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Optimization of ultrasound processing parameters for preservation of matured coconut water using a central composite design

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ORIGINAL ARTICLE

Abstract

Mature coconut water (MCW) is a natural beverage and a main by-product of various coconut processing industries such as virgin coconut oil, coconut chips, coconut milk, etc. In spite of huge benefits, MCW's short shelf life limits its market potential. In this context, the present study investigates the effect of ultrasonic processing parameters, such as amplitude (50%, 60% and 70%) and time (5 min, 10 min and 15 min), on microbial population and quality profile (pH, total soluble solids, total sugars, reducing sugars and non-reducing sugars) of MCW. Central composite design was used to create a multiple linear regression model for each response and to optimize ultrasound processing parameters. The optimal treatment parameters to ensure microbial safety and preserve the nutritional quality of MCW were 60% ultrasonic amplitude and treatment time of 10 min. Total sugars, reducing sugars, non-reducing sugars and microbial load of MCW determined at optimized conditions were 4.92%, 2.804%, 2.13% and 4.79 log cfu/mL, respectively. Ultrasonic treatment was found to be effective in inhibiting microbial growth and maintaining non-reducing sugars of MCW.

Keywords: ultrasonic treatment, coconut water, central composite design, total sugar, microbial load

Introduction

Coconut water is found in the cavity of coconut, and its nutrients (Prithviraj *et al*., 2021) and volume varies depending on nut maturity (Pandiselvam *et al*., 2019). It takes 11–12 months for nuts to develop (Beegum *et al*., 2022). Coconut water is mainly classified as tender coconut water (TCW) and mature coconut water (MCW). TCW is a natural and nutritious drink obtained from 6 to 7 months old matured fruit whereas coconut meat and MCW are main edible products obtained from 12 months old matured fruit. MCW is a major by-product of coconut meat processing industries such as desiccated coconut powder, coconut milk powder, coconut chips and virgin coconut oil. MCW possesses sugars and functional electrolytes that are beneficial for health (Sunil *et al*., 2020; Yong *et al*., 2009). A single matured coconut contains an average of 50–250 mL of coconut water (Beegum *et al*., 2018). In India, the annual coconut production in 2016–2017 from an area of 2.08 million ha was 23.90 billion nuts (Subramanian *et al*., 2018). A large volume of coconut water left unutilized during copra and coconut meat processing is estimated at 2.4-billion liters, which is not only wasted annually but also contributes to environmental pollution (Chauhan *et al.*, 2014).

Food science researchers have attempted to develop various value-added products, such as vinegar, squash, jelly, flavored drink, sparkling wine, coconut champagne and coconut water concentrates, from MCW. However, the nutritional profile, microbial safety and potential market price of these products depend on the initial quality of MCW. The traditional method used for collecting MCW spoils it within a day because of the proliferation of bacteria, such as *Escherichia coli*, which could be as high as 10^6 cfu/mL (Balter *et al.*, 2005). Exposure to air affects coconut water's sensory and nutritional qualities (Duarte *et al.*, 2002). A new and potential strategy is required to improve the shelf life of MCW and preserve its natural freshness, flavor and aroma.

Coconut water available in the market is treated with high temperature and short-time thermal process. Thermally processed coconut water by adding biopreservatives has extended shelf life, but it creates a negative impact on sensory attributes and quality profile (Haseena *et al.*, 2010; Pandiselvam *et al.*, 2022). Thermal processing is effective in inactivating enzymes and providing an antimicrobial effect (Atalar *et al.*, 2019); however, the use of nonthermal technology is gaining more prominence for preserving natural beverages without affecting their original quality (Prithviraj *et al.*, 2021).

Several novel nonthermal technologies, such as cold plasma (Dong *et al.*, 2021; Gavahian *et al.*, 2020), ultraviolet light, ozone, pulsed light (PL), ultrasound, pulsed electric fields, high-pressure processing (HPP), high-pressure homogenization, ionizing radiation, and ozone (Kongruang and Kleesuwan, 2020), have been tried for extending the shelf life of fruit juices. Choosing an appropriate processing technique must be considered keeping in view the biological and chemical safety of final products (Mousavi Khaneghah, 2021). In recent years, ultrasound (US) technology has gained attention because of its safety and ability to maintain the original flavor of food (Jiang *et al.*, 2020). The cavitation effect of ultrasound results from the generation, growth and implosion of tiny bubbles (Ercan and Soysal, 2011). Cao *et al.* (2019) showed that ultrasound treatment at intensity of less than 450 W/cm^2 within 8 min was effective in maintaining the quality of bayberry juice.

Optimization in food processing entails determining the best quality criteria (product and process efficiency) while saving time and money (Baş and Boyacı, 2007; Sevda *et al.*, 2012; Witek-Krowiak *et al.*, 2014). Achieving optimum conditions requires evaluating interactions and effects of different factors, and this can be done only through experimental studies. In conventional optimization, the one-factor-at-a-time approach is used to optimize multivariable system. Use of conventional methods requires different experiments, and it does not represent the combined effect (Behera *et al.*, 2018). Response Surface Methodology (RSM) is the most widely used experimental design because of its minimal number of experiments and fast experimental speed (Nwabueze, 2010; Xu *et al.*, 2022). The central composite design (CCD) model is a key component of RSM and is more accurate and requires no three-level factorial experiment to build a second-order quadratic model.

To our knowledge, no study is available on the application of ultrasound treatment for the preservation of MCW. Also, no optimization has been conducted for the ultrasonic treatment of MCW. In this context, the present study aims to optimize the process parameters of ultrasound treatment to preserve the physicochemical quality of MCW and ensure its microbial safety.

Materials and methods

Raw material

Mature coconuts (Ver. West Coast tall), aged 11–12 months, were obtained from the coconut nursery of Tamil Nadu Agricultural University, Coimbatore, India. Damage-free sound coconuts were selected for the extraction of water.

Collection of coconut water

Dehusked coconuts were split into two halves by using stainless steel cutter and the water was collected in a stainless steel container after filtering through a muslin cloth. Treatments were carried out immediately after collecting MCW to maintain quality of the product.

Ultrasonic treatment

Mature coconut water (80 mL) was taken in a 100-mL sterile glass beaker for ultrasonic treatment. A 20-kHz, 230-volt ultrasonic processor (VCX1500; Sonics and Materials Inc., Newtown, CT, USA), as shown in Figure 1, was operated at 1,500 W. A 25-mm diameter probe was immersed (about 2 cm) in the center point of the sample container. Ultrasonication was carried out at amplitudes of 50%, 60% and 70% at 28°C. A thermocouple was inserted in MCW to measure and maintain constant temperature throughout the experiment. Treatment periods (5, 10 and 15 min) were studied with 5-s on and 5-s off pulse cycle. As shown in Figure 1, MCW was constantly

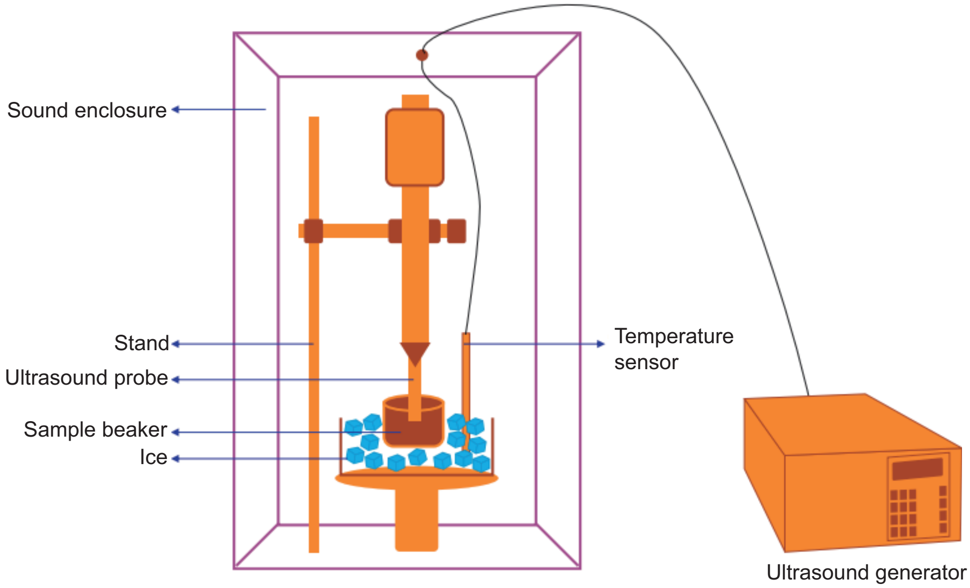

Figure 1. Experimental setup for ultrasonic treatment.

cooled to maintain a temperature of 28°C by immersing the sample glass beaker in an ice bath during the process of ultrasonication.

Method of analysis

Determination of pH and total soluble solids

The pH and TSS values of MCW were measured using a digital pH meter (accuracy: ±0.1 pH brand: Enric, Ahmedabad, India) and refractometer (Erma Inc., Tokyo Japan), respectively.

Determination of total sugars, reducing sugars and non-reducing sugars

Amount of total sugar in MCW was determined by using Dubois *et al.*'s (1956) procedure. The reducing sugar content of MCW was determined using the Nelson–Somogyi method as described by Ranganna (1986). The amount of non-reducing sugar was calculated using the following formula: Non-reducing sugar (%) = total sugar (%) – reducing sugar (%).

Determination of microbial load

The microbiological analysis of MCW was carried out by the total plate count method. The serially diluted samples were enumerated using the pour plate method. Duplicate diluted samples were poured onto plate count agar, and incubated for 24–48 h at room temperature for detecting microbial load (Purkayastha *et al.*, 2012).

Response surface methodology experiments

Response surface methodology (RSM) was employed to establish the optimum conditions of ultrasonic amplitude and treatment period for retention of non-reducing sugars and microbial stability of MCW. Design expert software was used to analyze data and construct models (version 13.0.5.0, Stat-Ease Inc., Minneapolis, MN, USA). Two-factor, three-level CCD was used to develop multi-regression models. Table 1 highlights the range and center point values for two independent variables, namely ultrasonic amplitude and treatment period. In CCD, effect of each independent variable, and interaction between independent variables were investigated and reported.

Results and Discussion

Model fitting

Response surfaces were used to determine the best model to demonstrate the influence of independent variables on dependent variables (Aydar*et al.*, 2018). Table 2 shows the significant effect of independent variables (ultrasound amplitude [%] and sonication time [min]) on quality profile (pH, TSS, total sugars, reducing sugars

Table 1. Independent variables and their level used for central composite design.

Independent variables	Coded levels				
	–α	–1	0	+1	+α
Amplitude (A)	45.8579	50	60	70	74.1421
Time (B)	2.92893	5	10	15	17.0711

Table 2. Ultrasound-based experimental design used for preservation of mature coconut water.

Run	Independent variables		Dependent variables					
	Ultrasound exposure time (min)	Ultrasound amplitude (%)	pH	TSS (Bx)	Total sugars (%)	Reducing sugars (%)	Non-reducing sugars (%)	Microbial load (log cfu/mL)
1	5	50	5.3	5	4.80	2.56	2.24	5.32
2	5	70	5.3	5	4.96	2.96	2.00	4.92
3	15	50	5.3	5	4.68	2.63	2.05	4.90
4	15	70	5.3	5	4.78	3.04	1.74	4.70
5	10	45.8579	5.3	5	4.76	2.58	2.18	5.12
6	10	74.1421	5.3	5	4.85	3.01	1.84	4.78
7	2.92893	60	5.3	5	5.01	2.86	2.15	5.19
8	17.0711	60	5.3	5	4.82	2.86	1.96	4.73
9	10	60	5.3	5	4.96	2.82	2.14	4.72
10	10	60	5.3	5	4.90	2.70	2.20	4.79
11	10	60	5.3	5	4.92	2.82	2.10	4.82
12	10	60	5.3	5	4.89	2.78	2.11	4.82
13	10	60	5.3	5	4.93	2.83	2.10	4.84
	Control		5.3	5	5.12	2.40	2.72	6.92

and non-reducing sugars) and microbial populations. Experimental design for the optimization of ultrasonic process parameters was chosen based on a previous study (Vivek *et al*., 2016). From the experimental data, coefficients of polynomial equation were calculated to predict responses. The results of 13 sets of experiments were analyzed and the interpretation was provided. F-value, *P*-value and significance of each variable on performance parameters of ultrasound treatment on dependent parameters are given in Table 3. Results of statistical analysis (ANOVA) revealed that the experimental data could be represented well with a linear and second-quadratic polynomial model, with coefficient of determination (R^2) values for total sugars, reducing sugars, non-reducing sugars and microbial load being 0.8938, 0.9036, 0.9476 and 0.9764, respectively. The proximity to unity R^2 in our study shows that the influence of ultrasound amplitude and ultrasound exposure time on response variables could be adequately described by linear and quadratic polynomial models. The results suggested that the regression model could fit dependent variables, namely total sugars, reducing sugars, non-reducing sugars and microbial load values, considerably, and the error analysis indicated that the lack of fit was insignificant for these dependent variables.

Response surface analysis

Effect of ultrasonic treatment on pH and total soluble solids of mature coconut water

The pH and TSS values of fresh and processed samples did not differ significantly (Table 2). Similar results were reported for grape juice by Aadil *et al*. (2013) that its pH did not change with ultrasonication (at processing conditions of 28-kHz frequency and temperature of 20°C) for 60 and 90 min. Furthermore, Saeeduddin *et al*. (2015) found that pears treated at processing conditions of 20-kHz frequency and 70% amplitude showed no significant changes in TSS and pH. A nonsignificant change in the pH of lime juice was observed after treating it for 60 and 90 min (Bhat *et al*., 2011) at a processing condition of 25-kHz frequency at 20°C. Also, no significant changes in pH were observed in apple juice (Kenari and Belgheisi, 2019) treated with an ultrasonic bath (for 15–60 min at 40°C and 60°C temperatures) and ultrasonic probe (for 10–20 min at 40°C and 60°C temperatures), and in tomato juice (Starek *et al*., 2021) at ultrasound intensity of 28 W cm^{-2} and 40 W cm^{-2} and frequency of 20 kHz.

Effect of ultrasonic treatment on total sugars and reducing sugars of matured coconut water

The plot of total sugars and reducing sugars as affected by ultrasonic amplitude and treatment time is shown in Figure 2. Upon sonication, a slight reduction was observed in total sugars. Yuan *et al*. (2009) also reported of having decreased total sugars in apple juice treated with an ultrasonic frequency of 20–24 kHz and an ultrasonic power of 60–900 W. Although reduction in browning after ultrasound treatment could be attributed to a decrease in sugar content, a slight reduction was observed in total sugars despite no significant change in TSS. However, the reducing sugar contents of MCW showed an increasing trend upon sonication and this could be due to high shear force upon cavitation

Table 3. F-values, *P*-values and significance of each variable on performance parameters of ultrasound treatment on dependent parameters.

	Total sugars		Reducing sugars		Non-reducing sugars		Microbial load	
	F-value	*P*-value	F-value	*P*-value	F-value	*P*-value	F-value	*P*-value
A	11.54	0.0115	92.71	<0.0001	70.67	<0.0001	95.36	<0.0001
B	24.8	0.0016	1.04	0.3325	34.35	0.0006	135.96	<0.0001
AB	0.5539	0.4810	-	-	0.6518	0.4460	0.6518	0.0378
A^2	21.73	0.0023	-	-	16.24	0.0050	16.24	0.0012
B^2	1.13	0.3230	-	-	7.08	0.0324	7.08	0.0009
Lack of fit	3.72	0.1183	0.8917	0.5722	1.10	0.4453	0.2761	0.8406
R^2	0.8938		0.9036		0.9476		0.9764	
Adj. R^2	0.8180		0.8843		0.9102		0.9595	

which resulted in the release of entrapped sugar molecules from the cell wall and membrane structure of MCW (Cruz-Cansino *et al.*, 2016; Zou and Jiang, 2016). Owing to the mechanical action of ultrasound, solvent's penetration power also increased, which eventually increased the diffusion process from material to solvent (Rostagno *et al.*, 2003). This could be due to the hydrolysis of non-reducing sugars into reducing sugars during ultrasound process. Increase in the percentage of reducing sugars of MCW upon sonication was in agreement with the results of previous studies which reported that sonication increased the contents of reducing sugars in carrot juice (Jabbar *et al.*, 2014), melon juice (Fonteles *et al.*, 2012) and apple juice (Abid *et al.*, 2014).

According to the results, the error analysis indicated that the lack of fit was not significant for reducing sugars and total sugars. The polynomial regression equations for reducing sugars and total sugars are given as under:

$$\text{Reducing sugars} = 1.70276 + 0.0177264 \times A + 0.00375 \times B;$$

$$\text{Total sugars} = 1.96172 + 0.093341 \times A + 0.0167825 \times B - 0.0003 \times AB + -0.0007125 \times A^2 - 0.00065 \times B^2,$$

where A is the amplitude and B is the time.

Effect of ultrasonic treatment on non-reducing sugars of matured coconut water

Sugars in coconut water are an important source of ergogenic aid, since it is the main source of energy for humans (Kailaku *et al.*, 2015). In sugars, non-reducing sugar is one of the important quality parameters of MCW that distinguishes young coconut water from MCW (Burns *et al.*, 2020). The response surface plot of the effect of ultrasonic amplitude and treatment time on non-reducing sugars demonstrates a significant decrease in non-reducing sugars with an increase in ultrasonic amplitude and treatment time (Figure 2). This could be due to the hydrolysis of sucrose (non-reducing sugars) into fructose and glucose after sonication (de Souza Soares *et al.*, 2019). Fonteles *et al.* (2012) reported that ultrasound-treated cantaloupe melon juice showed an increase in the content of reducing sugars. Samples treated at 60% amplitude for 10 min were found to have optimum content of non-reducing sugars. In the early stages of coconut maturation, almost all sugars, such as glucose and fructose (over 75%), are reducing sugars, but in their later stages (mature coconut), non-reducing sugars (sucrose) become more prominent (Jackson *et al.*, 2004). Therefore, in the case of MCW, retention of non-reducing sugars is considered. Even though maximum non-reducing sugars were retained at low amplitude and time (50% amplitude and 5-min time), this did not bring about the expected reduction in microbial load. Hence, by considering quality parameters and microbial load, the samples treated at 60% amplitude for 10 min were found to be optimum. Ultrasonic amplitude and treatment time had a significant ($P < 0.05$) effect on the non-reducing sugars of MCW ($R^2 = 0.9476$, and CV = 2.10), which indicated that the quadratic model fits considerably (Table 3).

The polynomial regression equation for non-reducing sugars is as follows:

$$\text{Non-reducing sugars} = 0.3128 + 0.0701146 \times A + 0.0380325 \times B - 0.00035 \times AB - 0.0006625 \times A^2 + -0.00175 \times B^2,$$

where A is the amplitude and B is the time.

Effect of ultrasonic treatment on microbial load of matured coconut water

Ultrasound destroys microorganisms by releasing energy from acoustic phenomenon (Hashemi Moosavi *et al.*, 2021).

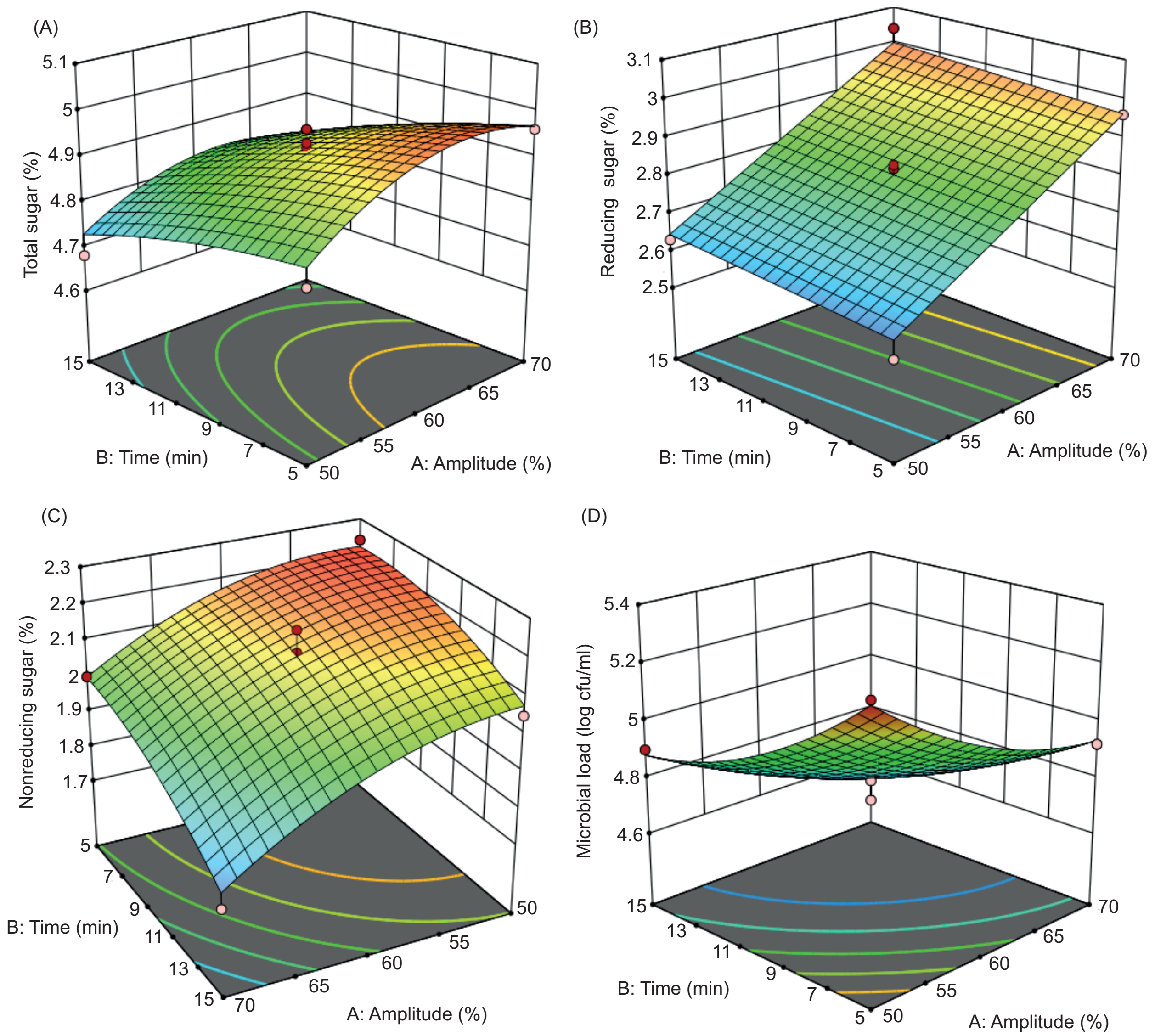

Figure 2. Effect of ultrasonic amplitude and treatment time on (A) total sugars, (B) reducing sugars, (C) non-reducing sugars and (D) microbial load of MCW.

The plot of microbial load as affected by ultrasonic amplitude and treatment time is given in Figure 2. Use of ultrasonic treatment significantly ($P < 0.05$) reduced the growth of microbial population. Independent variables (ultrasonic amplitude and treatment time) showed a significant reduction in microbial load ($R^2 = 0.9764$, and CV = 0.7992). It was observed that microbial load decreases with an increase in ultrasonic amplitude and treatment time. Maximum reduction in microbial load was observed at 70% amplitude and 15-min treatment time, and minimum reduction in microbial load was observed at 50% amplitude and 5-min treatment time. It could be probably due to increased permeability of membranes and DNA damage via free radical production because of acoustic cavitation (Pratheepa and Kamalanathan *et al.*, 2020). In comparison with the control sample, the total number of microorganisms was reduced by 2.14 log cfu/mL at 70% ultrasound amplitude and a treatment time of 15 min. Cruz-Cansino *et al.* (2016) also reported that treatment at higher amplitude for longer period was effective in achieving microbial reduction in cactus pear juice. Overall, microbial population in sonicated sample was significantly lower than that in non-sonicated sample (control).

The polynomial regression equation for microbial load is given below:

$$\text{Microbial load} = 9.64126 + -0.11621 \times A - 0.158063 \times B + 0.001 \times AB + 0.0007725 \times A^2 + 0.00329 \times B^2,$$

where A is the amplitude and B is the time.

Table 4. Optimized combination and desirability analysis.

	Amplitude (%)	Time (min)	Total sugars (%)	Reducing sugars (%)	Non-reducing sugars (%)	Microbial load (log cfu/mL)
Predicted results	60	10	4.920	2.804	2.130	4.798
Experimental result	60	10	4.92	2.79	2.13	4.798

Optimization of ultrasonic treatment conditions

The numerical optimization was executed in the design of expert of 'version 13.0.5.0', which provided a desirability function of 0.821. The goals selected for the optimization of ultrasonic parameters for MCW were maximum values for total sugars and non-reducing sugars, and minimum values for reducing sugars and microbial load. Each independent variable (ultrasonic amplitude and treatment time) was given equal importance of '5'. Accordingly, '3' was assigned to total sugars, reducing sugars, non-reducing sugars, and microbial load based on their relative contributions to final product quality. The optimized data and results are shown in Table 4. For the ultrasonic treatment conditions for MCW to be optimal, total sugars and non-reducing sugars must reach maximum levels, while reducing sugars and microbial load must attain minimum levels. The combined optimized conditions of ultrasonic process parameters for MCW were 60% amplitude and 10 min treatment time. The response values at optimized conditions were 4.92% total sugars, 2.804% reducing sugars, 2.13% non-reducing sugars, and 4.798 log cfu/mL microbial load. The predicted results were verified by experimental test.

Conclusion

In this study, we evaluated the application of ultrasound for the preservation of MCW. The results of this study showed that ultrasonic process parameters had a significant effect on microbial population and quality of MCW. According to the current study, linear and quadratic models adequately described and predicted changes in total sugars, reducing sugars, non-reducing sugars, and microbial load as functions of independent variables (ultrasonic amplitude and treatment time). In conclusion, CCD is an effective technique for optimizing ultrasonic conditions for MCW. Based on the desirability function, optimum conditions were determined through numerical optimization. In this study, sonication treatment decreased the concentration of non-reducing sugars, total sugars and microbial population whereas increased the content of reducing sugars. It was observed that applying ultrasound at 60% amplitude for 10 min was the most effective treatment for reducing microbial population and maintaining quality parameters of MCW. It is necessary to study and optimize the effect of other ultrasonic conditions, such as frequency and temperature, on MCW in the future studies.

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