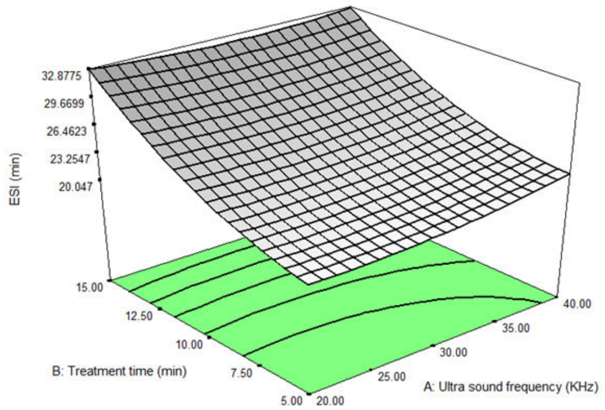
Emulsion Stability Index (ESI)

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). ESI (min) of coconut milk at different ultra sound frequency and treatment time is presented in figure 2. Maximum ESI value of 36.15 min was attained for 20 KHz frequency, 15 min treatment time and 10 % fat content. Minimum ESI value of 18.64 min was attained for 20 KHz frequency, 5 min treatment time and 8 % fat content. From the figure 2, it was observed that the ESI of coconut milk increased significantly ($p < 0.01$) with increase in treatment 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)[19]. Sui et al. (2017)[20] reported that the emulsion stability index of sonicated (20 KHz-150 W) soya bean protein isolate increased from 42.06 min to 221.03 min with increase in treatment time from 12 min to 24 min, respectively.

The best fit second order quadratic equation (R2 value = 0.874) for ESI of ultra sonicated coconut milk is as follows (4.2).

$$\text{ESI (min)} = + 24.20 + 0.58 *A + 5.76 *B + 1.68 *C + 0.72 *A^2 + 2.13 *B^2 - 3.88 *C^2 -0.65 *A *B\ 0.96 *A *C + 0.71 *B *C$$

Figure 2: ESI of coconut milk at different ultrasound frequency (20, 30 and 40 KHz) and treatment time (5, 10 and 15 min)



Creaming index

Creaming index of coconut milk at different ultra sound frequency, treatment time and fat content is presented in figure 3(a) and figure 3(b). Creaming index of coconut milk was maximum (45.24 %) at 20 KHz, 10 min treatment time and 8 % fat and minimum (11.54 %) at 20 KHz, 15 min treatment time and 10 % fat. From the figure 3(a), it was observed that the creaming index of coconut milk decreased significantly ($p < 0.01$) with increase in treatment time. From the figure 3(b), creaming index of coconut milk decreased significantly ($p < 0.05$) with increase in fat content. Creaming index test of ultrasonicated coconut milk was presented in plate 4.1. Decrease in creaming index of coconut milk indicated the increase in stability. This may be due to reduction of fat or protein globule size and prevention of flocculation by the cavitation effect. Lad and Murthy (2012)[21] reported that the sonication (20 KHz with 250 W power) of coconut milk protein and oil emulsion had significant effect on creaming index due to reduction in oil droplet size.

The best fit second order quadratic equation (R2 value = 0.972) for creaming index of ultra sonicated coconut milk is as follows.

$$\text{Creaming index (\%)} = + 19.24 - 1.78 *A - 4.69 *B - 12.33 *C + 2.53 *A^2 + 0.17 *B^2 + 7.38 *C^2 + 4.49 *A *B + 1.69 *A *C - 0.66 *B *C$$

Figure 3(a): Creaming index of coconut milk at different ultrasound frequency (20, 30 and 40 KHz) and treatment time (5, 10 and 15 min)

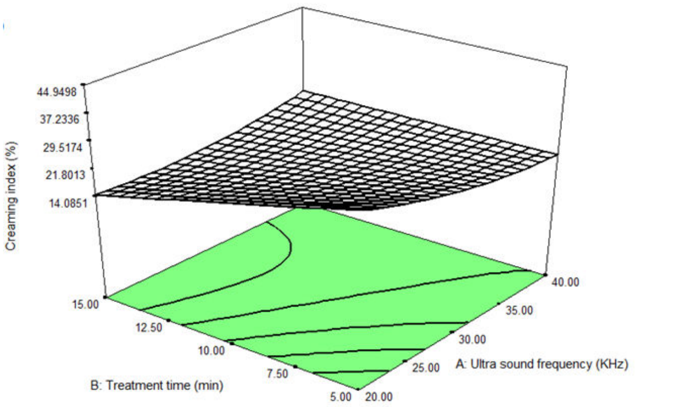
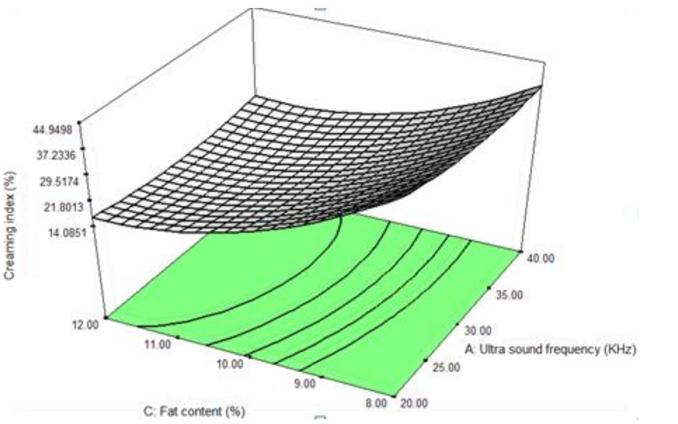


Figure 3(b): Creaming index of coconut milk at different ultrasound frequency (20, 30 and 40 KHz) and fat content (8, 10 and 12 %)



Particle size

The fat globule size (nm) of sonicated coconut milk at different ultra sound frequency, treatment time and fat content is presented in figure 4. The mimum fat globule size (102.8 nm) was obtained for 20 KHz frequency, 15 min treatment time and 10 % fat and the maximum fat globule size (2517.8 nm) was obtained for 40 KHz frequency, 10 min treatment time and 12 % fat. From the figure 4, it was observed that the size of fat globule decreased significantly ($p < 0.01$) with increase in treatment time. This might be due to breakage of fat globules during cavitation process. The micrographs of sonicated coconut milk were represented in plate 1.

The best fit second order quadratic equation (R2 value = 0.981) for fat globule size of ultra sonicated coconut milk is as follows.

$$\text{Fat globule size (nm)} = +892.50 + 171.8 * A - 238.5 * B + 664.25 * C - 33.85 * A^2 - 171.06 * B^2 + 479.43 * C^2 + 140.48 * A * B + 366.9 * A * C + 60.4 * B * C$$

Figure 4: Fat globule size of coconut milk at different ultrasound treatment time (5, 10 and 15 min) and fat content (8, 10 and 12 %)

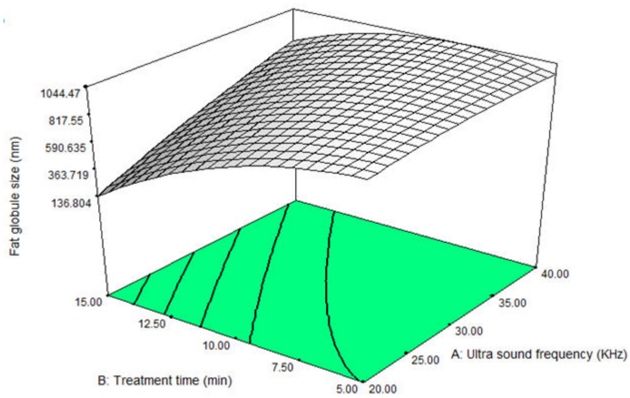
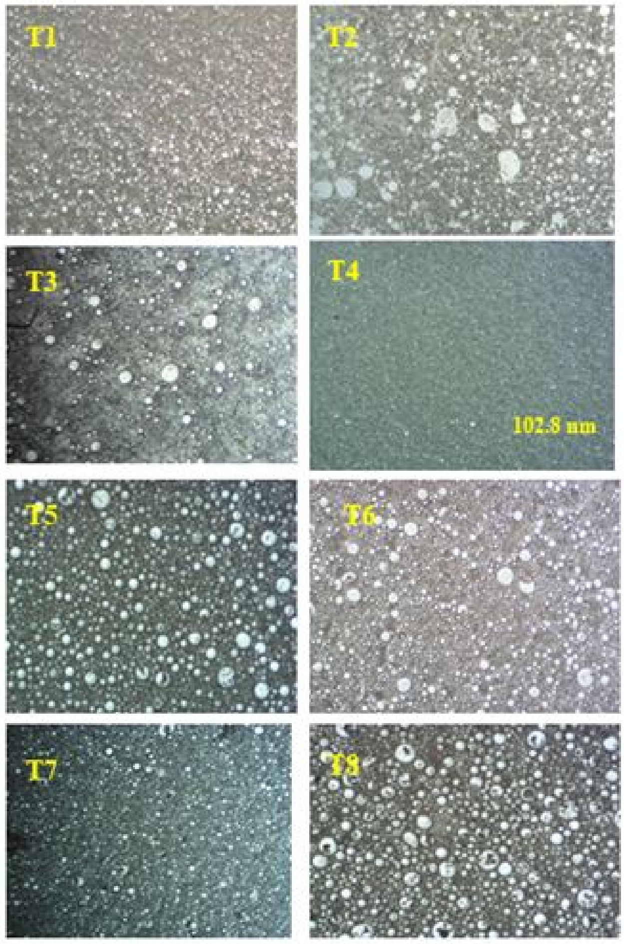
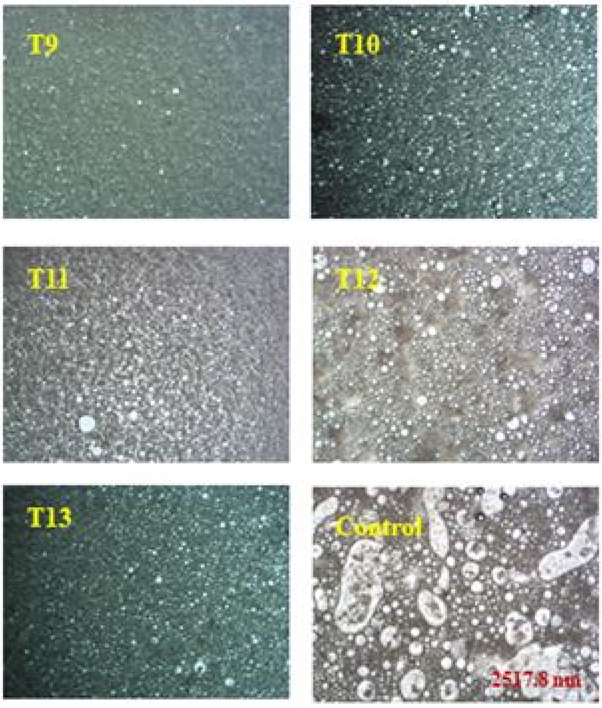


Plate 1: Micrographs of sonicated coconut milk





Optimization of ultra sound treatment parameters was done for getting the stable coconut milk. Among the three frequencies 20 KHz showed better results, due to formation of larger cavity bubbles during sonication. When the frequency increased to 30 and 40 KHz, the size of cavity bubbles decreases due to change in wave length (Golmohamadi et al., 2013). The numerical optimization by the Box Behnken method gave desirable quality of coconut milk at 20 KHz ultra sound frequency, 15 min treatment time and 10 % fat content. The experimental and predicted response variables for optimized treatment parameters are presented in the table 3.

Table 3: Experimental and predicted dependent variables for optimized treatment parameters

Response variables	Experimental values	Predicted Values
Maximum - Acoustic energy density (KJ/100 ml)	4.259	4.168
Maximum - Emulsion stability index (min)	30.982	32.545
Minimum - Creaming index (%)	14.348	15.89
Minimum - Fat globule size (nm)	217.8	106.53

CONCLUSION

This work reveals some of the ways that ultra sonic treatment can affects the properties of coconut milk. The particle size was reduces along the ultrasound frequency increases by the cavitation phenomenon. Among the three frequencies 20 KHz showed better results, due to formation of larger cavity bubbles during sonication. When the frequency increased to 30 and 40 KHz, the size of cavity bubbles decreases due to change in wave length. Finally it was cocluded that the optimum stable coconut milk was achieved at 20 Khz frequency, 15 min treatemnt time and 10 % fat content.

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