Study on model plant based functional beverage emulsion (non-dairy) using ultrasound – A physicochemical and functional characterization

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1. Introduction

Owing to perceived health benefits, functional drinks have become a trend among conscious consumers. The most critical factors in consumer’s decisions to buy functional drinks are suitability, affordability, and health benefits. Functional beverages are accompanied by several health benefits, such as cardiovascular health, prevention of cancer, digestive tract health, immune protection, weight management, bone health, enhancement of athletic endurance, energy, hydration, etc [1]. The common functional beverages in market are yoghurt & dairy drinks, sports & performance drinks, fortified functional fruit drinks & juices, plant beverages and ready to drink teas. However, consumers reduced or started avoiding bovine milk products due to conditions like such as lactose intolerance, cow’s milk protein allergy, problems with cholesterol and phenylketonuria, also for life style adoptions such as remaining vegetarian or choosing vegan diet or for apprehensions on hormone and health benefits. Functional beverages are accompanied by several health benefits. Functional beverages are accompanied by several health benefits. Functional beverages are accompanied by several health benefits.

The commonly available dairy alternatives in the market are soy and tree nut products. These are considered to be the most common allergens, hence there comes the importance of other plant based beverages [3]. Apart from soy and tree nut, the other plant sources popular in market are oats, rice, hemp, etc. Leaving soybean of the legumes or tree nut products. These are considered to be the most common allergens, hence there comes the importance of other plant based beverages [3]. Apart from soy and tree nut, the other plant sources popular in market are oats, rice, hemp, etc. Leaving soybean of the legumes or tree nut products. These are considered to be the most common allergens, hence there comes the importance of other plant based beverages [3]. Apart from soy and tree nut, the other plant sources popular in market are oats, rice, hemp, etc. Leaving soybean of the legumes or tree nut products. These are considered to be the most common allergens, hence there comes the importance of other plant based beverages [3]. Apart from soy and tree nut, the other plant sources popular in market are oats, rice, hemp, etc. Leaving soybean of the legumes or tree nut products. These are considered to be the most common allergens, hence there comes the importance of other plant based beverages [3]. Apart from soy and tree nut, the other plant sources popular in market are oats, rice, hemp, etc. Leaving soybean of the legumes or tree nut products. These are considered to be the most common allergens, hence there comes the importance of other plant based beverages [3]. Apart from soy and tree nut, the other plant sources popular in market are oats, rice, hemp, etc. Leaving soybean of the legumes or tree nut products. These are considered to be the most common allergens, hence there comes the importance of other plant based beverages [3]. Apart from soy and tree nut, the other plant sources popular in market are oats, rice, hemp, etc. Leaving soybean of the legumes or tree nut products. These are considered to be the most common allergens, hence there comes the importance of other plant based beverages [3]. Apart from soy and tree nut, the other plant sources popular in market are oats, rice, hemp, etc. Leaving soybean of the legumes or tree nut products. These are considered to be the most common allergens, hence there comes the importance of other plant based beverages [3]. Apart from soy and tree nut, the other plant sources popular in market are oats, rice, hemp, etc. Leaving soybean of the legumes or tree nut products. These are considered to be the most common allergens, hence there comes the importance of other plant based beverages [3]. Apart from soy and tree nut, the other plant sources popular in market are oats, rice, hemp, etc. Leaving soybean of the legumes or tree nut products. These are considered to be the most common allergens, hence there comes the importance of other plant based beverages [3].Apart from soy and tree nut, the other plant sources popular in market are oats, rice, hemp, etc. Leaving soybean of the legumes or tree nut products. These are considered to be the most common allergens, hence there comes the importance of other plant based beverages.

This study reports the development of non-dairy functional beverage emulsion employing ultrasound (US) of 20 kHz at 130 W and 195 W at processing times of 2 to 8 min using chickpea milk extract and bioactive, flaxseed oil (4%). The pre-emulsion was formed with high shear homogenizer followed by main sonication process. The sonicated emulsions were stored at 4 ± 2 °C till 14 days and characterized for physicochemical and functional properties. A comparative study was carried out using conventional high shear homogenizer (UT) at 10,000 RPM for 5 min. Upon optimization, 130 W – 8 min, 195 W – 6 min and 195 W – 8 min sono-emulsions showed creaming stability of 100%; with particle sizes as 1.12, 0.97 and 0.78 µm; and zetapotential values as – 40.4 mV, – 37.52 and – 36.91 mV, respectively. The improvement in protein solubility by 86% proved the emulsifying capability of chickpea proteins, which had partially denatured upon physical effects of acoustic cavitation producing stable and finer emulsion droplets. The reduced sedimentation values of sonicated chickpea extract in comparison to UT showed improvement in physical stability of plant-based milk. Oxidative stability is observed for 130 W – 8 min sono-emulsions with no change in conjugated dienes, indicating the absence of process generated free radicals. The US process did not have any effect on reduction of stachyose content. But extracted chickpea milk had lower amount of stachyose in comparison to raw chickpeas, reducing the flatulence problem, mainly due to adaptation of high temperature pressure cooking process.
could deliver nutrients as a stable emulsion. Flaxseed is rich in non-polar alpha-linolenic acid (ALA), an omega-3 fatty acid and anti-oxidants like tocopherols and beta-carotene. The Omega-3 fatty acids promotes brain development and function, wound healing, etc., [4].

The stable delivery of the non-polar functional ingredient in a beverage is a challenge, because commercial beverage uses huge amounts of external food additives like emulsifiers and stabilizers to produce a stable emulsion of oil in water throughout the recommended shelflife. Conventionally high shear mixers and homogenizers are used with loaded amount of chemical stabilizers and emulsifiers for stabilizing plant beverages. Ultrasound (US) is also one of the important techniques used for stabilization and emulsification however its application in plant-based beverage, especially with that chickpea extract or chickpea milk is not found in literature and thus novel. The intense physical forces of low frequency high power US such as acoustic streaming, shock waves, micro jets, turbulence, and shear forces of US can be utilized for production of shelf stable emulsions through sono-emulsification [5]. Additionally, the US induced changes to proteins and other components of the beverage matrix can be tapped for producing shelf stable emulsions with no or minimal usage of external food additives. For example, the addition of transient ultrasonic mechanical energy causes a protein to undergo a variety sono-mechanical reactions causing a temporary or permanent change in the folded protein’s 3D structure leading to unfolding of protein and enhance its activity as a surfactant between oil and water interphase [6]. But the studies available in literature, on ultrasound and plant milk are mainly concentrated on microbial inactivation, protein structural stability and extraction in which soy milk, almond milk or coconut milk are used as key ingredient [7–9]. Thus broadly, the aim of this study is to employ 20 kHz US under various experimental conditions for stable delivery of the bioactive, flaxseed oil in non-dairy chickpea milk extract and to study the physicochemical and functional characteristics of the developed emulsion against particular storage time and storage temperature. The study also extends to understand the changes to raffinose, an oligosaccharide which is predominant in chickpea extract, knowing to cause flatulence.

2. Materials and methods

2.1. Raw materials

The chickpea and flaxseed oil were procured from the local market of Thanjavur, India. Xanthan gum and Sodium azide were purchased from Hi-media. The chemicals used for the experiments were of analytical grade purchased from Hi-media (Nasik, India). The raffinose standard for HPLC analysis was purchased from Sigma-Aldrich (Bangalore, India).

2.2. Preparation of chickpea milk

The boiled chickpea milk was prepared by soaking the raw chickpea for 12 to 16 h, then the soaked water was removed and chickpea was washed in normal water. Then the soaked chickpea was cooked in a household pressure cooker with distilled water at 80 °C for 30 min and the cooking water was discarded to reduce the anti-nutritional compound. Next, the cooked chickpea along with filtered water in the ratio of 1:4 (1 part of raw chickpea: 4 parts of water) was ground in colloidal mill for 10 min. The resulting slurry was filtered using a muslin cloth to obtain chickpea milk. The materials trapped in the muslin cloth were disposed later [10,11].

2.3. Emulsification by ultratrrax and ultrasound

The emulsion was prepared by using 4 % flaxseed oil (w/v) in 96 % chickpea extract and xanthan gum (0.1 %) (w/v). The emulsion was prepared in two steps. In the first step, a pre-emulsion was prepared using a high-shear probe homogenizer with chickpea extract, flaxseed oil (4 %) and xanthan gum for 1 min at 10000 rpm. To prepare the control (unsonicated sample), the emulsion was homogenized for 5 min at 10000 rpm using the high-shear probe homogenizer (Ultraturrax IKA T18 Basic, Wilmington, NC, USA). Henceforth, the unsonicated emulsion sample is called control and is processed by high shear homogenizer called as Ultratrrax, only. In second step, the final emulsion was produced using a probe sonicator using high intensity ultrasound. A 50 ml of the pre-emulsion was poured into a glass vessel and during US treatment it was surrounded by an ice-water bath to maintain sample temperature near to 25 ± 2 °C. The probe sonicator with 20 kHz, 650 W (6 mm diameter horn, labman sonicator) ultrasound at 130 W and 195 W nominal applied power at 2, 4, 6 and 8 min was used. The sono-emulsified samples were stored in a refrigerator condition (4 ± 2 °C) for 14 days by adding sodium azide (0.02 % w/w) mainly to avoid microbial spoilage during storage studies. Higher US power of more than 195 W at 20 kHz and processing time of more than 8 min was not used, because of the notable burnt smell in the product as this might possibly reduce the nutritional and sensory qualities of the product. The storage studies, functional and physicochemical measurements were performed on fresh and stored samples [11,12]. The reason for selecting the storage study or shelflife period of 14 days at a specific storage temperature of 4 ± 2 °C relied on the pH of the food product, which is 7.07 for the pasteurized chickpea milk extract beverage emulsion made with flaxseed oil. Thus the shelf stability of product is checked on Day 1 and Day 14 at 4 ± 2 °C mainly, and few times the intermediate day of storage especially Day 7 is also checked.

2.4. Particle size and zeta potential

The particle size and zeta potential value of sonicated and unsonicated samples were taken on day 1, day 7 and day 14 by storing them at 4 ± 2 °C. Nano plus (model-Micromeritics) was used to determine size distribution of the emulsion droplets using by dynamic light scattering technology. The refractive index (RI) of flaxseed oil and that of chickpea milk is 1.37 and 1.47, respectively. During measurement, the sample is dispersed in water and measurements are taken. D(10), D(50) and D(90) are diameter of particle sizes. D(10) refers to portion of particles with diameters smaller than this value is 10% by volume. D(50) represents volume mean diameter; D(90) means that 90% volume of the total particles are smaller than this diameter. D(50) value is used mostly in result and discussion section. The zeta potential of the emulsion was determined using Zetaziser (Malvern Instruments, U.K.).

2.5. Creaming stability

The phase separation and creaming were visually examined in the emulsions. To improve the visibility of separated phases, Sudan III dye was used. A magnetic stirrer was used for 2.5 h to combine 0.0025 % Sudan III dye with flaxseed oil at room temperature. Instead of plain flax seed oil, oil dye mixture was used in making US treated emulsions. A 10 ml freshly prepared emulsion samples were transferred into a clear test tube with screw cap lids and stored at 4 ± 2 °C. The creaming of emulsions was observed after 1, 7 and 14 days of storage. Some of the samples showed separation during storage, resulting in a cream layer on top surface. The total height of the emulsion sample (HoE) and the height of the cream layer (HoC) were measured. The creaming index (CI) can be expressed as a ratio of the cream height to the total height of the emulsion in the tube [5].

\[ CI = \frac{HoC}{HoE} \times 100 \] (3.5).

2.6. Sedimentation

For determining dry sediments in emulsified samples 10 g of freshly prepared emulsion was added into 15 ml transparent tube and centrifuged at 4,200 rpm for 5 min at room temperature (25 °C). The centrifuge tube was turned upside down for 10 s and the supernatant was discarded. Wet sediment was transferred to a dry petri plate and dried at
105 °C until it reached a stable weight (W in g) [13].

Dry sediment (%) = (W/10) × 100 (3.6).

2.7. Solubility of protein

The solubility of protein before and after ultrasonic treatment was assessed to examine the effect of ultrasonic treatment on proteins of the emulsion system. The emulsion was made without flaxseed oil by following all of the other processing steps in section 3.3.1.

To find solubility, the emulsions were mixed for 1 h in magnetic stirrer before being centrifuged for 20 min at 12,000g at 25 °C. Then kjeldahl method was used to determine the protein content of the supernatant [14].

2.8. Viscosity

Rheological properties of emulsion samples were determined using a Modular Compact Rheometer (MCR 52, Anton Paar, Co. Ltd) equipped with a temperature-control unit. The rheometer had a bob-cup configuration. Measurements were carried out in the shear rate range of 1 to 100 s⁻¹ at constant temperature of 25 °C. 20 ml of each sample was transferred in to cup and the measurement was taken immediately. The apparent viscosity was determined as a function of shear rate. The viscosity was recorded for day1, day 7 and day 14 samples stored at 4 ± 2 °C [15],

\[ \eta = k \cdot \gamma^{-1} \] (3.7),

\( \eta \) - apparent viscosity (Pa s).

k – consistency coefficient (Pa sn).

\( \gamma \) - shear rate (s⁻¹).

n - flow behavior index (dimensionless).

2.9. Conjugated dienes (CD)

The production of CD was used to assess the lipid oxidation in the emulsions. A 200 μl of emulsion sample was mixed with 10 ml of 2:1 isooctane/2-propanol solution and vortexed for 1 min immediately. It is then centrifuged at 5000 g for 5 min and filtered through a 0.25 μm syringe filter. The separated organic phase was observed in UV–Vis spectrophotometer for absorbance at 232 nm. The filtration was done right before the measurement of absorbance to eliminate proteins from the sample, which could cause spectral interference in the same area. The CD value was determined for the samples on day 1, day 7 and day 14 stored at 4 ± 2 °C [16].

2.10. Quantification of oligosaccharide by HPLC

Extraction of oligosaccharide from chickpea extract.

2 ml of chickpea extract was mixed with 1.5 ml of double distilled water, and the diluted sample was incubated for 10 min at 60 °C. Then 1 ml of acetonitrile, 0.25 ml of 500 mM aqueous potassium ferrocyanide and 0.25 ml of 500 mM aqueous zinc acetate were added. Then the materials was gently mixed and then kept at room temperature for 1 h. The precipitate so formed was removed by centrifugation at 10,000 g, 8 min and 20 °C. The extracted oligosaccharides was then filtered out of the supernatant using a 0.45 μm nylon membrane. After that, the filtrate was analyzed using high-performance liquid chromatography (HPLC) [17].

Separation and Quantification of oligosaccharide by HPLC.

A HPLC equipped with refractive index (RI) detector and NH2 column was used. The isocratic solvent system was used. A 70:30 of solvents involving acetonitrile and water at 1 ml/min was employed. The column and RI-cell temperatures were kept at 40 °C. Then the above mentioned chickpea extract filtrate was injected in a 10 μl amount and evaluated using HPLC [17].

2.11. Statistical analysis

All studies were carried out in triplicate, and all findings were expressed as mean ± standard deviation. For statistical analysis, Minitab 17.3.1 program was used. One-way variance analysis (ANOVA, with tukey comparison) with a confidence level of 95 % and significance level p < 0.05 was performed.

3. Results and discussion

3.1. Physicochemical characterization of non-dairy beverage emulsion

3.1.1. Particle size and zeta potential

The stability of an emulsion is determined by the particle size of the emulsion. Table 1. shows the diameter of emulsions processed at 130 W and 195 W power and at different processing time 2, 4, 6 and 8 min and upon storage at 4 ± 2 °C. The data in Table 1 (a) shows a gradual decrease in D(50) particle size from 0 min to 8 min treatment time for both the power treatments of 130 W and 195 W. The UT emulsion (control) showed maximum particle size of 8.9 μm and 8 min sono-emulsified sample at 130 W showed minimum particle size of 1.12 μm. Similarly, a higher and significant decrease in particle size was observed among the 195 W treated samples with increase in treatment time and the lowest size was 0.78 μm at 8 min of treatment time. While comparing both the powers, i.e., 130 W and 195 W, the 195 W treated samples showed a lower particle size than 130 W. It was due to the intense shear forces produced by the acoustic cavitational collapses created by higher power ultrasound at 195 W in comparison to lower power of 130 W [18].

The D(50) particle size values were also taken in day 7 and 14 for the sono-emulsions to check the stability with time as represented in Table 1 (a). All the 130 W power treated samples creamed off mostly on day 7 and day 14, except 8 min treated sample which was stable with particle size of 1.23 μm and 1.35 μm respectively in day 7 and day 14, respectively. Similarly, 195 W treated samples at processing times of 4, 6 and 8 min were stable till day 7 with no much significant change in particle size from day 1, while on day 14 only the 6 min and 8 min treated samples showed stability against creaming with D(50) particle size of 1.26 and 1.11 μm, respectively.

The stability of sono-emulsions on Day 14 processed at conditions of 130 W – 8 min, 195 W – 6 min and 195 W – 8 min is also proved with the D(90) value, viz., 1.56, 1.37, 1.21 μm, respectively from Table 1(b). D(90) refers to 90% volume of the total particles of emulsion that are smaller than D(90) diameter contributing to stability. The decrease in particle size with increase in ultrasound power input enables the size of acoustic cavitation bubbles to get larger and the intensity of the collapse to increase, resulting in higher shear forces in the surrounding environment. The higher shear forces lead to increase in breakdown of bigger flaxseed oil emulsion droplets further into smaller ones, thus reducing their size ultimately. The particle size decreases with increase in applied ultrasound treatment power and time. Also upon storage, the oil droplets coalescence which is type of emulsion instability, that causes creaming off and increase in particle size are noted for lower power and lower treatment time [19]. Similar results was observed in studies with soy protein isolate and whey protein emulsion of oil in water using ultrasound [12].

The zeta potential value is a useful measure for evaluating emulsion stability. The emulsions with a low zeta potential are more likely to flocculate or coagulate, those with a high zeta potential are electrically stabilized. In Fig. 1 it was observed that that zeta potential value of all samples were negative which may be due to negative charge of chickpea protein at pH higher than its isoelectric point [20]. The best emulsion stability would be achieved if zetapotential is of −30 mV [21–24]. The samples treated with 130 W of US power for 2, 4, 6 and 8 min showed a gradual increase in zeta potential value i.e., −31.25, −33.1, −35.4 and −40.4 mV on day 1 in comparison to 0 min untreated control sample
Zeta potential (mV)
-45 -40 -35 -30 -25 -20 -15 -10 -5 0 5
0 2 4 6 8
Zeta potential (mV)
Sonication time (min)
-40 -35 -30 -25 -20 -15 -10 -5 0 5
0 2 4 6 8
Zeta potential (mV)
Sonication time (min)

Table 1
(a) D(50) and (b) D(90) particle size of emulsions produced US at 130 W and 195 W for 0, 2, 4, 6 and 8 min treatment times on day 1, day 7 and day 14.

| Sonication time (min) | D(50) at 130 W (µm) | Day 1 | Day 7 | Day 14 | D(50) at 195 W (µm) | Day 1 | Day 7 | Day 14 |
|----------------------|---------------------|-------|-------|--------|---------------------|-------|-------|--------|
| 0 (Control/UT)       | 8.9 ± 0.07          | C     | C     | C      | 8.9 ± 0.01          | C     | C     | C      |
| 2                    | 2.51 ± 0.00         | C     | C     | 1.71 ± 0.00 | C     | C     | C      |
| 4                    | 2.14 ± 0.00         | C     | C     | 1.29 ± 0.01 | 1.41 ± 0.00 | C     | C      |
| 6                    | 1.57 ± 0.01         | C     | C     | 0.97 ± 0.01 | 1.13 ± 0.00 | 1.26 ± 0.00 | C      |
| 8                    | 1.12 ± 0.00         | 1.23 ± 0.00 | 1.35 ± 0.01 | 0.78 ± 0.02 | 0.95 ± 0.01 | 1.11 ± 0.05 | C      |

| Sonication time (min) | D(90) at 130 W (µm) | Day 1 | Day 7 | Day 14 | D(90) of 195 W (µm) | Day 1 | Day 7 | Day 14 |
|----------------------|---------------------|-------|-------|--------|---------------------|-------|-------|--------|
| 0 (Control/UT)       | 9.1 ± 0.03          | C     | C     | C      | 9.1 ± 0.03          | C     | C     | C      |
| 2                    | 2.73 ± 0.02         | C     | C     | 1.86 ± 0.00 | C     | C     | C      |
| 4                    | 2.36 ± 0.00         | C     | C     | 1.43 ± 0.01 | 1.60 ± 02 | C     | C      |
| 6                    | 1.82 ± 0.00         | C     | C     | 1.11 ± 0.00 | 1.25 ± 00 | 1.37 ± 00 | C      |
| 8                    | 1.29 ± 0.03         | 1.41 ± 02 | 1.56 ± 00 | 0.88 ± 0.03 | 1.19 ± 01 | 1.21 ± 00 | C      |

C refers to cream separation.

![Fig.1 (a)](image1)

![Fig.1 (b)](image2)

Fig. 1. Zeta Potential values of emulsion prepared at 130 W and 195 W at different sonication times of 0, 2, 4, 6 and 8 min on (a) day 1 (b) day 14 of storage at 4 ± 2 °C.

which was –25.8 mV. Similarly, 195 W - US treated samples for same treatment time showed significant increase in zeta potential compared to control sample (–25.8 mV), the zeta values for 2, 4, 6 and 8 min are –34.2, –38.52, –37.52 and –36.91 mV. On day 14 zeta potential readings for stable sono-emulsified samples are taken. Along with this zeta potential values for UT treated sample and 4 min samples are also taken to show the drop in zeta potential value at the time of creaming during storage, mainly to show the instability. Thus, on day 14, 130 W for 8 min treated sample showed zeta potential value of 39.62 mV and was stable but 130 W for 4 min sonicated sample showed –25.26 mV and was unstable. Likewise, the untreated UT treated emulsion had a drop from a low value of –25.8 to –18 mV denoting its instability and loss of electrostatic potential. Similarly, day 14 zetapotential readings for 195 W treated sample at 6 min and 8 min showed zeta potential of 35.92 and 35.12 mV, respectively indicating stability.

Thus the intense shear forces of US in the oil droplet’s micro environment helps in movement and adsorption of chickpea proteins of the base matrix to the oil-water interface creating a higher negative charge on it leading to stability of flaxseed oil droplets in chickpea milk extract, especially in the case of sonicated samples [16,21]. Thus the value of zeta potential increased with increase in power and time of ultrasound processing. The emulsions with a low zeta potential are more likely to flocculate or coagulate, those with a high zeta potential are electrically stabilized. The creaming stability data (Fig. 3) for both 130 W and 195 W at process times of 0 to 8 min also supported zeta potential results both on day 1 and day 14 of storage. Also, when the sample creamed off, a significant change in zeta potential value is also noted (refer Fig. 1). As ultrasound treatment’s power and time is increased, there is increased possibility of opening up of protein structure resulting in increase in hydrophobicity of proteins by partial denaturation at the bubble-liquid interfaces of acoustic cavitation and making them a surfactant followed by adsorption of protein at the oil and water interfaces, resulting in higher zeta potential value and thus stability. However this can be proved only with increase in hydrophobicity or solubility of protein [9,16,25]. Further, our data on increase in solubility of proteins of the sonicated-emulsions supports this argument as mentioned in Fig. 4. As a result, phase separation in the US emulsion system was thus avoided.

3.1.2. Creaming stability

The creaming stability of emulsions prepared at 130 W and 195 W of US power at treatment times of 0, 2, 4, 6 and 8 min was measured on day 1, day 7 and day 14 of storage days at 4 ± 2 °C and data is reported in Fig. 2. The creaming index (CI) or creaming stability serves as a measure for the level of oil droplet aggregation and separation from the water phase in an emulsion [26]. From the data, it is clear that the 130 W sonicated emulsions on day 1 showed stability of 100% in case of 2, 4, 6 and 8 min treatment time except for control UT emulsion which showed 95% stability. But on day 7, it was observed that at 130 W all sonicated samples except for 6 min treatment time, all the other treatment times of 0, 2, 4 and 6 min exhibited reduction in creaming stability with values of 82.5 %, 86.25 %, 90.83 % and 93.75 %, respectively. The 8 min – 130 W
sonicated emulsion showed a stability of 100% on day 7. Similar, is the case with 195 W sonicated emulsions, i.e., all samples showed a creaming stability of 100% except the 0 min treated UT emulsion sample which showed a stability of 96%. With storage for 7 days at 4 ± 2°C, the creaming stability decreased for 195 W – 2 min and 195 W – 4 min sonicated samples and is 82.5% and 94.6%, respectively but the 6 min – 195 W and 8 min – 195 W sonicated emulsions showed a stability of 100% on day 7. However, on day 14, only the 130 W-8 min sonicated emulsion sample and the 195 W sonicated 6 min and 8 min samples showed a stability of 100% as shown in Fig. 3. This is mainly due to combination of smallest emulsion droplet formation and presence of proteins on the emulsion interfaces, contributing to stability [27].

The emulsion that was not ultrasonically prepared, exhibited the lowest creaming stability of 96% on the day 1, 82% on day 7 and 76% on day 14. It is thus proved that the high shear homogenizer, UT has neither created smaller particle size nor improved the emulsification properties of protein to make it a surfactant and thus the stability. On visual examination, the phase separation of UT emulsions started just after 2 to 3 h of preparation. This could be related to the inhomogeneous distribution of oil droplets in unsonicated emulsion’s, which induced agglomeration of oil droplets leading to coalescence and creaming with time. On the other side, ultrasonically treated emulsions were more

Fig. 2. Creaming stability of emulsions at (a) 130 W (b) 195 W in different treatment times of ■ 0 min, □ 2 min, △ 4 min, ■ 6 min and ▣ 8 min on day 1, day 7 and day 14 of storage at 4 ± 2°C.
stable during storage, this may be due to high intensity ultrasound emulsifying capacity to reduce flocculation, prevent creaming which results in distribution of oil droplets [28]. The xanthan gum which is used as a stabilizer in the formulation may have possibly contributed to increase in creaming stability by increasing the viscosity and by keeping oil particles in suspended state. However, from the above discussed results it is clear that without sonication, only presence of xanthan in the chickpea milk matrix cannot contribute to stability of emulsions. Thus, sonication is important in imparting stability for emulsions, mainly by reducing oil droplet size, by partially denaturing the chickpea proteins and by helping in adsorption of these proteins at o/w interfaces due to the intense shock waves, turbulences and shear forces created by the phenomenon of acoustic cavitation. [15]. So, it is understood that for making stable chickpea flaxseed emulsions, a combination particle reduction technique like US, surfactants like chickpea protein and stabilizer like xanthan are required.

3.1.3. Solubility of protein

Protein solubility is considered to be a key factor in deciding emulsifying ability of a component. The Fig. 3 shows solubility of sonicated boiled chickpea milk prepared at 130 W and 195 W of US power in 0, 2, 4, 6 and 8 min. The result indicates that sample treated at 130 W of US at 2, 4, 6 and 8 min showed an increase in solubility compared to unsolicited boiled chickpea milk. For unsolicited boiled chickpea milk sample, the solubility was 1.75 % and for other sonicated boiled chickpea milk prepared at 130 W for treatment times of 2, 4, 6, 8 min, the values were 2.58, 2.97, 3.03 and 3.26 %, respectively. Similarly, for 195 W sonicated boiled chickpea milk, the solubility of 2, 4, 6 and 8 min treatment times were 2.97, 3.15, 2.91 and 2.85 %, respectively. In case of both the US power treatment there is a significant increase in solubility to about 86 and 63% for 130 W – 8 min and 195 W – 8 min in comparison to the unsolicited boiled chickpea milk samples.

The increase in solubility of sonicated samples may possibly be due to reduction in particle size of the component, which can enhance solubility, allowing for an increase in surface area and enabling efficient chickpea protein adsorption at the oil-water interface [29]. Likewise, sonication partially unfolded proteins and expose more hydrophilic and hydrophobic groups increasing solubility and emulsifying ability. Similar kind of result is observed in study on soy protein isolate and sunflower oil emulsion [14]. Hence proved that proteins of boiled/pasteurized chickpea extract when sonicated turns into emulsifier and is also supported by higher and increasing values of the zeta potential based on the Fig. 1. A similar observation was observed in a study carried by Wang et al., where the solubility of chickpea protein isolate has increased after sonication [30]. Also, within error limits of the experiment for both the 130 W and 195 W power treatments, the increase in sonication time showed an increasing trend for solubility, though the change is insignificant different sonication times.

4. Functional characterization of non-dairy functional beverage emulsion

4.0.1. Sedimentation

Sedimentation characteristics of plant milk is regarded as an important functional property among others like viscosity, colour, appearance, turbidity, etc. It determines the overall sensorial acceptability of non-dairy plant milks in the market. Usually addition of excessive amounts of stabilizers and emulsifiers addresses the instability issues of commercial non-dairy plant milks, otherwise these non-dairy milk alternatives undergo phase separation resulting in an unstable inhomogeneous undesirable product. Such instability issues are solved by novel physical techniques like ultra-high pressure homogenizer, ultrasound, high pressure homogenizer, etc., [31–33].

Thus, in our work to determine the functional characteristics of non-dairy beverage emulsion manufactured by the process of ultrasonication, stability of product is tested based on sedimentation values of the samples. The Fig. 4 represents the percentage of sediment in samples treated with 130 W and 195 W power US for 0, 2, 4, 6 and 8 min of treatment times. The results indicate that the UT emulsion (control) sample have sedimentation of 7.63 % while the 2, 4, 6 and 8 min sonicated emulsions showed 7.15, 6.84, 6.85 and 6.56 % respectively for 130 W samples. The results indicated that 130 W sono-emulsions showed a significant decrease in sedimentation at all process times in comparison to UT emulsion sample. Likewise, the 195 W sono-emulsions at different process times of 2, 4, 6 and 8 min shows sedimentation of 6.48, 6.51, 6.35 and 6.37 % and are also significantly lower to value of 7.63 % of control UT emulsions prepared by high shear homogenization.

The lower sedimentation percentage means increase in stability of all particles in the sonicated samples compared to unsolicited sample (UT emulsion treatment). The sedimentation mechanism can be explained according to stokes law that is the unsolicited samples will have large particle size which will sediment faster with gravity than sonicated samples with smaller particle size upon storage. Similar result was observed in the peach juice treated with ultrasound in which sedimentation decreased and emulsion became more stable with sonication [34]. Even a small amount of sedimentation reduction is a big achievement in a market sample such as a beverage emulsion or a pulse based functional drink [35]. Thus, US is seen as a good tool for reducing the sedimentation.

![Fig. 3. Solubility of chickpea extract prepared at 130 W and 195 W in 0, 2, 4, 6 and 8 min of treatment times.](image)

![Fig. 4. The sediment percentage in emulsion treated with 130 W and 195 W at different treatment times of 0, 2, 4, 6 and 8 min.](image)
4.0.2. Viscosity

Another important parameter which addresses the instability of non-dairy milk extract is viscosity. In non-dairy milks, the increase in viscosity of the matrix happen with reduction in particle size of the components even at ambient temperature of processing like 30 °C, ultimately contributing to increase in colloidal stability of the particles and thus a stable emulsion [31].

According to Fig. 5, at 130 W for 0, 2, 4, 6 and 8 min of treatment time, the viscosity values of samples were 0.74, 0.78, 0.83, 0.79 and 0.85 Pas, respectively. The values showed an increase with increase in process time though the increase between the values are insignificant. Similarly, at 195 W for 0, 2, 4, 6 and 8 min of treatment time the viscosity values of samples as per Fig. 5 were 0.74, 0.83, 0.82, 0.88 and 0.88 Pas, respectively and it has also shown an increase with increase in sonication time like 130 W sonicated samples. Though there is statistically insignificant increase in viscosity values, within error limits of the experiment as per Fig. 5 there is an apparent increasing trend observed.

Fig. 5. Viscosity of chickpea extract and flaxseed oil emulsion on Day 1, Day 7 and Day 14 prepared with (a) 130 W and (b) 195 W for 0, 2, 4, 6 and 8 min of treatment time, respectively.
for the viscosity values at both the power of 130 W and 195 W with increase in process time for the sonicated samples in comparison to the control UT sample (0 min sample). The reason for this insignificant increase in viscosity values may possibly be due to increase in the number of finely divided smaller emulsion droplet particles created by the physical effects of acoustic cavitation, contributing to stability of sonicated emulsions with increase in sonication time as per Fig. 2. Similar results were observed in studies on avocado puree and rice flour samples treated with ultrasound in which viscosity increased when compared to unsonicated samples, due to reduction in particle size of the materials. The reduction in particle size by ultrasound results in a larger interfacial area and a reduced mean distance between particles, resulting in stronger inter-particle interactions and thus the milder increase in viscosity of our sonicated samples [35-37]. Likewise, the decrease or the change noted in the viscosity of sonicated emulsions across all the treatment times and powers on day 7 and day 14 as shown in the Fig. 5 is statistically insignificant and thus such sonicated beverage emulsions can be marketed as a shelf stable homogeneous product.

4.0.3. Conjugated dienes (CD)

Conjugation dienes measurement has proven to be a useful tool for analysing lipid oxidation, another functional characteristic of beverage emulsions which has high amount of dispersed oily component. Due to the rearrangement of the double bonds, conjugated dienes are commonly formed during the synthesis of hydro peroxides (primary oxidation product) from unsaturated fatty acids during oxidative deterioration of food samples [38]. The flaxseed oil which is used as a functional bioactive in our study has huge amounts of unsaturated fatty acids and though we are aware that the process of ultrasonication at lower frequencies is known to produce insignificant or no free radicals, it is important to study the oxidative quality of the flaxseed oil chickpea extract emulsion in our study [39]. The Fig. 6 shows CD value of flaxseed oil chickpea extract emulsion prepared with 130 W and 195 W for 0, 2, 4, 6 and 8 min treatment time on day 1, day 7 and day 14 upon storage at 4 ± 2 °C. With 130 W - US treatment carried for 2, 4, 6 and 8 min, the CD values were 0.36, 0.36, 0.39 and 0.41, respectively in comparison to 0.35 for 0 min or unsonicated UT emulsion on day 1. Though there was small increase noted in CD values with increase in sonication time, it was statistically insignificant indicating the absence of free radical generation during ultrasonication [16]. Also, the CD values of all sonicated samples is contributed by the CD value of plain untreated flaxseed oil, which is already 0.34. Similar is the case with 195 W sonicated emulsions at lower processing times of 2 min and 4 min, i.e., CD value is 0.37 and 0.40, respectively. But at longer processing time of 6 min and 8 min at higher power level of 195 W, there is significant increase in CD value to 0.43 and 0.45 in comparison to 0.35 of unsonicated UT sample. This may possibly be because of prolonged treatment time in 195 W treatment which may possibly have led to increase in temperature in the localised cavitation collapse zones of the matrix during US treatment [38]. Upon storage of these samples at 4 ± 2 °C the CD reading were recorded on day 7 and day 14 and from Fig. 6 they are 0.34, 0.35, 0.35, 0.38 for 130 W – 2, 4, 6 and 8 min of treatment times and 0.34, 0.37, 0.40, 044 for 195 W – 2, 4, 6 and 8 min of treatment times. Upon storage, both the 130 W and 195 W treated sample exhibited a decrease in CD value with storage time of 7 days and 14 days for all the 0, 2, 4, 6 and 8 min treatment times. The decrease in noted in CD values which is observed on day 7 and day 14 were due to breakdown of primary oxidation products to secondary oxidation products [38,39].

4.0.4. Quantification of oligosaccharide by HPLC

Raffinose and stachyose are two main oligosaccharides present in chickpea that cause flatulence in humans, which is one of the most discussed functional disadvantages of pulse and pulse based products like chickpea extract which is used as the base matrix for preparation of non-dairy beverage emulsion. Among the above listed oligosaccharides, stachyose concentration is more in chickpea compared to other oligosaccharide concentration [40]. So, the analysis of concentration of stachyose in the unsonicated chickpea extract is important for determining the functional quality of extract, as it is used as main base matrix. Similarly, the sonication induced effects on reduction of stachyose content in the shelf stable samples (130 W – 8 min and 195 W – 6 min) was also important and thus studied. The amount of stachyose in raw chickpea ranges between 1.6 % and 3.1 % as per literature [41,42] but the stachyose content of unsonicated chickpea milk extract is only 0.7% according to Fig. 8 prepared from HPLC analysis. This observed lower value of the unsonicated chickpea extract is mainly because of the process adapted for extraction of chickpea milk, i.e., the process of boiling and pressure cooking which had resulted in the leaching out of oligosaccharides [43,44]. Moreover, from the Fig. 7, it is also observed that there is no significant difference in the stachyose content between the sonicated and unsonicated chickpea extracts. This observation highlighted that the process of sonication has not imparted any changes to the oligosaccharide molecules or its concentration, especially that of stachyose in the sonicated chickpea extracts.

5. Conclusion

Using ultrasound technique, a novel plant based functional beverage emulsion as an alternative for dairy based milk beverage was developed using chickpea milk extract and the bioactive, flaxseed oil. The sonicated beverage emulsion at 130 W – 8 min was physically shelf stable till 14 days of storage at 4 ± 2 °C in comparison to conventional high
shear processed UT samples. The creaming stability of 130 W – 8 min optimized sample was 100% against 76.7% for the control UT sample on day 14. The particle size of stabilized emulsion droplet was 1.12 µm for 130 W – 8 min. The stability of beverage emulsion was also supported by the good negative electrostatic charge on the emulsion droplets, which is −40.4 mV for 130 W – 8 min. The partial denaturation of chickpea protein and its role as emulsifier in sonicated emulsions was confirmed with the increase in solubility of protein in comparison to unsonicated, conventionally processed emulsion. Thus, the achievement of shelf stability in sono-emulsions is mainly due to the physical effects of acoustic cavitation causing particle size reduction of all the components of base matrix, leading to absence of phase separation and stratified layers of enormously varying particle sizes. In this study, the safety of US in food processing is reaffirmed with good oxidation status of flaxseed oil emulsion droplets. Moreover, the study has found that the sono-processing parameters didn’t have any effect of stachyose content of chickpea milk. But the advantageous lower content of stachyose observed in the sono-processed functional beverage in comparison to raw chickpea is mainly due to the pre-processing conditions which are adapted in the study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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