Progress Report of the Project done at Tezpur University
Sanctioned by Coconut Development Board,
Government of India

A. Name of the Project:- Processing of coconut milk, development of beverage from curcumin enriched nanoemulsified coconut milk (partially defatted) and pineapple juice and evaluation of their health implications

B. Name of the Principal Investigator:-

Dr. Charu Lata Mahanta from Tezpur University, Tezpur

Dr. Ramaprasad TR from CFTRI, Mysore

C. Name of the Institute undertaking the Project:- Tezpur University

D. Project Duration:- 02 years + three months extension

E. Date of Commencement of the project:- 01/04/2019

F. Date of termination of the present term of the project:- 30/06/2021

G. Objective(s) Accomplishment Summary:-

H.

Sl. No.	**Objectives of the project (as per MoA)**	**Status (Accomplishment of Objectives)**
1	a) To evaluate the physicochemical properties of coconut milk. b) To develop and characterize curcumin enriched nanoemulsified coconut milk (partially defatted) and pineapple juice beverage. (to be done at Tezpur University)	1. The objectives of the project have been completed.

Milestones for Implementation of the Project

Sl.No.	Milestones (As per MoA)		Status (Accomplishment of Milestones)	Outstanding Milestones and reason(s) for delay if any
	Item	**Duration**		
1	Procurement of chemicals, recruitment of JRF/Project Assistant. Purchase of equipment	**01/04/2019 to 30/09/2019**	1. Chemicals fully supplied by vendor against order. 2. Project Assistant has been recruited	None
2	Standardisation of blended beverage of coconut milk and pineapple juice and study of the properties of the beverage	**01/05/2019 to 30/06/2021**	Standardisation of blended beverage of coconut milk and pineapple juice has been done and properties have been studied.	None
3	Characterization of the coconut milk, defatted coconut milk, pineapple juice, blended beverage of defatted coconut milk and pineapple juice, nanoemulsion of virgin coconut oil, water and surfactant, and curcumin enriched nanoemeulsified blended beverage of coconut milk and pineapple juice	**24/11/2020 to 30/06/2021**	1. Biochemical analysis of coconut milk, pineapple juice and blended beverage, sensory evaluation, colour, antioxidant property, and microbial inactivation studies were done. 2. Nanoemulsion and curcumin enriched nanoemulsion in blended beverage were studied for particle size, PDI, mineral composition, proximate composition (minus protein), FE-SEM, FTIR, LCMS were done.	None

Note: Detail progress report is attached separately.

(Signature of the Principal Investigator)

Place: Tezpur

Date: 30/09/2021

Detail Progress Report

Project Title: Processing of coconut milk, development of beverage from curcumin enriched nanoemulsified coconut milk (partially defatted) and pineapple juice and evaluation of their health implications

Objectives

a) To evaluate the physicochemical properties of coconut milk.

b) To study the health implications (nutritional benefits/adversities) of coconut milk in normal and metabolically challenged conditions.

c) To evaluate the physicochemical properties and the health implication (nutritional benefits/adversities) of coconut milk in normal and metabolically challenged conditions.

d) To develop and characterize curcumin enriched nanoemulsified coconut milk (partially defatted) and pineapple juice beverage.

1.0 Introduction

Coconut milk is an important ingredient for Asian cuisine as well as in other parts of the world. The composition of coconut milk varies according to variety, age, growing environment of the coconut, cultural practices, method of preparation, and the process conditions used in extraction, for example, the amount of added water and the temperature used for extraction (Tangsuphoom and Coupland, 2005). Coconut milk is a milky white oil-in-water emulsion extracted from coconut flesh. It plays an important role in many traditional foods of Asian and Pacific regions. Separation of the emulsion into an aqueous phase and cream phase commonly occurs and leads to an unacceptable physical defect of either fresh or processed coconut milk. For typical canned coconut milk process, the addition of suitable emulsifiers and homogenization for reducing fat globule size are required prior to heat treatment to retain the emulsion stability (Chiewchan et al., 2006). There is a need for diversification of coconut milk used in our daily diet. Pineapple (*Ananas comosus* var Smooth cayennes) is one of the common fruits in many tropical and subtropical countries. It is best consumed fresh or in the form of a juice. Pineapple juice contributes to healthy living because it is a good source of vitamins, phenols, organic acids and carbohydrate (Bamidele and Fasogbon, 2017). Blending of coconut milk and pineapple juice offers scope for development of a new food product and this blended beverage can be used as a vehicle to deliver curcumin as a nanoemuslion. Thus, the curcumin enriched nanoemulsion incorporated in coconut milk and pineapple juice can have health benefitting properties in the body. The developed beverage and beverage incorporated with curcumin enriched nanoemulsion have been characterized in this report.

2.0 Materials and methods

Coconuts and pineapples were purchased from local market near Tezpur University, Assam. The pineapples were packed into pouches and stored at –20^{o}C until extraction of juice.

2.1 Sample preparation

2.1.1 Blended beverage of partially defatted coconut milk and pineapple juice

Coconut was dehulled and made into two parts. Coconut was grated from each half of the shell using a scraper. Lukewarm water (1 part) was added to the grated coconut (3 parts) to extract the milk and the whole was filtered through a muslin cloth. The residue in the cloth was further squeezed to extract the milk to the extent possible. The coconut milk was centrifuged at 7000 rpm for 15min and the upper fat layer was removed. The milk was analyzed for fat content using Rose Gottlieb method (AOAC, 2000).

Pineapple was peeled and cut into small pieces and the juice was extracted in a juicer grinder and filtered through muslin cloth. The residual pulp was squeezed to extract the juice to the extent possible.

Coconut milk and pineapple juice were blended in different ratios and all the beverages were standardized to pH 3.5 with citric acid.

2.1.2 Serum separation measurement of blended beverage

Based on some preliminary studies, 0.5% of gum acacia (GA) was added to the blended beverage and homogenized and effect on sedimentation of homogenized beverage was evaluated during storage at 5°C for a period of 20 days (Silvia et al., (2019)

2.2 Development of development of curcumin enriched nanoemulsified coconut milk and pineapple juice

The nanoemulsion was prepared by slightly modified method using different composition of coconut oil, non-ionic surfactant Tween 80 and water. Tween 80 was preferred as surfactant owing to its high hydrophilic–lipophilic balance (HLB) value which is favourable for formulating oil-in-water nanoemulsion (Ghosh et al., 2013). Firstly, oil phase was prepared by dissolving curcumin powder (40 mg) to 100 ml of total emulsion in coconut oil and aqueous phase including surfactant were mixed and homogenized by a high-speed homogenizer at 13500 rpm for 5 min. Then, nanoemulsion was prepared using a high-pressure homogenizer at different pressures from 200-500 bar for 5 cycles (Joung et al 2016; Sari et al. 2015). The parameters of homogenization and concentration of Tween 80 (5-20%) and virgin coconut oil (5-20%) were optimized (process for obtaining virgin coconut oil is given in Section 2.1.1. The experimental design is given in Table 1**.** The number of passes during high pressure homogenization was selected by trial-and-error method and **5 passes** was selected as the best condition.

The well mixed coarse emulsion was homogenized in a two-stage high-pressure homogenizer (GEA, Lab homogenizer Panda Plus 2000, Italy) for 5 pass as per the experimental design (Table 1). Five hundred millilitres of coarse emulsion was processed at each experiment set, and pressure of the second stage was adjusted to about 1/10 of that of the first high-pressure, which generally used in industrial practice to achieve better homogenization (Chutia & Mahanta, 2021).

2.2.1 Virgin oil extraction

Virgin coconut oil (VCO) was produced according to method of (Marina et al., 2009). Endosperm of mature coconut milk was grated and made into viscous slurry and squeezed through cheese cloth to obtain coconut milk. To obtain VCO through the chilling (CH) technique, the coconut milk was centrifuged at 3,500 rpm. The coconut cream obtained was refrigerated for 48 h and was then subjected to mild heating (50ºC) in a thermostat oven for 2 h. The coconut cream was subjected to centrifugation at 3,500 rpm to separate the oil from the cream. To obtain VCO through the fermentation technique, coconut milk was left standing at room temperature for 12 h. The oil settled on the upper layer was pipetted out and used for further analysis.

Table 1: Real and coded values of the independent parameters of the processes

Experimental Variables	Code	Coded levels		
		-1	**0**	**+1**
Oil (%)	A	5	12.5	20
Stabilizer (%)	B	5	12.5	20
Pressure (bar)	C	200	350	500

2.2.2 Experimental design

In this study, designing of the experiments and its optimization condition was performed using Response surface methodology (RSM). For modelling the experimental process, face centered composite design (FCCD) was used. The predicted model was fitted to a second-order quadratic polynomial. Design-Expert Version 7.1.2 (Stat-Ease, Inc. MN) was used for the experimental design process. As per the design, a total of 19 experimental runs were carried out to find the suitability of the model.

The experiment variables, given in Table 1, were coded according to Eq. (1) (Chutia & Mahanta, 2021)

$$y_j = \frac{Y_j - Y_{j0}}{\Delta Y_j} \quad (1)$$

where Y_j and y_j indicates the actual and coded values of the "j" experimental variable, Y_{j0} is actual value of the "j" experimental variable at the central point, and ΔY_j is the step change of the dimensionless value

2.2.3 Optimization

Optimization of nanoemulsion production processes were performed by setting the desired goal of output parameters of particles size and PDI value as minimum for the two response parameters. A second order polynomial equation was used to predict the response and optimization was performed based on higher value of desirability reported (Chutia & Mahanta, 2021).

2.2.3 Addition of curcumin enriched nanoemulsions in blended beverage of coconut milk and pineapple juice

Curcumin enriched nanoemulsion **(**0.9375 g) was incorporated into 100 g of blended juice to produce nanoemulsified beverage.

2.2.4 Codes given to the different samples prepared

The samples were coded as given in Table 2.

Table 2. Sample codes

S. No	Sample	Abbreviations
1	Coconut milk	CM
2	Defatted coconut milk	DCM
3	Pineapple juice	P
4	Coconut milk and pineapple juice blend	CP
5	Curcumin enriched nanoemulsion	CRN
6	Nanoemulsified coconut milk and pineapple juice	CPN
7	Gum acacia treated blended beverage	CPGA

2.2.5. Effects of storage time on emulsion physical stability

Emulsion stability was evaluated during storage in darkness at 6±2°C and 25±4°C. The emulsion stability index (ESI) was determined monitoring the extent of gravitational phase separation for 28 days at an interval of 7 days according to Chutia and Mahanta, (2021).

2.2.6 Freeze drying of blended beverage and curcumin nanoemulsion enriched blended beverage

Blended beverage and curcumin enriched nanoemulsified coconut milk and pineapple juice were freeze dried for animal study at (CFTRI) Central Food Technology Research Institute, Mysore using lyophilizer. The heat plate temperature of the freeze dryer was 20 °C and the condenser temperature was –40 °C for 24h. Total of 28 L of beverage and emulsion were used to produce 1.5 kg of dried blended beverage sample (Baeghbali et al. 2016).

2.3 Analysis of the coconut milk: pineapple juice blended beverage

2.3.1 TSS content

The total soluble solid content was determined by using a portable hand refractrometer (0-32ºBrix) by placing a drop of sample solution on its prism (Ranganna 1986).

2.3.2 pH value

This was determined directly by digital pH meter (PB700 EuTech).

2.3.3 Total titratable acidity

TTA was determined by titration 5ml of sample with 0.1N NaOH solution, using 1% phenolphthalein indicator (Ranganna 1986).

%Total acid = (T*N*E*100)/(V*100)

Where, T = titre,

N= Normality of NaOH

E= Equivalent weight of acid

V= Volume of sample taken for estimation

2.3.4 Color measurement

The color of the juice blends was measured using Hunter Lab Colorimeter (Ultrascan, VIS-Hunter Associates Lab.) that was calibrated with a white tile.

2.3.5 **Antioxidant properties**

Antioxidant property like total phenol content, total flavonoid content, DPPH scavenging activity of blended juice was determined by using method of Hossain and Rahman (2010); Martos *et al*. (2010). FRAP and metal chelation activity of juice were measured by the method of Saikia *et al*., (2016).

2.3.6 Sensory evaluation

Sensory analysis was performed with 10 panellists for sensory parameters of aroma, taste and colour and overall acceptability using 9 point hedonic scale.

2.3.7 Assessment of bacterial inactivation time in beverage

Three different types of bacterial strains were taken for this study which included *Bacillus cereus, Listeria monocytogene and Staphylococus aureus.* The bacterial strains were harvested from stock culture in the Luria Bertani (LB) broth at 37 ± 1°C for 18 ± 1h. After harvesting, the overnight grown culture were centrifuged on 6000 rpm for 10min at 25ºC and the supernatant was washed with phosphate buffer saline (PBS), washing with the PBS step was followed twice. After washing the bacterial culture were mixed into the pineapple juice and coconut milk blend in the ratio of 1:10 and kept for 30 min to acclimatize. The above mentioned and inoculated above mentioned grown culture after that thermal treatment were done at different time and temperature combinations 60ºC,70ºC,80º C and 90ºC for 5sec, 10s, 20s, 40s, 60s, and 120s, respectively. Inoculations of each bacterial cultures were done individually for individual bacteria and effect of treatment were studied (Topalcengiz, 2019).

2.3.8.1 Microbiological enumeration and enrichment

After thermal treatment, the population of tested microorganisms in centrifuge tubes were diluted and spread plated in duplicate. Thermally treated juice were plated on LB agar for *L. monocytogenes, B. cereus and S. aureus* and incubated at 37 ± 1°C for 24 ± 1h. For enumeration, serial dilutions of thermally treated juice were done and spreading of serially diluted juice were done on the LB agar and kept in 37ºC for 18-24 h. After incubation, grown colonies were counted and effect of heat on lethality on microorganism in juice was studied.

2.4 Characterization of nanoemulsions

Droplet size distribution and polydispersity index (PDI) of coconut oil nanoemulsion was determined using a DLS (Nanoplus, Zeta and nanoparticle analyser) Branco et al., 2020.

2.4.1 Proximate analysis

The moisture, ash fat, protein and amylose content of blended juice and nanoemulsified juice were determined according to AOAC (2000).

2.4.2 DPPH radical scavenging assay

Briefly, 0.2 mM solution of DPPH in 80% ethanol was prepared and 50 μL of the solution was added to 150 μL of nanoemulsion described by (Joung et al., 2016). After incubating the samples in the dark for 30 min, the absorbance was measured at 517 nm against blank samples lacking scavenger.

2.4.3 Field emission scanning electron microscopy (FE-SEM)

The morphology study of dried juice samples with and without nanoemulsion was carried out using the FE-SEM (ZEISS, SIGMA, Germany) after gold coating of samples (Mary et al. 2019).

2.4.4 Atomic absorption spectroscopy for determination of the minerals

The samples were subjected to hot digestion with nitric acid and hydrogen peroxide. A solution composed of 5 mL of samples, 2 mL of 30% H_2O_2 and 1 mL of 65% HNO_3 was heated to a temperature of 75 °C in a thermodigester until discoloration of the sample. After discoloration, the liquid was made up to 20 mL with ultrapure water and filtered through qualitative filter paper, following the methodology of Dutra et al. (2018). Concentration of Na, K, Ca, Mg, Mn, Fe, and Zn were determined.

2.4.5 Chromatographic analysis of phenolic acids

Twelve mL of 80% methanol (v/v) was added to 1 g of blended beverage and curcumin enriched nanoemulsified coconut milk and pineapple juice was filtered through a 0.45-µm pore-size membrane filter before injection (Irkin et al. 2015). The phenolic compounds that were present was determined using LC/MS equipment (410 Peostar Binary LC with 500 MS IT PDA Detector).

2.4.6 GC-HRMS analysis of virgin coconut oil

Analysis of FAME was performed on a GC-HRMS EI/CI source time of flight analyser mass range -10-2000amu, mass resolution - 6000 with a FID detector and Head space injector. Helium was used as the carrier gas at a flow rate of 1.99 mL/min and a split ratio of 1:10 (Moigradean et al. 2013).

3. Results and Discussion

3.1 Fat content in coconut milk

The fat content in defatted coconut milk was 0.3 % (Table 3). The defatted milk was used for blending with pineapple juice.

Table 3 Fat content in coconut milk and defatted coconut milk

Sample	Fat (%)
Coconut milk	21.2±0.03
Defatted coconut milk	0.3±0.02

3.2 Biochemical properties of blends of coconut milk and pineapple juice

The pH, °Brix, titratable acidity and ascorbic acid content of the coconut milk and pineapple juice blends that were blended in different ratios are presented in Table 4. pH of blended juice decreased with increase in the concentration of pineapple juice and titratable acidity correspondingly increased.

Table 4. Biochemical properties of blends of coconut milk and pineapple juice

Coconut milk : Pineapple juice	pH	°Brix	Titratable acidity (%)
100:0	6.09±0.02	11.0±0.00	0.40±0.01
80:20	5.49±0.01	11.0±0.02	0.41±0.05
70:30	5.3±0.02	11.5±0.01	0.42±0.01
60:40	5.09±0.01	11.0±0.01	0.44±0.03
50:50	4.89±0.00	12.5±0.03	0.44±0.01
40:60	4.76±0.03	11.0±0.02	0.51±0.01
30:70	4.59±0.01	11.9±0.01	0.53±0.00
20:80	4.45±0.07	10.8±0.01	0.63±0.02
0:100	3.88±0.01	10.8±0.00	0.80±0.00

DCM=Defatted coconut milk, P= Pineapple juice

3.3 Mineral content in the non- emulsified and emulsified beverage

The seven different minerals determined in the samples are given in Table 5. K, an essential mineral for controlling the salt balance in human tissues, was the most abundant in defatted coconut milk i.e. 963.85mg/L and coconut milk 662.35mg/L and most abundant in pineapple juice observed by Lu et al. (2014). Mg is the second abundant mineral in coconut milk (68.26mg/L) and pineapple juice (114.45mg/L). Na is third prominent mineral in coconut milk and Ca is the third preponderant mineral in pineapple juice and fourth in coconut milk. The blended beverage is therefore rich in calcium. Some trace elements (e.g., Fe, Zn and Mn) in plants are known to be very low. As shown in (Table 4) Fe and Mn are in lesser amounts (9.08mg/L to 0.90mg/L and 5.75mg/L to 1.45mg/L for coconut milk and defatted coconut milk) than Zn and Mg in the blended beverage.

3.4 Antioxidant properties of the blends of coconut milk and pineapple juice

The antioxidant activity and the total phenolic content of the blends of defatted coconut milk and pineapple juice indicate that the blends have high phenolic content, flavonoid content and high antioxidant activity (Table 6). The blends showed high content of phenolic acid. The blends exhibited good antioxidant properties as determined by different assays.

Table 5. Mineral composition of the juice samples

Samples	Na (mg/L)	Ca (mg/L)	K (mg/L)	Fe (mg/L)	Zn (mg/L)	Mn (mg/L)	Mg (mg/L)
CM	44.18	22.50	662.35	9.08	33.04	5.16	68.26
DCM	39.305	14.50	963.85	0.90	5.00	1.45	64.70
P	17.13	85.00	618.30	1.76	9.92	5.75	114.45
CP	27.89	52.20	415.42	2.14	10.74	3.52	72.70

Table 6. Antioxidant activity of blended beverage

Sample ratio C:P	Total phenolic content (GAE mg/L)	Total flavonoid content (QEmg/L)	DPPH (%)	Metal chelation capacity (%)	FRAP (µM/100g)
100:0	17.90±0.01	3.51±0.1	66.89±0.01	47.15±0.1	15.01±0.01
80:20	15.00±0.03	4.02±0.1	76.67±0.02	50.97±0.01	15.49±0.02
70:30	15.98±0.01	4.40±0.01	83.02±0.01	52.46±0.02	16.61±0.20
60:40	18.79±0.01	4.79±0.02	85.33±0.01	56.58±0.10	16.77±0.10
50:50	19.50±0.04	5.48±0.20	86.05±0.02	67.79±0.10	17.11±0.10
40:60	20.70±0.04	3.75±0.10	87.00±0.01	64.92±0.00	17.89±0.20
30:70	22.48±0.01	3.67±0.04	87.78±0.04	60.10±0.10	18.93±0.01
20:80	24.61±0.02	3.39±0.04	88.67±0.04	52.67±0.10	20.53±0.10
0:100	26.21±0.05	3.38±0.05	89.65±0.03	52.94±0.10	21.53±0.10

C=defatted coconut milk, P= Pineapple juice

3.6 Stabilizing capability of GA

Addition of GA was able to prevent a layer separation at the bottom till 25 days. For the raw beverage, at the beginning stage of storage, stability of beverages rapidly reduced because larger sized particles collide, aggregate, and decant rapidly. It was observed that GA increased the viscosity of beverages, reduced the mobility and coalescence of the particles and acted as emulsifying and stabilizing agent.

3.7 Colour of blends of coconut milk and pineapple juice

The colour of all blends was yellow in colour that varied with the proportion of pineapple juice (Table 7). Lightness and yellow colour decreased as pineapple juice content was increased.

Table 7. Color measurement using Hunter Lab

Coconut milk: Pineapple	L*	a*	b*
80:20	60.22±0.001	-2.2±0.001	4.27±0.01
70:30	58.24±0.001	-2.51±0.01	4.68±0.002
60:40	55.72±0.002	-2.76±0.002	6.25±0.001
50:50	55.79±0.001	-3.23±0.01	6.04±0.02
40:60	53.84±0.002	-3.67±0.001	6.64±0.01
30:70	51.07±0.01	-4.02±0.02	6.34±0.01
20:80	44.73±0.02	-3.91±0.001	6.63±0.001

3.8 Sensory evaluation of the blends of coconut milk and pineapple juice

Sensory analysis was performed with 10 panellists for sensory parameters of aroma, taste and refreshing appeal and overall acceptability using 9 point Hedonic scale (Table 8). The blend with the maximum proportion of coconut milk that scored near to the blends with higher pineapple juice was selected, i.e., the blend with 50:50 ratio of defatted coconut milk and pineapple juice was selected and was pasteurised at different time and temperature combinations to determine the thermal death time of different microorganisms.

3.5 Thermal inactivation of microorganisms

Fig. 1 shows the thermal death time for *S.aureus* in coconut milk:pineapple juice blend (50:50 ratio). Figs 2-3 show the thermal inactivation of *S.aureus* at different temperature for 5 and 120 s. Bacterial enumeration at 10s, 20s, 40s, 60s are not presented. Bacterial growth reduced with increase in time and temperature of heat treatment. D value or thermal death times indicates the time required to reduce the microorganism by one log cycle which also indicates the thermal death behaviour of the microorganism. The D values of the *S. aureus* microorganism were determined for different time and temperature with and without the effects of lactic acid. The D value for 60, 70, 80 and 90 ^{0}C for blend without lactic acid was 62.18, 45.24, 42.55 and 28.81s respectively. D value for blend with 1% lactic acid was 42.02, 38.47, 33.33 and 27.32s, respectively. D values for the blend without lactic acid was higher than the blend with lactic acid. *Bacillus cereus* and *L. monocytogenes* did not show any growth at 60ºC for 5s (Fig.4).

Table 8. Sensory scores of blends of coconut milk and pineapple juice

Sample	Sensory parameters			
concentration	Aroma	Taste	Color	Overall acceptability
20:80	6.2	5.9	6.5	6.3
30:70	5.8	7.3	7.6	7.0
40:60	7.0	7.2	7.6	7.6
50:50	7.8	7.6	7.2	7.9
60:40	8.0	8.3	8.0	8.0
70:30	8.0	8.2	8.4	8.1
80:20	8.4	8.1	8.5	8.4

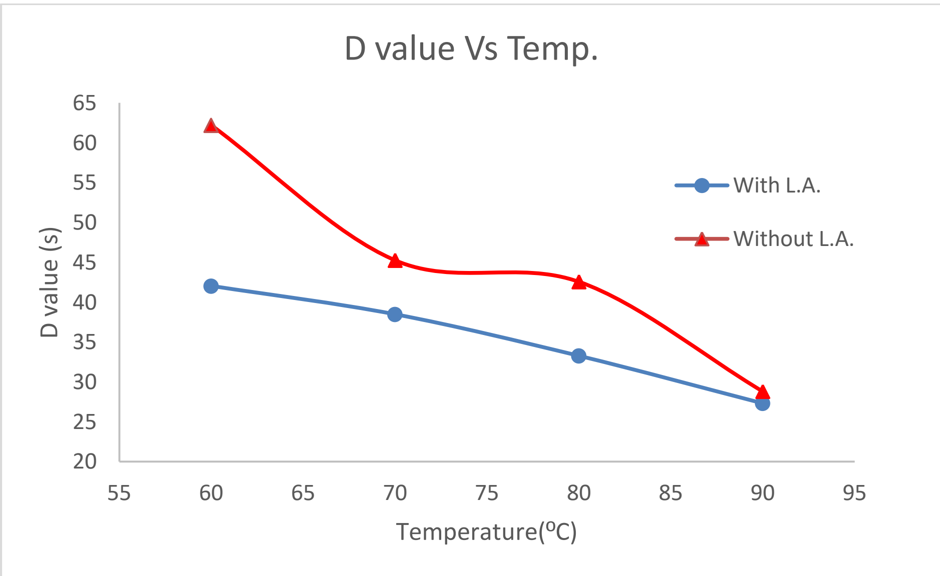


Fig.1. Thermal death time for *S.aureus* in coconut milk:pineapple juice blend (50:50 ratio)

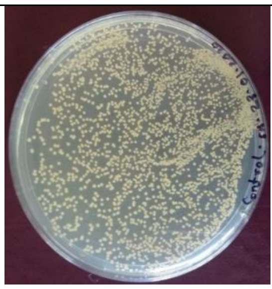
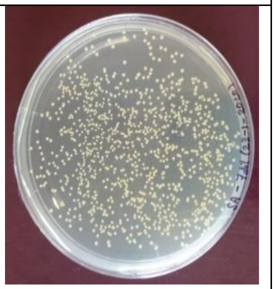
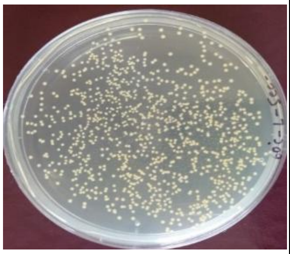
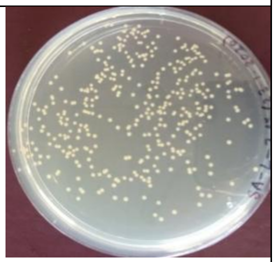
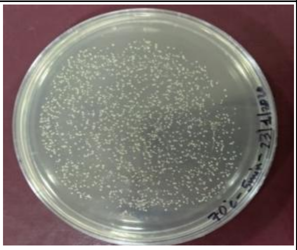
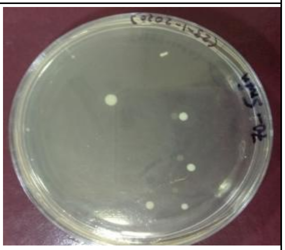
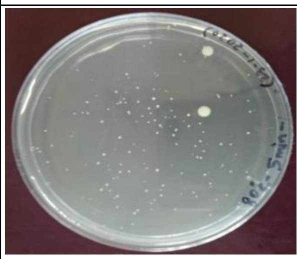
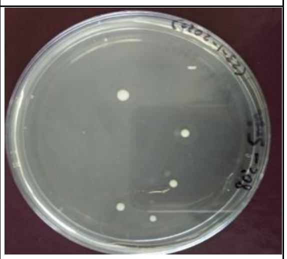
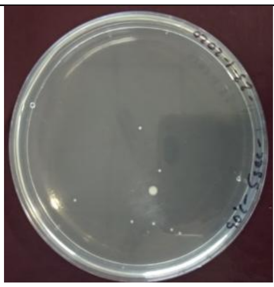
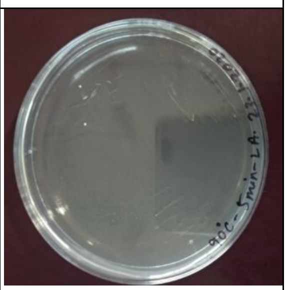
	Without lactic acid	With lactic acid
	 Control	 Control
60ºC		
70ºC		
80ºC		
90ºC		

Fig.2. Plates showing *S.aureus* growth at different temperatures for 5s after two dilutions of blends with and without lactic acid

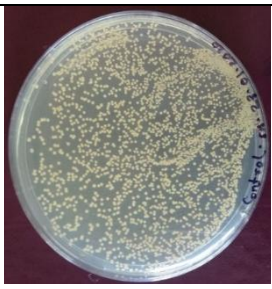
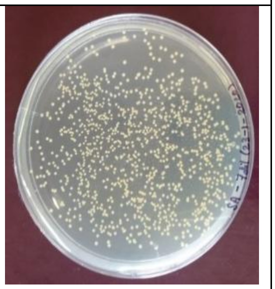
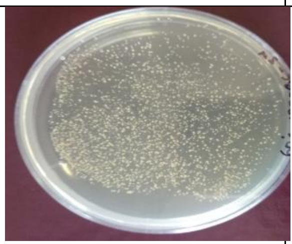
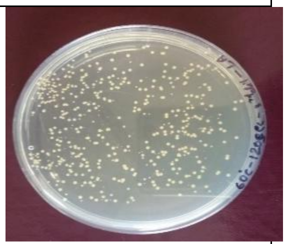
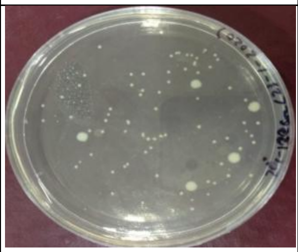
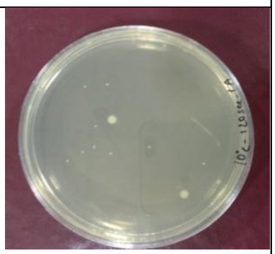
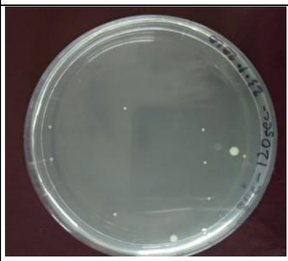
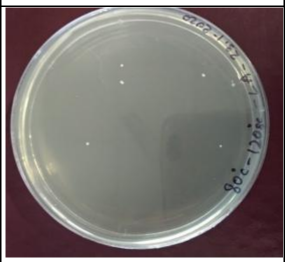
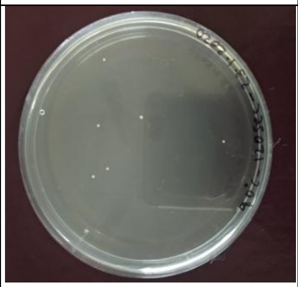
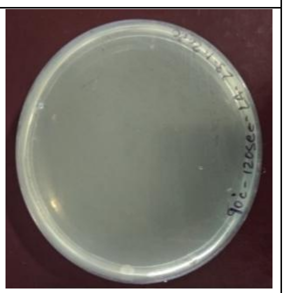
	Without lactic acid	With lactic acid
	 Control	 Control
60ºC		
70ºC		
80ºC		
90º		

Fig.3. Plates showing *S.aureus* growth at different temperatures for 120 s after two dilutions of blends with and without lactic acid

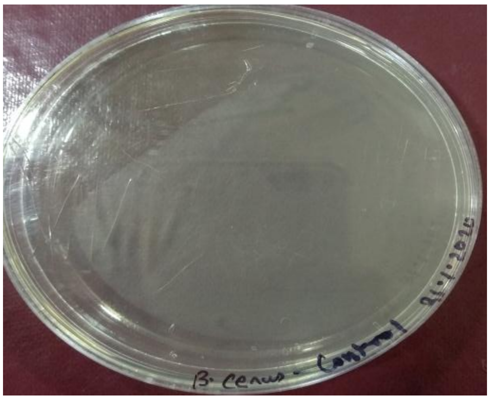
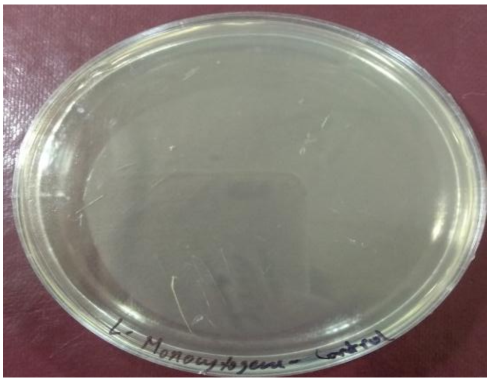
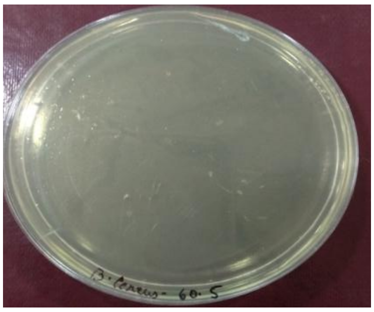
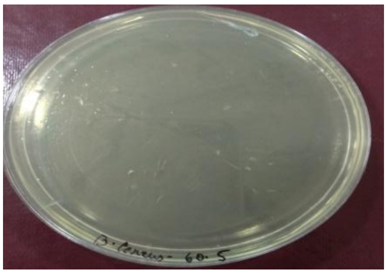
Plates for *Bacillus cereus*	Plates for *L. monocytogenes*
	
Control : Raw sample with citric acid	Control:Raw sample with citric acid
	
Plates with treatment temp 60ºC for time 5s	Plates with treatment temp 60ºC for time 5s

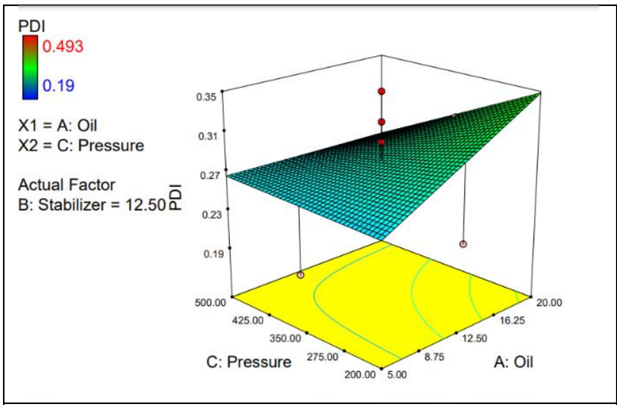
Fig. 4. Plated showing no growth of *Bacillus cereus* and *L. monocytogenes* at 60ºC for 5s

From the thermal death time figure, temperature of 90^{o}C for 30 s was selected for thermal treatment of coconut milk: pineapple juice in the ratio of 50:50. The blend treated this way will be used for developing nanoemulsion incorporated beverage.

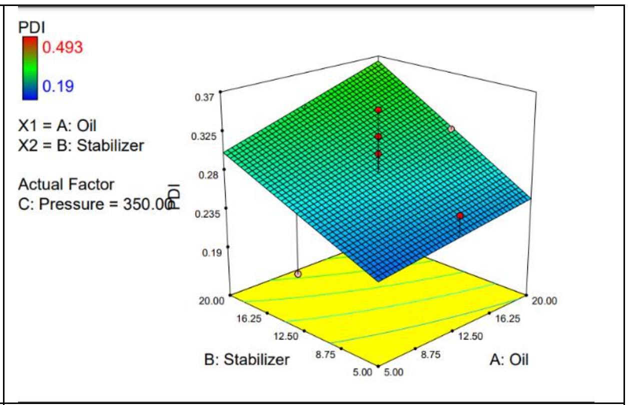
3.9 Naoemulsion preparation and effect of parameters on size and PDI value

Effects of oil content on particle size and PDI of the nanoemulsion

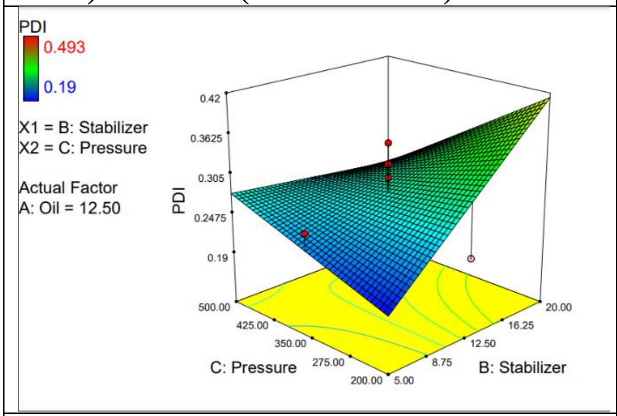
In this study, as shown in Fig 5A, 5B, 5D and 5E, the emulsion size was found to slightly decreased in particles size and PDI with an increase of oil content up to 7%, thereafter increase the size and PDI values were observed with further increase in oil content. The overall effect of oil content was found to be significant for size (p=0.0194 < 0.05) and non-significant for PDI value (p=0.1294>0.05) (Table 9 and 10).



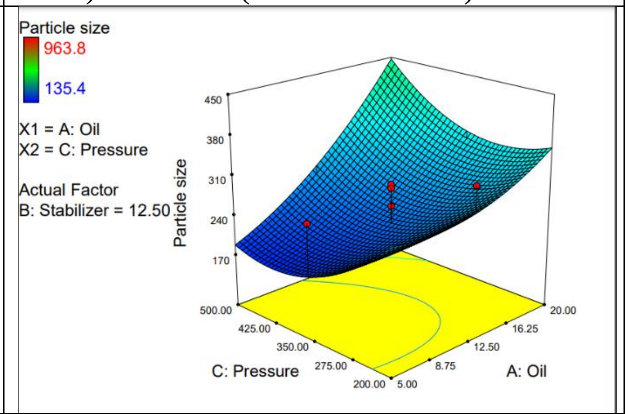
A) PDI value (Pressure and Oil)



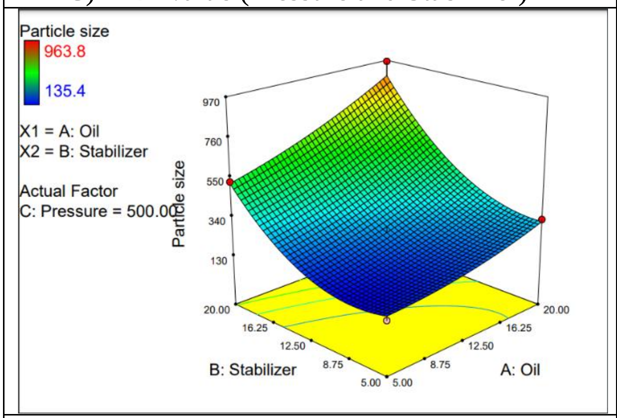
B) PDI value (Stabilizer and Oil)



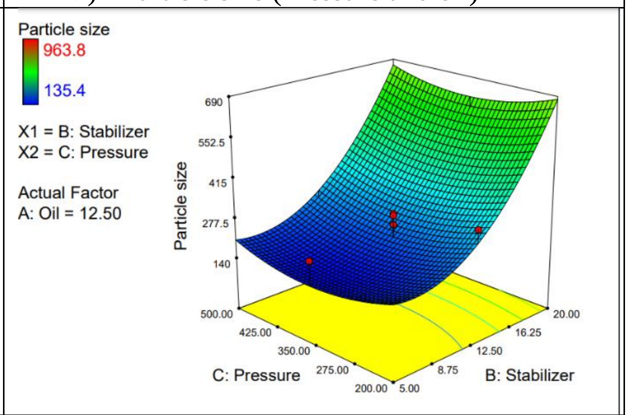
C) PDI value (Pressure and Stabilizer)



D) Particle size (Pressure and oil)



E) Particle size (Stabilizer and oil)



F) Particle size (Pressure and stabilizer)

Fig. 5. Effects of pressure, oil and stabilizer on particles size and PDI value.

Effects of stabilizer content on particle size and PDI of the nanoemulsion

In this study, size of droplet and PDI value was seen to gradually increase with an increase in stabilizer content from 5 to 20% (Fig. 5B, 5C, 5E and 5F). For both particles size and PDI value, the effect of stabilizer was found to be significant.

Effects of pressure content on particle size and PDI of the nanoemulsion

In the industry, homogenization pressures normally used in the range varied between 200 and 500 bar (Wang et al., 2008). So, in this study pressure was chosen in the industrial range only. The effects of pressure on responses, particles size and PDI was found to be non significant (p>0.05 (Table 9 & 10). The particle size and PDI value decreased as high pressure increased from 200 bar to approximately 260 bar (Fig. 5A, 5C, 5D and 5F) followed by an increase in size and PDI with further increase in pressure, where other parameters were remain same. Similar trend was observed by Chutia & Mahanta, (2021).

Modeling and validation

The quadratic equations fitted with designed experimental data (FCCD design) to explain the effect of input parameters is represented in Eqs. (2 & 3)

Particles size= 235.76+ 81.97*A+ 234.52*B- 4.03*C +52.07*A*B+ 47.76*A*C-1.78*B*C+31.70*A*A+ 150.95*B*B+ 53.90*C*C (2)

PDI value= 0.28+ 0.022*A+ 0.049*B- 0.020*C + 0.009375*A*B - 0.023*A*C- 0.061*B*C (3)

Table 9: ANOVA table of nanoemulsion preparation process (Particles size)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	Remarks
Model	8.638+005	9	95983.22	11.52	0.0006	Significant
A-Oil	67185.89	1	67185.89	8.06	0.0194	
B-Stabilizer	5.500E+005	1	5.500E+005	66.02	<0.0001	
C-Pressure	162.65	1	162.65	0.020	0.8920	
AB	21687.16	1	21687.16	2.60	0.1411	
AC	18247.19	1	18247.19	2.19	0.1730	
BC	25.45	1	25.45	3.055E-003	0.9571	
A2	2745.60	1	2745.60	0.33	0.5800	
B2	62259.35	1	62259.35	7.47	0.0231	
C2	7937.92	1	7937.92	0.95	0.3545	
Residual	74980.21	9	8331.13			
Lack of Fit	66417.80	5	13283.56	6.21	0.0507	Not significant
Pure Error	8562.41	4	2140.60			
Cor Total	9.388E+005	18				
R^2				0.9201		

There was a good correlation between the predicted values, calculated by using the model equation and experimental value, indicating the good fitting of the model. R^2 value was 0.92 and 0.757 for particles size and PDI value, respectively, which indicated the good correlation between the input and response variables. Lack of fit for both response parameters was found to be non-significant and the model was highly significant (0.0006) (Table 9) and 0.0036 (Table 10) for particles size and PDI, respectively, this validated the models i.e. models could explain the experimental data with high efficiency.

Table 10: ANOVA table of nanoemulsion preparation process (PDI)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	Remarks
Model	0.067	6	0.011	6.23	0.0036	Significant
A-Oil	4.752E-003	1	4.752E-003	2.65	0.1294	
B-Stabilizer	0.024	1	0.024	13.18	0.0035	
C-Pressure	3.881E-003	1	3.881E-003	2.16	0.1669	
AB	7.031E-004	1	7.031E-004	0.39	0.5429	
AC	4.186E-003	1	4.186E-003	2.34	0.1524	
BC	0.030	1	0.030	16.67	0.0015	
Residual	0.022	12	1.793E-003			
Lack of Fit	0.017	8	2.104E-003	6.21	0.0507	Not significant
Pure Error	4.680E-003	4	1.170E-003			
Cor Total	0.089	18				
R2				0.7570		

3.10 Optimization

As shown in Table 11, optimized conditions consisting of 6.86% oil, 5 % GA and 257.32 bar pressure gives the maximum desirability level of 0.965. At these conditions, predicted value of particles size and PDI values were 191.798 and 0.19, whereas the optimized experimental values were 192.24 and 0.21, respectively.

Table 11: Optimization condition of nanoemulsion

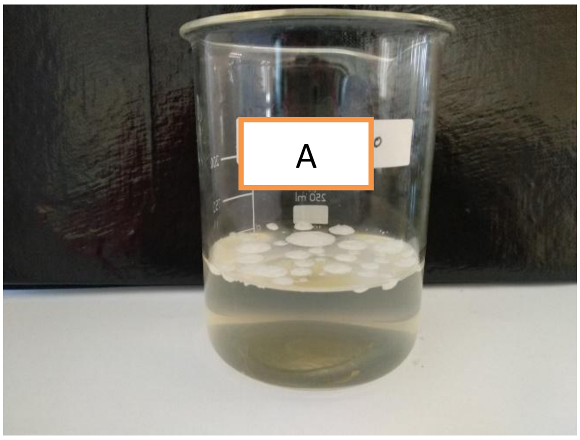
Oil content (%)	Stabilizer (%)	Pressure (bar)	Particle size	PDI	Desirability
6.86	5	257.32	191.798	0.19	0.965
6.95	5	256.50	191.807	0.189	0.965
6.66	5	259.12	191.82	0.19	0.965
6.16	5	263.85	192.5	0.192	0.965

3.11 Physical activity of optimized nanoemulsion

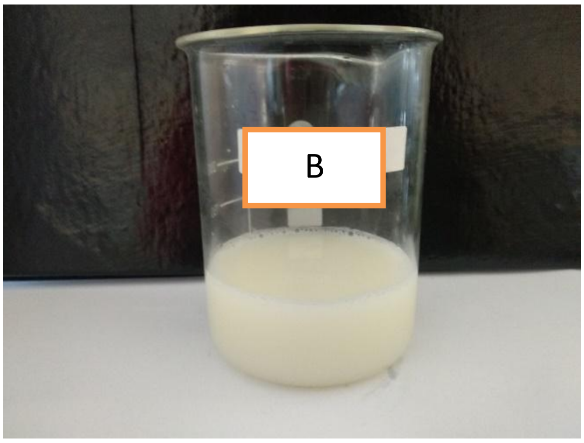
Nanoemulsion stability was evaluated during storage in darkness at 6±2°C. The emulsion stability index (ESI) was determined monitoring the extent of gravitational phase separation during 28 days according to Chutia & Mahanta, (2021). During the storage, 25° C emulsion separated within 14 days whereas no separation was observed upto 28 days.

3.12 Characterization of nanoemulsion

Fig. 6a shows the mixture of oil, surfactant and water before homogenization and Fig. 6b shows the homogenized mixture. The physicochemical properties of nanoemulsions were significantly dependent on the ratio of oil, surfactant, and water in the mixture. In this study Tween 80 was used as surfactant owing to its high hydrophilic–lipophilic balance (HLB) value which is favourable for formulating oil-in-water nanoemulsion (Ghosh et al., 2013).



(a) Nanoemulsion before homogenization



(b) Nanoemulsion after homogenization

Fig 6. Mixture of virgin coconut oil, Tween 80 and water before homogenization (a), and after homogenization (b).

Various ratios of oil, surfactant, and homogenization pressure were tested to optimize the O/W emulsion formations. Nanoemulsion were stabilized and optimized with 5 cycles of high-pressure homogenization. Addition of curcumin converted the colour of the nanoemulsion from white (Fig. 7a) to yellow (Fig 7b). The droplet size and PDI of curcumin enriched nanoemulsion (CRN, Fig. 3b) ranged from 135nm to 963nm with PDI vales ranging from 0.281 to 0.378. The smallest particle size was observed in the nanoemulsion with 5% coconut oil and 5% surfactant with 200Mpa pressure and this nanoemulsion showed DPPH scavenging activity of 100.50±0.45%. The droplet size of CRN was significantly dependent on the ratio of oil, surfactant and homogenising pressure. In order to form stable nanoemulsions, the amount of aqueous phase should be at least 2 to 3 times higher than the total amount of oil phase and surfactant, which is also supported by Joung et al. (2016).

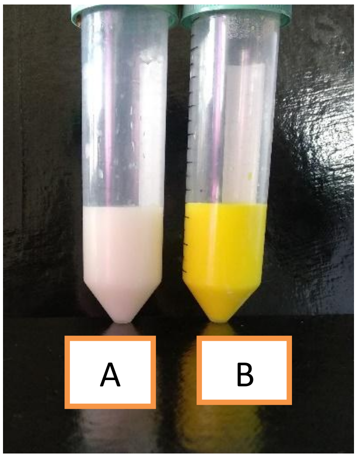


Fig 7. (a) Nanoemulsion without curcumin, and (b) Nanoemulsion with curcumin

3.13 Proximate analysis

Moisture, ash, fat, protein, crude fiber and carbohydrate content of freeze dried CP was 2.70±1.20%, 4.94±0.33%, 0.01±0.00%, 10.11±0.2%, 3.54±0.60% and 78.74±2.80%, respectively and for CPN was 2.98±1.11%, 5.8±0.05%, 0.44±0.10%, 10.13±0.1%, 3.65±0.20% and 77.00±2.45%, respectively (Table 12). The ash and fat content is slightly higher in nanoemulsified juice compared to blended juice without nanoemulsion.

Table 12. Proximate analysis freeze dried powder blended beverage with and without nanoemulsification

Sample	Moisture (%)	Ash (%)	Fat (%)	Protein %	Crude fiber (%)	Carbohydrate by difference (%)
CP	2.70±1.20	4.94±0.33	0.01±0.00	10.11±0.90	3.54±0.60	78.74±2.80
CPN	2.98±1.11	5.8±0.05	0.44±0.10	10.13±0.70	3.65±0.20	77.00±2.45

3.14 FTIR spectroscopy of the samples

FTIR of the freeze-dried samples were performed and the functional groups were determined as presented in Fig. 8 and Table 13.

(a) (b)

Fig.8 Freeze dried (a) CP and (b) CPN.

CP: Blended beverage of defatted coconut milk and pineapple juice;

CPN: Nanoemulsified blended beverage of defatted coconut milk and pineapple juice

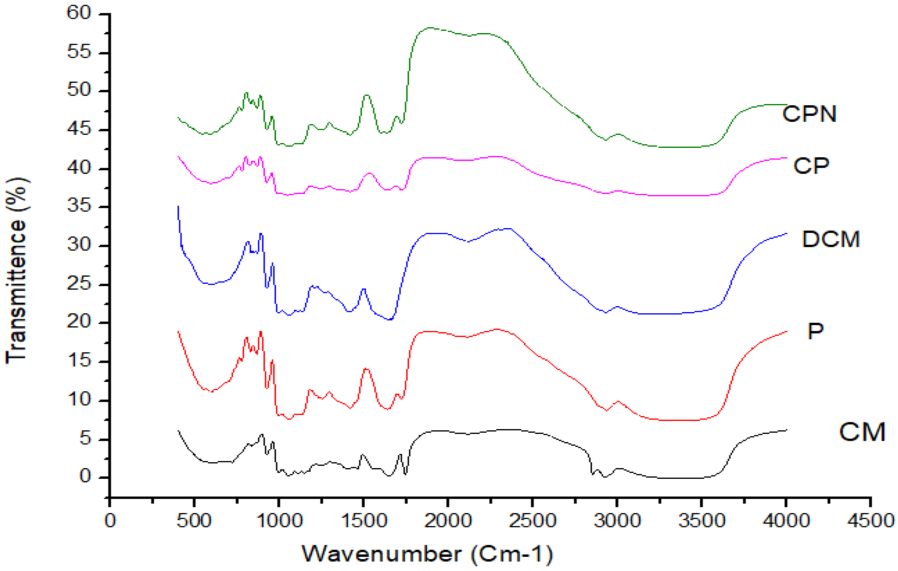
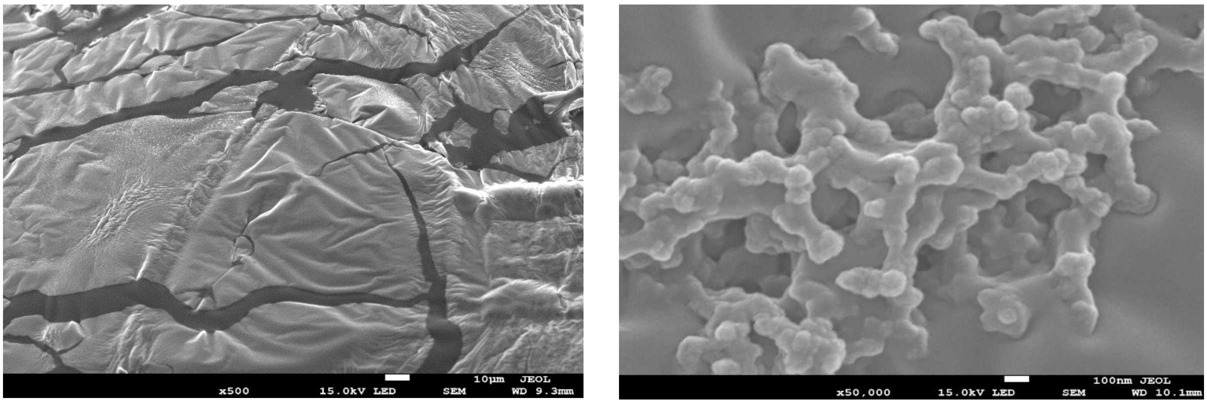


Fig 9. FT-IR spectra of CM- Coconut milk, DCM- Defatted coconut milk, P- Pineapple juice, CP- coconut milk and pineapple juice and CPN- nanoemulsified blended juice.

Table 13. FT-IR analysis showing functional group stretching with wavenumber

Sample name	Wavenumber (cm^{-1})	Functional group
CM	3247-3511	N-H
	1051-1107	C-C
	1641	CO
DCM	3157-3312	O-H
	1648	CO
P	3272-3518	N-H
	1641	CO
	1055-1247	C-C
CP	3249-3480	N-H
	1051-1247	C-C
	1641	CO
CPN	3272-3518	N-H
	1055-1247	C-C
	1641	C=C

3.15 FE- SEM analysis of freeze dried nanoemulsified juice



(a) **(b)**

Fig 10. FE SEM images of freeze-dried juice of (a) CP and (b) CPN

In the FE-SEM image of juice without nanoemulsion, no particle structures were seen, whereas after the addition of nanoemulsion to the juice, the agglomerated particles were visible (Fig.10). Though specific shapes were not observed from the FE-SEM image, it confirmed the formation of nanoemulsion in the beverage (Mary et al. 2019).

3.16 Chromatographic analysis of the phenolic acids in the samples

The phenolic compounds determined in the developed samples along with their retention time are presented in Table 14.

Table 14. LC-MS analysis of phenolic compounds in blended juice and nanoemulsified juice

Sample	RT	Phenolic compounds
CPN	1.029	Dihydrocaffeic acid 3-O-gluconoid
	1.807	Quercetin ($C_{15}H_{10}O_7$)
	7.167	Thermoxanthin-13(carotenoid)
	26.805	Gallic acid
CP	1.387	Dihydrocaffeic acid 3-O-glucuronide
	7.165	Thermozeaxanthin-13 (Carotenoid)
	21.9	Sesaminol 2-O-triglucoside

Gallic acid, dihydrocaffeic acid 3-O gluconoid, thermoxanthin (carotenoid) and quercetin were found in nanoemulsified juice and dihydrocaffeic acid 3-O-glucuronide, thermozeaxanthin-13 (carotenoid) and sesaminol 2-O-triglucoside were found in blended beverage of coconut milk and pineapple juice.

3.17 GC-HRMS of virgin coconut oil

Fig.15 gives the different chromatographic peaks of fatty acids present in virgin coconut oil.

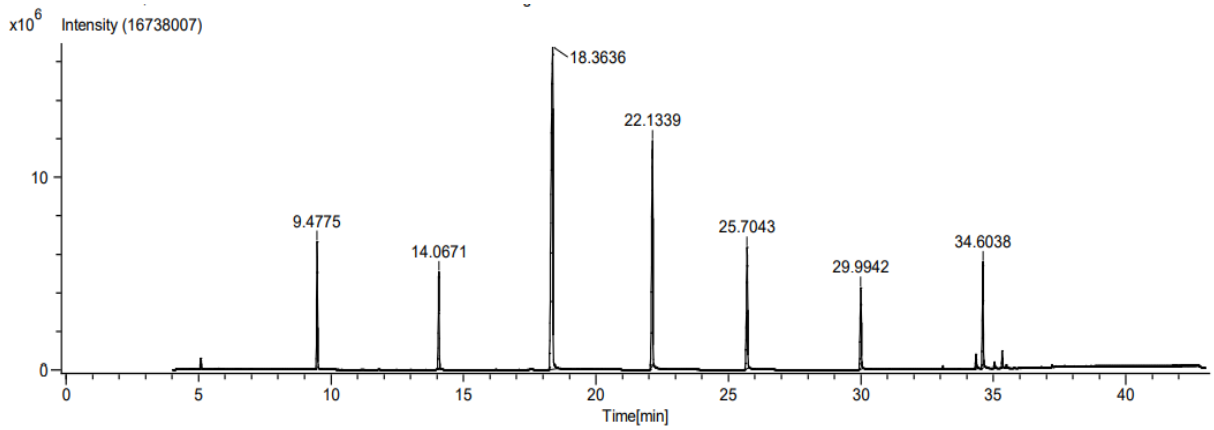


Fig 11. GC-HRMS peaks of virgin coconut oil

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Table 15. Fatty acids in virgin coconut oil

S.No	Peak time (min)	Fatty acids
1	5.0811	Caproic acid methyl ester
2	9.4775	Caprylic acid methyl ester
3	14.0671	Capric acid methyl ester
4	18.3636	Lauric acid methyl ester
5	22.1339	Myristic acid, methyl ester
6	25.7043	Palmitic acid, methyl ester
7	29.9942	Stearic acid
8	33.0917	Arachidic acid methyl easter
9	34.6038	Hexadeconoic acid, 10-hydroxy-methyl ester
10	35.0434	Octadeconoic acid,9,10-dihydroxy-methyl ester
11	35.3365	Octadeconoic acid,9,10-dichloro-methyl ester

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(Charu Lata Mahanta)

PI

GFR 19 – A

(See Rule 212 (1))

Utilization Certificate

(Period: 01-04-2021 to 30-06-2021)

Sl.	Letter No. & Date	Amount
1.	**NIL**	**NIL**

Certified that out of **Rs. NIL** of Grants- in-aid sanctioned during the year 2021-2022 in favour of Registrar, Tezpur University, Napaam-784028, Assam, and **Rs. 73,497.00** left on account of unspent balance of the previous year 2020-2021, a sum of **Rs. 4,85,858.00** has been utilized during the extended period from 01-04-2021 to 30-06-2021 for the purpose for which it was sanctioned.

2. Certified that I have satisfied myself that the conditions on which the grants- in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised that following check s to see that the money was actually utilized for the purpose for which it was sanctioned.

Kinds of check s exercised.

1. Vouchers and Books of Accounts
2. Grants-in-Aid Register

Chandan Lata Mahanta

Signature of Principal Investigator with date

Signature of Registrar/ Accounts Officer
Finance Officer
Tezpur University

Signature of Head of the Institute
Registrar
Tezpur University

Statement of Expenditure (01-04-2021 to 30-06-2021)

S. No.	Particulars	Amount Sanctioned (₹)	Amount Sanctioned (₹)	Total grant sanctioned (₹)	Expenditure (₹)				Total Expenditure (Rs)	Balance amount to be received (₹)
		1st Installment	2nd Installment		2019-2020	2020 - 2021	01-04-2021 to 30-06-2021			
							Actual	Committed		
1	Equipments	4,52,000	-	4,52,000	-	4,19,879	-	-	4,19,879	32,121
2	Recurring Expenses	8,05,000	4,00,926	12,05,926	1,79,710	9,98,464	41,386	3,90,440	16,10,000	(-) 4,04,074
3	Institutional charges	80,500	40,092	1,20,592	-	1,06,968	-	54,032	1,61,000	(-) 40,408
Grand Total		13,37,500	4,41,018	17,78,518	1,79,710	15,25,311	41,386	4,44,472	21,90,879	(-) 4,12,361

Charu Lata Mahanta

Name and Signature of Principal Investigator

Date:

28/9/21

Signature of Competent financial/ audit authority with seal

Finance Officer
Tezpur University

Date:

नारियल विकास बोर्ड
(कृषि मंत्रालय, भारत सरकार), केरा भवन, कोची - 682 011, भारत
COCONUT DEVELOPMENT BOARD
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निदेशक Director : 2375237
सचिव Secretary : 2377737
कार्यालय Office : 2376265
2377266

फा.सं F.No.1345/2018 TMoC 145654

दि. Date: 20.04.2021

सेवा में To

Dr. Ramaprasad T.R.
Scientist
Dept. of Biochemistry
CSIR-Central Food Technological Research Institute
Mysuru 570 020
Karnataka

विषय Sub: Project titled "Processing of coconut milk, development of beverage from curcumin enriched nanoemulisiifed coconut milk (partially defatted) coconut" reg:-
Ref: Your office letter dated 15.03.2021.

महोदय Sir,

With reference to the above, this is to inform that the competent authority has approved your request for extending the project period upto June 2021 (3 months) for completing the work without any additional financial commitment.

Hence it is requested to kindly complete the project by June 2021 and submit the final report to this office.

भवदीय Yours faithfully,

उप निदेशक Deputy Director

Copy for information to:
1. Prof. Charu Lata Mahanta, Dept. of Food Engineering & Technology, School of Engineering, Tezpur University, Tezpur-784028.
2. The Director – Central Food Technological Research Institute (CFTRI), Council of Scientific and Industrial Research (CSIR), Mysuru - 570 020, Karnataka