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Research Article

Effect of probe ultrasonication and β-Cyclodextrin on bitterness reduction and enhancement of biochemical properties of *Citrus limon* Burm juice

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Abstract
Lemon is a globally cultivated citrus fruit crop that has gained a significant market share in recent years. Lemon tends to taste bitter on prolonged storage periods, leading to undesirable changes in juice quality. Therefore, the study aimed to enhance lemon juice quality by reducing bitterness through combined probe ultrasonication and β-cyclodextrin treatment. Ultrasonic treatment of 20 kHz, an incubation period ranging from 20 to 80 minutes, was applied to the juice and its effect on pH, total titratable acidity (TA), total soluble solids (TSS), ascorbic acid, total phenolic content (TPC) and antioxidant activity was evaluated. Batch debittering of limonin was carried out by adding β-cyclodextrin at concentrations ranging from 0.25% to 2.50% for 20, 60 and 80 minutes of sonication treatment. The study also examined the influence of sonication treatments on microbial load, revealing significant reductions corresponding to 60 minutes of sonication. The sound waves generated at 20KHz from ultrasonication, including β-cyclodextrin, could help to break down the cyclodextrin molecules and improve the encapsulation efficiency of the target compound. Moreover, increased water-soluble polyphenolic compounds due to cyclodextrin addition can enhance the lemon juice processability at an industrial scale. The present study emphasized the development of cost-effective β-cyclodextrin debittered lemon juice in combination with ultrasonic treatment that improved juice quality at room temperature (25 °C±1 °C).

Keywords: Bitterness, β-cyclodextrin, Burm juice, *Citrus limon,* Quality, Ultrasonication

INTRODUCTION

Citrus fruits are one of the world's most widely grown fruit crops, with production and consumption taking place in virtually every country. According to the Food and Agricultural Organization of the United Nations (FAO, 2020) data, global citrus production reached approximately 141 million metric tonnes in 2020, with the top citrus-producing countries being China, Brazil, Mexico, India and Spain (Source: http://www.fao.org/economic/est/est-commodities/citrus-fruit/en/). Some of the popular citrus fruits grown in India include sweet orange, mandarin, lime and among these Assam lemon locally known as kaji nemu or *Citrus limon* burm, an important fruit crop in the North-eastern state of India. Citrus is grown on 57.2 thousand hectares in the North-Eastern part of India, which is thought to have one of the highest genetic diversity reservoirs (Baruah *et al.,*2018; Baldelli *et al.,*2016). Assam lemon is a highly aromatic lemon with thick and rough skin that is rich in essential oil with a distinct flavor and aroma that contains a variety of polyphenols and other bioactive compounds with possible health advantages and can help people avoid cardiovascular and other degenerative disorders (Ekawati *et al.,*2019). Like many other citrus fruits, the bitterness of Assam lemon is primarily caused by limonin and naringin, which shows a detrimental effect on juice quality on storage. Several innovative technologies have been investigated for improv-

ing shelf life and preserving the nutritional and organoleptic qualities of fresh fruits or their products, including radiation processing, hydrothermal treatments, osmotic dehydration, pulsed electric field and other applications (Sridhar *et al.,*2021, Purewal *et al.,*2021; Ros-Chumillas *et al*., 2007; Valero *et al.,*2007; Tiwari *et al.,*2009). One of these technologies is sonication treatment, a newly developed method for debittering that is thought to be low-cost, easy to use, dependable, environmentally benign, and very successful (Bermudez-Aguirre, 2017). Ultrasonication is a process in which high-frequency sound waves are applied to a liquid to create cavitation and this phenomenon creates extraction of compounds from the liquids including the bitter compounds from the food. The high-frequency sound waves disrupt the product's cell walls, releasing enzymes that catalyse the breakdown of polyphenols into smaller, less bitter compounds. Ultrasonication can help reduce bitterness in citrus juices by breaking down bitter compounds and increasing the dispersion of bitter particles, reducing the size of the particles and increasing the surface area (Jerman *et al.,*2010; Valdramidis *et al.,*2010; Dolatowski *et al.,* 2007). Ultrasonication is already implied as an alternative to heat treatment in fruit and vegetable juice processing without affecting its nutritional properties (Bhat *et al.,*2011; Cheng *et al.,*2007). In some studies, ultrasonic treatment does not have any effect on reducing the bitterness and no impact on the TSS, TA, and pH of bayberry juice, pear, and orange juices subjected to ultrasonication (Cao *et al.,*2018; Valero *et al.,*2007; Saeeduddin *et al.,*2015). However, sonication effectiveness depends on the juice concentration and processing condition that has been observed in strawberry juice, pomelo juice, coconut shell powder, pomegranate peel and citrus peel in various applications in improving the debittering efficiency and reducing the processing time (Rodrigues *et al.,*2007; Gupta *et al.,*2021; Khan *et al.,*2010, Machado *et al.,*2019).

Cyclodextrins (CDs) are cyclic oligomers widely employed in food production as additives for various purposes, such as enhancing sensory characteristics, extending shelf life, and encapsulating components (Gonzalez Pereira *et al*.,2021). To enhance the stability and reduction of bitterness, the inclusion of β-cyclodextrin (β-CD) in lemon juice may stabilize the unstable compounds. β-CD are cyclic polymers of D-glucopyranose units linked by α-1-4 bonds, where 6,7 and 8 glucose units are identified as α, β, γ CD, which form hydrophobic central and hydrophilic external layers (Jansook *et al.,*2017; Chaudhari *et al.,*2019). CD forms inclusion complexes with fats and flavours and is also used for removing or masking undesirable flavour in food and for flavour protection and delivery (Hu *et al.,*2014; Dos *et al.,*2017). The effectiveness of binding complexes of CD depends on the complex association constant usually 10^{-1} to 10^{-4} moleK^{-1}, pH and guest host ratio of the complex formed (Linde *et al.,*2011; Dos *et al*.,2019). The extended use of CD in foods, pharmaceuticals, cosmetics and several other industries has been approved by the US Food and Drug Administration (FDA) as a food additive and is generally recognized as safe (GRAS) (Anaya-Castro *et al.,*2017).

Hence, the present study aimed to treat Assam lemon juice with ultrasonication and inclusion of β-CD, its effect on bioactive properties, and reducing limonin content to improve the juice quality.

MATERIALS AND METHODS

Materials

Fresh, matured lemon fruit samples, free from any defect, were collected from Mangaldai, Assam, India. The fruits were transported to the Department of Bioengineering & Technology, working laboratory under sterile conditions and stored in a refrigerator at 4 °C until further use.

Chemicals

All the chemicals and solvents were procured from Sigma, Loba Chemie Pvt. Ltd., Merck, Qualigens Fine Chemicals, HiMedia Laboratories Pvt. Ltd., Mumbai, India and other reputed local firms and were of the highest purity and analytical grade. Chemicals used for microbial analysis were purchased from Merck.

Juice extraction from lemon

The fruits were cleaned and washed thoroughly with running tap water and rinsed with distilled water. The peel was removed with a sharp stainless steel knife, and the fruit was rewashed with distilled water. The juice was extracted using a screw press juice extractor and filtered using Whatman no. 1 filter paper to remove any dirt particles. The juice was processed in four parts: one with control raw juice, another with ultrasonicated juice, β- CD treated juice for pH, acidity and total soluble solids analysis and the other with the addition of β- CD and ultrasonicated juice.

Ultrasonic treatment of juice

The ultrasonic treatment was given to lemon juice samples using a probe-based ultrasonicator. The sonicator (PCi analytics) was operated at a constant frequency of 20 kHz using a 19 mm diameter probe attached to the transducer and maintained at fixed pulsation. A 100 ml clean sterile glass beaker was filled with the juice sample and placed into the treatment chamber of the instrument. Further the juice was clarified and filtered by coarse sintered glass Buchner funnel under reduced pressure and transferred to a screw-capped glass bottle and pasteurization was conducted at 95 °C for 2 min. The juice was immediately cooled in an ice-cold water bath and then refrigerated at 4 °C for further

study. All sample preparations were carried out in triplicates (Tiwari *et al.,*2009).

Batch debittering procedure

Different concentrations of β-CD at 0.25, 0.5%, 0.75%, 1%, 1.5%,2% and 2.5% (w/v) were added to freshly prepared juice, and the mixture was constantly stirred for 1 hour at room temperature (24 ± 2 °C) for complete solubilization of the β-CD and the resulting mixture was stirred magnetically. Based on lower limonin content, a final 2.5g of β-CD was added to 100 ml of juice (Deshaware et al.,2018).

Determination of pH, titratable acidity (TA) and total soluble solid (TSS)

The pH of the juice samples was determined using a handheld pH meter (Eutech, Singapore), which was calibrated using buffer solutions at pH 7, pH 4 and pH 9. The pH of the juice samples was measured and recorded by dipping the hand help pH meter.

Total titratable acidity of the juice samples was determined using the titration method mentioned by association of official analytical chemistry. Briefly, 10 ml of the juice samples were titrated with a standardized 0.1 N sodium hydroxide (NaOH) solution using 2-3 drops of phenolphthalein as an indicator until a pink colour appeared that persisted for 30 seconds. This was expressed using the following formula in terms of the percentage of citric acid. mentioned in Rangana (2017).

Acidity (Citric acid %) = Volume of the titre X Normality of NaOH X Equivalent weight 0.064 / Volume of the sample X 100 X 100 Eq.1

The TSS of the juice samples was determined using a digital refractometer (Bellingham Stanley, UK). The refractometer was calibrated and cleaned using distilled water. Few drops of the juice were added to the reading well of the refractometer and the values were recorded as ºbrix.

Estimation of limonin content

Estimated by the Colorimetric method mentioned by Vaks *et al.* (1981) with slight modifications. 5 ml of centrifuged juice/extract made to 25 ml with distilled water was extracted with petroleum ether (Boiling Point at 60-80 °C) in a separatory funnel to extract the coloring matter. The petroleum ether was discarded and aqueous solution was extracted with chloroform(3×25ml). The chloroform extract was washed with distilled water (4×50ml). The volume was made to 50 ml with chloroform. A known quantity of this solution was used for the determination of limonin by developing color with Burnham's reagent (0.1 g of 4-dimethyl amino benzaldehyde dissolved in 3 ml of glacial acetic acid and mixed with 2.4 ml of 70% percholoric acid), followed by stirring vigorously on an electric stirrer.

Determination of ascorbic acid

Ascorbic acid was determined by the titration method based on 2,6- Dicholorophenol indophenol (DCPIP) method by AOAC. The dye solution was prepared by weighing exactly 50 mg of 2,6-dichlorophenolindophenol in 150 ml of distilled water with 42 mg sodium bicarbonate. The solution was then made up to 200 ml with distilled water. The dye was later standardized by titrating with a 5 ml standard ascorbic acid solution (0.1 mg/ml) added with 5 ml 3% metaphosphoric acid (HPO_3) and titrating till pink color appeared. The dye factor (mg of ascorbic acid per ml of the dye) is determined by using the formula:

$$\text{Dye Factor} = \frac{5}{\text{titre}} \quad \text{Eq.2}$$

For estimation 20 ml of the juice was made to 100 ml with 3% HPO_3, and 3 ml aliquot of the sample was taken and titrated with the standard dye to a pink endpoint which persisted for 15s. The ascorbic acid content of the sample was determined using the following equation:

mg of ascorbic acid per 100ml = Titre x Dye factor X Volume made up X 100/ Aliquot of extract taken for estimation X Volume of sample taken for estimation Eq.3

Total phenolic content estimation (TPC)

The total phenolic content of the juice was estimated using the Folin-Ciocalteau assay with slight modification, Saikia *et al*.,(2014). A 100 to 500 µL of the standard and 200 µL of the sample were taken in different test tubes and 2 ml water was added to it followed by 0.3 ml of Folin-Ciocalteau's phenol reagent and 0.8 ml 20 % sodium carbonate added after 5 min and the final volume was made up to 5 ml. After incubating the samples for 30 min at room temperature, the absorbance was measured at 765 nm in a UV-Vis spectrophotometer. Using a standard solution of gallic acid, a calibration curve was prepared and the absorbance was measured at 765 nm in a UV-Vis spectrophotometer (Cary 60 UV-Vis, Agilent) The results of phenols were expressed in µg gallic acid equivalent (GAE) per millilitre of the sample.

Total flavonoid content estimation(TFC)

The total flavonoid content of the juice was determined using the methods mentioned by Miliauskas *et al*. (2004). A 100 µL of the sample was taken and diluted to 1 ml with 900 µl of methanol added. The diluted samples were added with 10 ml of distilled water, followed by the addition of 0.3 ml of 5% $NaNO_2$. After 5 minutes, 0.3 ml of 10% NH4Cl was added, followed by 2 ml of 1M NaOH 6 minutes later. The sample was further mixed with 2.4 ml distilled water and incubated for 1 h. A standard curve was prepared using quercetin hydrate standard (0 - 250 µg/ml). The absorbance was

recorded at 415 nm in a UV-Vis spectrophotometer. Results were expressed as µg quercetin equivalent per millilitre of the sample.

Determination of 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH)

Radical scavenging activity of the juice samples was measured by determining the inhibition rate of DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical method mentioned by Saikia *et al.,* (2014). A methanolic solution of 0.1 mM DPPH radical was added to 200 µl of extract. The mixture was then incubated in the dark for 30 min at room temperature. 1 mM DPPH solution with added 200 µl was used as blank. The absorbance was measured at 517 nm in a UV-Vis Spectrophotometer. The results were expressed in terms of radical scavenging activity.

$$\text{Radical scavenging activity (\%)} = \frac{A_o - A_s}{A_o} \times 100 \quad \text{Eq.4}$$

Where, A_o is absorbance of control blank; As is absorbance of samples.

Microbiological analysis

In this study, juice samples with raw juice as control and sonicated with β-CD used for total plate counts (TPC) and yeast and mold were determined to ensure the microbial safety of the treated and non-treated juice samples. In this process, Bacteriological analytical manual technique (FDA, 2001) was used to determine the microbial load of the samples with spread plate methods using plate count agar. The plates were incubated at 37 °C for 24 hours in an incubator. The total yeast and mold count was enumerated using the spread plate method using potato dextrose agar (PDA) media. The plates were incubated at 25 °C for 5-6 days in an incubator in an inverted position. The results were analyzed as colony-forming units (CFU) per milimeter of lemon juice.

The tests were performed in triplicates and results were expressed as log CFU per milimetre of juice.

Sensory evaluation based on hedonic rating

For sensory evaluation, 15 individuals from Gauhati University Campus between the age group 20-40 years were selected as 20 age group for participation, 30 age group as panelists, and 40 age group as individuals for sensory evaluation of the samples. The sensory analysis was determined by Nayak *et al.* (2017) with slight modifications. A powdered 1g sample was mixed with 10 mL of water and kept at refrigerator temperature before being served to the panellists. Participants were asked to grade each sample on a 9-point hedonic scale concerning color, taste, aroma and overall acceptability. The 9-point hedonic ratings were as follows:

Disliked extremely – 1	Liked slightly – 6
Disliked very much – 2	Liked moderately – 7
Disliked moderately – 3	Liked very much – 8
Disliked slightly – 4	Liked extremely – 9

Statistical analysis

All measurement was taken from samples prepared and expressed as mean ± standard deviation (SD) of triplicate (N=3). The data analysed with IBM SPSS v.20.0 software. Duncan's one-way ANOVA test was used to compare the significant differences.

RESULTS AND DISCUSSION

Effect of ultrasonication and β-CD on pH, acidity and total soluble solids (TSS)

The results of ultrasonication showed no significant effect on pH and total soluble solids ($p>0.05$) at different treatment times (20, 40,60 and 80 min) in lemon juice, as presented in Table 1. Similar findings were reported in Mandarin juice (Cheng *et al*.,2020), kiwifruit juice (Wang *et al.,*2019), prebiotic-rich strawberry juice (Cassani *et al.,*2020) and tomato juice (Adekunte *et al.,*2010), where there were no significant changes observed in pH and total soluble solids ($p>0.05$). The °Brix and pH increased in jambul *(Syzygium cumini* L.) squash on ultrasonic treatment (Nadeem et al.,2022).

Table 1. Quality parameter of pH, acidity and total soluble solids (TSS) on ultrasonication

Sample	Ultra-sonicated juice		
	pH	TA (%)	TSS (^{o}brix)
Control (RJ)	3.59 ± 0.12^a	4.49 ± 0.19^a	5.00 ± 0.22^a
20 min	3.41 ± 0.41^a	4.65 ± 0.27^a	5.14 ± 0.13^a
40 min	3.43 ± 0.15^a	4.52 ± 0.22^a	5.40 ± 0.17^a
60 min	3.47 ± 0.38^a	4.99 ± 0.31^b	5.20 ± 0.28^a
80 min	3.41± 0.21^a	4.85 ± 0.22^b	5.11± 0.27^a

RJ: raw juice; TA: titratable acidity; TSS: total soluble solids; Values are the means ± SD of triplicates with the same letters in the same column showing insignificant difference ($p>0.05$); Data on the same row with different superscript (a, b, c,) are significantly different at $p < 0.05$

Significant changes were observed in acidity ($p<0.05$) in sonication at 60 and 80 mints. This study is in agreement with Nguyen *et al.* (2018) in mulberry juices and Significant changes in acidity due to the destruction of cell walls and an increase in extraction efficiency on ultrasonication treatment lead to significant changes ($p<0.05$) with increasing treatment time. β-CD commonly acts as an encapsulating agent, providing stability in a solution. It shows significant changes ($p<0.05$) in pH at 0.25 % to 2.5% concentration and acidity when it forms an inclusion with the juice as it reduces acidity and shows a significant effect on its total soluble content, as shown in Table 2. The increase in acidity is due to the breaking down of pectin substances, which is also reported by Karangwa *et al. (*2012) in blended carrot orange juice quality in addition to β-CD where increased acidity was observed in hydroxyl propyl β-CD. Similar study resulted in the increase in titratable acidity in kagzi lime juice reported by Bala *et al.* (2017) significant acidity changes on the addition of β-CD monomer.

Effect of addition of β-CD and ultrasonication treatment in limonin reduction

There was a clear relationship between the concentration of β-CD used in the batch process and the effectiveness of limonin complexation with ultrasonic treatment (Table 3). The outcome also showed that binding was already saturated at 60 minutes. It was determined that a batch process running at room temperature (30 ° C) required 2.5% polymer and 60 min of contacting time reduced the limonin content. It was observed that 2.5% of β-CD still produced palatable juice with limonin levels that were below the threshold for bitterness (5 ppm) and showed a significant change ($p<0.05$). Therefore, when ultrasonication and β-CD inclusion were combined, the sound wave generated during ultrasonication could help to break down the CD molecules and improve the encapsulation efficiency of the target compound. The increased stability provided by the β-CD inclusion could improve the ultrasonication process's effectiveness.

Effect of addition of β-CD and ultrasonication treatment on ascorbic acid

In citrus juice ascorbic acid or vitamin C helps preserve the color and flavor of the juice by preventing oxidation and the formation of brown pigment that can make the juice taste unpleasant. Ascorbic acids also provide numerous health benefits, protect the body's cells from damage caused by free radicals and help prevent cancer and cardiovascular diseases. In the present investigation, sonication treatments led to a considerable rise in the ascorbic acid level ($p<0.05$) (Table 4). Increasing treatment time caused loss of ascorbic acid content,

Table 2. Quality parameter pH, acidity and total soluble solids (TSS) on addition of β-CD at different concentrations

Sample	pH	TA (%)	TSS (^{o}brix)
Control	3.64 ± 0.01^{a}	4.98 ± 0.19^{cd}	5.00 ± 0.31^{d}
0.25% β-CD	3.31 ± 0.22^{b}	4.65 ± 0.09^{cd}	5.01 ± 0.17^{d}
0.5% β-CD	3.32 ± 0.11^{b}	4.52 ± 0.13^{cd}	5.14 ± 0.19^{d}
0.75 % β-CD	3.01 ± 0.24^{c}	4.16 ± 0.15^{d}	5.39 ± 0.11^{d}
1.00% β-CD	3.03 ± 0.32^{c}	5.13 ± 0.42^{c}	7.10 ± 0.23^{c}
1.50% β-CD	3.09 ± 0.11^{c}	5.18 ± 0.21^{ab}	9.16 ± 0.16^{b}
2.00% β-CD	2.98 ± 0.27^{c}	5.21 ± 0.28^{b}	9.46 ± 0.05^{ab}
2.5 % β-CD	2.91 ± 0.24^{c}	5.98 ± 0.27^{a}	9.66 ± 0.09^{a}

TA: titratable acidity; TSS: total soluble solids; Values are the means ± SD of triplicates with the same letters in the same column showing insignificant difference ($p>0.05$)

Table 3. Reduction of limonin with β-CD and ultrasonication treatment (ppm)

Conc. of β-CD (% w/v)	Reduction of limonin with β-CD (ppm)	Reduction of limonin with β-CD and ultrasonication treatment time (minute) expressed in ppm		
		60 min	40 min	20 min
0.25	11.00 ± 0.45^{aAB}	11.00 ± 0.55^{aB}	11.03 ± 0.18^{bB}	11.52 ± 0.38^{aA}
0.5	11.09 ± 0.77^{aB}	10.01 ± 0.67^{aB}	11.42 ± 0.31^{aB}	11.87 ± 0.15^{aA}
0.75	11.01 ± 0.34^{aB}	10.00 ± 0.88^{bC}	10.92 ± 0.55^{bB}	11.92 ± 0.11^{aA}
1.00	10.90 ± 0.43^{bA}	8.81 ± 0.12^{cB}	10.84 ± 0.85^{bA}	10.70 ± 0.12^{bA}
1.50	9.09 ± 0.41^{cB}	8.00 ± 0.13^{dC}	9.90 ± 0.23^{cA}	9.93 ± 0.29^{bA}
2.00	8.05 ± 0.71^{dB}	6.05 ± 0.18^{eA}	8.15 ± 0.11^{dB}	8.91 ± 0.51^{cA}
2.5	7.01 ± 0.32^{eA}	5.07 ± 0.32^{fC}	6.22 ± 0.37^{eB}	7.04 ± 0.22^{dB}

β-CD: β-cyclodextrin: min: minute; Values are the means ± SD of triplicates with the same letters showing insignificant difference ($p>0.05$)

Table 4. Combined effect of ultra-sonication in limonin content in addition to β-CD

Samples	Ascorbic acid (mg/100ml)	TPC (µg GAE/ml)	TFC (µg QE/ml)	Radical scavenging activity (%)
Control (RJ)	50.03±0.19^{e}	0.983±0.38^{e}	0.991±0.48^{d}	70.36±0.65^{c}
2.5% β-CD+ RJ	52.46± 0.22^{d}	1.99±0.75^{cd}	1.91±0.39^{c}	71.04±0.11^{b}
20 min + β-CD+RJ	52.97±0.61^{d}	2.24±0.84^{bc}	2.06±0.97^{c}	71.28±0.81^{b}
40 min+ β-CD+RJ	55.07±0.28^{b}	2.48±0.46^{b}	3.17±0.48^{a}	72.04±0.65^{a}
60 min+ β-CD+RJ	57.28±0.16^{a}	3.08±0.14^{a}	3.04±0.21^{ab}	72.04±0.67^{a}
80 min+ β-CD+RJ	54.36±0.34^{c}	1.17±0.33^{d}	2.92±0.33^{b}	70.36±0.31^{c}

RJ: raw juice; TPC: total phenolic content; TFC: total flavonoid content; GAE: Gallic acid equivalents; QE: Quercetin equivalent; Values are the means ± SD of triplicates with the same letters showing insignificant difference (p>0.05)

and higher retention was observed in 60 minutes of treatment compared to the control raw juice and the other 20, 40, and 80 minutes of treatment time. These findings are comparable to those made in sonicated guava juice (Cheng *et al.,*2007) and mango juice (Santhirasegaram *et al.,*2013), and Phlasa drink (Nadeem *et al.,*2022) and kiwi fruit juice (Wang *et al.,*2019), where it was observed that the ascorbic acid level had increased significantly at 60 minutes of treatment ($p<0.05$) but decreased with longer treatment times. It might be attributed that ultrasonic cavitation caused the removal of dissolved oxygen from juice solution, resulting in the breakdown of ascorbic acid and ultimately improving the ascorbic content. On the other side, the addition of β-CD enhances the high retention of ascorbic acid ($p<0.05$) with 60 min of sonication treatment time (Table 4).

Effect of treatment on total phenolic (TPC), total flavonoid content (TFC), and antioxidant capacity

The effect of treatment time and methods on TPC and TFC content in the treated and non-treated juices is shown in Table 4. Rise in TPC and TFC at 40 and 60 showed significant changes ($p<0.05$) with 80 min of treatment time, where there are no changes ($p>0.05$) with juice with β-CD treated and with a combination of ultrasonic treatment at 20 min. In gooseberry juice, a significant increase ($p<0.05$) in TPC and availability of other bioactive compounds was observed on ultrasound processing juices (Abid *et al.,*2013). The present study supported the finding on ultrasonicated mulberry juice, where TPC increased within 60 minutes of treatment, as mentioned by Nguyen *et al*. (2018). This study is supported by Deshaware *et al. (*2018), where significant increases in TPC and antioxidant was observed ($p<0.05$) in bitter gourd juice at 2 % concentration of β-CD. Several studies reported an increase in TPC and antioxidant activity increased on the inclusion of β-CD, impact stability to the phenolic compounds by solubilization of insoluble polyphenols and binding stronger inclusion complexes present in the juice and increased its bioavailability (Aree *et al.,*2016).

Citrus fruits are known for their high antioxidant properties, which can be measured using DPPH assay. In this assay, DPPH, a stable free radical, is used as a reagent to measure the ability to scavenge free radicals. In the present investigation, the percentage of DPPH inhibition in the sonicated samples was significantly higher than in the control group. In comparison to control samples (71.65%), antioxidant activity was higher in samples that were sonicated for 20, 40 and 60 min and significantly reduced ($p<0.05$) with 80 min of treatment time (Table 4). A similar finding was observed in Strawberry juice, where 30 minutes of sonication improved the antioxidant activity significantly ($p>0.05$). The present investigation also supported DPPH radical scavenging activity improvement in sonicated apple juice (Abid *et al.,*2013). Iqbal *et al.* (2023) investigated the functional properties of sonicated citrus juices and found that sonication increases phenolic content, flavonoids, and free radical scavenging activity of juices. Total phenolics rose from 223.49 to 590.47 µg GAE/g, and DPPH values increased from 791.54 to 1251.93 µmol trolox/mL post-ultrasound. This technique enhances citrus juice quality, making it viable for industrial production with improved nutrient content.

These findings suggest that sonication treatment can improve antioxidant component extractability. The increase in the bioactive compound is described as the implosive collapse of bubbles in the liquid, which generate turbulence, high-velocity inter-particle collision and, as a result, accelerated diffusion that leads to an increase in extractable antioxidants, which are higher

Table 5. Effect of sonication and inclusion of β-CD in microbial count

Sample	TPC (log cfu/ml)	TYMC (log cfu/ml)
Control	1.9 ± 0.04^{a}	1.0±0.06^{a}
β-CD juice	1.8 ± 0.09^{b}	ND
40 min + β-CD	ND	ND
60 minute + β-CD	ND	ND

ND: not detected; TPC: total plate count; TYMC: Total yeast & mold count; CFU: colony forming unit; Values are the means ± SD of triplicates with the same letters showing the insignificant difference (p>0.05)

Table 6. Effect of sonication and inclusion of β-CD in sensory profile

Sample	Test parameter			
	Taste	Odour	Colour	Overall acceptability
Control (RJ)	8.00 ±0.01^{ab}	8.00 ± 0.11^{a}	7.44 ± 0.21^{b}	8.03 ± 0.44^{ab}
20 min+ β-CD	8.00 ±0.43^{ab}	8.22 ±0.18^{a}	8.00 ±0.13^{a}	7.81 ±0.11^{b}
40 min+ β-CD	8.03 ±0.32^{a}	8.35 ±0.28^{a}	8.00 ± 0.52^{a}	8.11 ±0.19^{a}
60 min+ β-CD	8.00 ±0.27^{b}	8.36 ±0.34 ^{a}	8.05 ±0.41^{a}	8.18 ± 0.35^{a}

RJ: raw juice; β-CD: β-cyclodextrin; Values are the means ± SD of triplicates with the same letters showing insignificant difference (p>0.05)

than control juices (Chowdhury et al.,2009). In the present investigation, the ultrasonically produced cavitation, which may have contributed to the observed changes, may be directly ascribed to the enhanced concentration of antioxidant capacity in sonicated juice. Therefore, out of four sonication times and inclusion of β-CD and sonication probe, an appropriate 60 minutes can improve the Assam lemon juice quality.

Effect of sonication and inclusion of β-CD in microbial count

The presence of microbial load in the food products causes the generation of undesirable flavours and initiates the production of bio-toxic compounds. Due to the production of toxins, the product loses its shelf life during storage, which makes it unsafe for human consumption. Since the reduction of microbial load in food products is also an important part of processing. In this context, sonication is a useful technique that reduces the bacterial count of fruit juices and helps meet FDA specifications (2009). In the present study, the addition of β-CD and ultrasonication treatment showed a significant reduction ($p<0.05$) of microbial load from 40 min to 60 min of treatment (Table 5). This minimizing effect was also observed in orange and tomato juices on ultrasonication treatment ((Adekunte *et al.,*2010; Valero et al.,2007). Similar reports were found in strawberry juice as ultrasound treatments significantly decreased ($p \leq 0.05$) counts of aerobic mesophilic bacteria, molds, yeasts, and coliforms at 35 °C compared to the untreated juice (Menelli *et al.*,2021). Changes in bitterness concentration can also affect microbial count on the storage and preservation of juice from pathogens. In this regard, the inclusion of β-CD helped to improve the reduction of harmful microorganisms. Hence, sonication combined with other physical treatments helped to improve juice quality and safety.

Effect of treatment on sensory properties based on Hedonic ratings

The mean sensory scores for various attributes of ultrasonication treatment with β-CD inclusion juice compared with raw juice and 20, 40 and 60 min of treated juice. The taste of raw juice was comparatively the same with 20 min of treatment and no significant difference is observed ($p>0.05$) (Table 6). The color of the juices was the same and there was no significant difference ($p>0.05$). Odor and overall acceptability were found to be better in 60 minutes of treatment time, a significant difference ($p<0.05$), which is highly acceptable compared to raw juice. The sensory analysts preferred 60 minutes of treated β-CD encapsulated juice, which enhanced its taste better as β-CD masks the bitter compound.

Conclusion

Effectiveness in complexing with β-CD and ultrasonication treatment provides chemical stability and is generally regarded as a safe status for processing aids. The present study revealed that β-CD is a promising candidate for debittering citrus juice in combination with sonication treatment with balanced time. The sonication treatments of lemon juice significantly ($p<0.05$) improved the overall selected attributes and β-CD masks the bitter taste of the juice. The combined treatment of ultrasonication with β-cyclodextrin, can potentially increase bioactive substances without compromising their sensory and nutritional aspects, improve juice quality and act as microbiologically safe. It is suggested that more research be carried out on the effects of sonication on juices in combination with other physical food processing techniques and enzyme activities in sonicated juice samples.

Conflict of interest

The authors declare that they have no conflict of interest.

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