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Optimization of ohmicsonication for overall quality characteristics of NFC apple juice

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Abstract

In this work, the feasibility of using ohmicsonication (OS) as a new hurdle technology for pasteurization of not-from-concentrate (NFC) apple juice was proposed. Optimization of OS conditions (ohmic heating [OH] temperature and sonication time) was done by response surface methodology with polyphenoloxidase (PPO) activity as endpoint evaluation of the NFC apple juice. The effect of OS as well as sonication (S), thermosonication, OH as an alternative to traditional technologies on inactivation of PPO and some quality parameters were studied. The obtained results showed that the highest inactivation of PPO was by OS treatment (98%). The highest bioactive profiles (ascorbic acid, total carotenoids, and total phenolic contents) and color values were obtained with the S and OS compared to other treatments. In addition, the lowest hydroxymethylfurfural and cloud values were with OS. Finally, OS could be applied as an alternative thermal process to produce NFC apple juice.

Practical applications

The results of this study showed that the lab scale ohmicsonication (OS) succeeded as an alternative thermal process to produce NFC apple juice. OS process showed less degradation of the bioactive profile and improved inactivation of polyphenoloxidase in comparision to other treatments. Ohmic heating (OH) is already used in many factories. Therefore, we proposed to include an ultrasound probe instead of the existing blenders in the mixing tank before OH line. This would improve the mixing process as well as getting the ultrasound benefits.

1 | INTRODUCTION

Apple juice (*Malus Domestica*) is a rich source of phytochemicals (such as phenolic compounds, carotenoids, and ascorbic acid) as well as other nutrients. These phytochemicals have numerous health benefits exemplified by reduction of the risk of many diseases (i.e., cancers, asthma, and diabetes) (Boyer & Liu, 2004). Phenolic compounds in apple juice are responsible for development of color and flavor (Rawson et al., 2011) and their concentration depends on many factors such as apple variety, and agricultural conditions as well as processing method (Will, Roth, Olk, Ludwig, & Dietrich, 2008). The presence of hydroxymethylfurfural (HMF) in the juice is due to excessive heat treatment and inappropriate storage (Zou & Jiang, 2016). Generally, fruit juices are susceptible to quality characteristic changes whether during processing steps or storage time that mainly related to enzymatic activities. The extraction of apple juice results in an enzymatic browning (due to the oxidation and dehydrogenation of polyphenols to o-quinones by polyphenoloxidase (PPO) activity) (Gong, Li, Liu, Cheng, & Wang, 2015). Not-from-concentrate (NFC) juice is one of the preferable juices processed by removal foreign impurities (i.e., insoluble pulp, skin, and seeds) without dilution or concentration. This NFC juice should then undergo heat treatment to control both microbiological load and enzymatic activities at safe limits, whilst maintaining as much of the taste, odor, and color as possible. The NFC juice should be treated by high-temperature short time (HTST) and deaerated by pressing N_2 gas instead of air inside the packages with hot closing. The juice is then stored at 0–2°C for at least 1 year (Clark, 2009).

Conventional thermal technology (i.e., pasteurization and sterilization) have commonly been used for juice processing in food plants. Nevertheless, this treatment could cause undesirable changes in taste and odor as well as damage of bioactive components and functional properties during juice processing (especially if treated at higher temperature than 80°C for a long time) (Mena et al., 2013). Hence, the food researchers seek to apply alternative recent technologies, for example, ohmic heating (OH) (thermal) and sonication (S) (non-thermal) capable of inactivating enzymes and microorganisms as well as preserving food nutritional values and physical and chemical characteristics (Chemat & Khan, 2011). OH is a fast heat treatment by applying alternating electric current to a food material. During OH treatment, the electrical energy is converted into heat in a rapid and uniform manner with minimal thermal degradation of nutrients (Lima, 2014). OH has achieved great success during, for example, fruit juice production such as pineapple, papaya, apple, mango, and orange to inactivate polyphenoloxidase, peroxidase (POD), and pectinmethylesterase (PME) (Abedelmaksoud, Mohsen, Duedahl-Olesen, Elnikeety, & Feyissa, 2018a, 2018b; Demirdöven & Baysal, 2014; Mohsen, Murkovic, El-Nikeety, & Abedelmaksoud, 2013). Sonication (S) was considered as a potential substitute technology while reducing 5-log in the microbial load of fruit juices as required by FDA, when high-power sonication generates cavitation bubbles resulting in pressure changes and resulting in the succeeding compression cycles of a propagated sonic wave (USFDA, 2001). Sonication alone has limited applications, however, it has been used successfully when mixed with other treatments. For example, combining sonication with heating at temperatures of 37 to 75°C (known as Thermosonication (TS)) inactivated both enzymes and microorganisms in juice as an alternative pasteurization by Lee, Kim, Cadwallader, Feng, and Martin (2013). Due to cavitation and heat, TS utilize lower temperatures compared to conventional heating (CH) treatments to achieve the same level of inactivation of enzymes and microorganisms (Abdullah & Chin, 2014). However, a combination of sonication with other treatments such as heat, ultraviolet irradiations, and pressure has previously been investigated (Leistner, 2000).

However, there is no publications already exist on the effects of ohmicsonication (OS) (combination of sonication with OH) as a substitute pasteurization process for obtaining the quality properties of juices until now. Therefore, the aim of this study was to study the effect of an optimized OS (OH temperature and sonication time) as a new potential combined technology on physical, chemical, and microbiological characteristics of NFC apple juice. We will compare OS to other technologies (S, TS, OH, and CH) to select the best treatment method that improves overall quality properties of NFC apple juice.

2 | MATERIALS AND METHODS

2.1 | Chemicals

The different chemicals, solvents, and reagents such as catechol, polyvinyl poly pyrrolidone (PVPP), sodium bicarbonate, 2,6-dichlorophenol-indophenol (DCPIP), fructose, sucrose, glucose, L-ascorbic acid, 5-hydroxy methyl furfural, aluminum chloride, sodium nitrate, butylated hydroxytoluene (BHT), acetone (p.a.), oxalic acid, hexane (p.a.), sodium carbonate, and Folin–Ciocalteau reagent from Sigma-Aldrich Chemical Co., Denmark, methanol (p.a. fromVWR nr. 20846.320) and NaOH 50% (w/w) (J.T. Baker 7067, New Jersey, USA) were used.

2.2 | Raw material

Fresh apple (*Malus Domestica*, *cv. Elstar*) fruits bought from a local supermarket in Copenhagen, Denmark, were washed and cut into quarters. The foreign impurities (i.e., seeds, overripe portions, and stems) were discarded and juice extraction was done as described previously (Abedelmaksoud et al., 2018b). The apple juice batch was divided into six parts named according to treatments: (1) fresh apple juice; (2) S; (3) CH; (4) TS; (5) OH, and (6) OS. All six parts were quickly cooled (4°C) using an ice bath and kept at −18°C.

2.3 | Processing methods

2.3.1 | Conventional heating

The juice samples (150 ml apple juice in a clean 250 ml glass bottle) were treated at 90°C for 60 s in shaker water bath (Julabo, SW22, Germany) as described by Abedelmaksoud et al. (2018b).

2.3.2 | Sonication and Thermosonication (S and TS)

S and TS of apple juice samples of 150 ml juice in a 250 ml glass bottle was carried out by an ultrasonic processor of 550W (Sonifier SFX550 Model, Mexico) at 20 kHz frequency with a 0.5-inch probe. Sonication treatment was conducted at 25°C by applying 100% of power (550 W) for a holding time of 8 min keeping pulse durations of 5 s. TS was done by setting the temperature at 60°C and others conditions similar to sonication. Overheating of the samples was stopped by circulating ice water out of the chamber of treatment.

2.3.3 | Ohmic heating

An ohmic heater (BCH ltd., Lancashire, UK) with an ohmic unit consisting of a holding cell made of W500 grade polyethylene–polypropylene with variable size adjustment and mountings for temperature loggers (K-type) was used. The ohmic heater had a maximal supply of 230 voltage using alternating current (60 Hz, sinusoidal) and a titanium electrode which had a high corrosion resistance in the chloride environments. A distance between the electrodes at 3.9 cm and a width of the chamber of 9.5 cm was set. According to Abedelmaksoud et al. (2018b) OH treatment was conducted after optimization of OH parameters by response surface methodology (RSM). OH at 40 V/cm and 80°C (±1°C) for a holding time of 60 s was selected as an optimum condition for inactivation of PPO with the best maintain for quality profiles in apple juice.

2.3.4 | Ohmicsonication

OS was conducted by a combination of OH and sonication treatments, where the apple juice samples were first treated with sonication for 8 min and then directly followed by OH at 40 V/cm, to 75°C for a holding time of 60 s. Set up of the OS is shown in Figure 1.

2.4 | Physical analysis

The electric conductivity (EC) and color values [L^* (lightness), a^* (redness), b^*(yellowness), and ΔE (total color differences)] of NFC apple juice samples was measured using the same method as Abedelmaksoud et al. (2018a). The viscosity was measured (at 22 ± 2°C) using a viscometer (Model DV-II, Brookfield Engineering Laboratories, Inc, Stoughton, MA, USA), with spindle number 5 and a speed of 100 rpm. The cloud value of apple juice samples was measured according to Versteeg, Rombouts, Spaansen, and Pilnik (1980). Size distribution particles of apple juice (20–30 ml) were detected with the Mastersizer (Model 2000, Malvern, UK) (El-Maksoud et al., 2018).

2.5 | Chemical analysis

2.5.1 | Sugar compounds

Glucose, fructose, and sucrose were analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) according to Mariotti et al. (2015). Two to five grams of homogenized apple juice was mixed with150 mL of Milli-Q water (60°C) in 250 ml conical flask and shaken for 3 hr in SW22 shaking water bath (Julabo, Germany) at 60°C. The volume was then completed to 200 ml by Milli-Q water (volumetric flask) followed by filtration (10 µm, Ø125 mm, Whatman Inc., USA). Triplicates using 1 mL filtrated (0.22 µm Whatman inc., USA) supernatant of each was transferred to a HPLC vial and placed in an AS-model autosampler (Dionex ICS- 3000, Thermo Fisher Scientific, Sunnyvale, CA, USA) at 10°C for sugar detection by HPAEC-PAD (injection volume of 25 µl). Separation was done as described by Mariotti et al. (2015) with a total run time of 25 min and an eluent flow of 0.45 ml/min with a linearity test in the concentration range of 0.0005–0.05 mM by means of glucose (rt = 6.02 min), fructose (rt = 7.06 min), and sucrose (rt = 8.01 min) standards with a recovery of 0.02 mM from each sugar in the juice between 85 and 115%.

2.5.2 | Hydroxymethylfurfural

HMF was determined according to a method of Kalábová and Večerek (2006) with modification for apple juice. A mixture of a half mL of juice with 1 ml of methanol were vortexed (Vortex Genie II, Scientific Industries, Bohemia, USA) for 10 min followed by centrifugation (Bioguge pico, Heraeus, D-37520 Osterode, Germany) at 13,000 rpm for 10 min. This extract was filtered (0.45 µm, Whatman Inc., USA) and directly used for HPLC analysis injecting a volume of 20 µl. The chromatographic determination was carried out on an Alliance apparatus manufactured by Waters Company, with a 2,996 diode array detector and separation on a Zorbax Eclipse XDB-C8 column (4.6 × 150 mm, 5 µm Waters, Milford, USA) at 30°C. For analysis, a mobile phase (10% of methanol in water) with a flow rate of 1 ml/min was used with detection of HMF in the UV region at 285 nm. Determination of HMF using the Empower software (Waters) was done by the external standard method. Retention time for the HMF was 3.17 min and the linearity of the HPLC method was tested in the concentration range of 0.01–200 mg/L by means of an HMF standard.

2.5.3 | Ascorbic acid

The 2,6-dichlorophenol indophenol (DCPIP) visual titration method with some modifications (Ranganna, 1986) were used for determination of the ascorbic acid content. Five milliliter of each sample was instantly added to 5 ml of 1% of oxalic acid (stops degradation of ascorbic acid) followed by titration with standardized dye

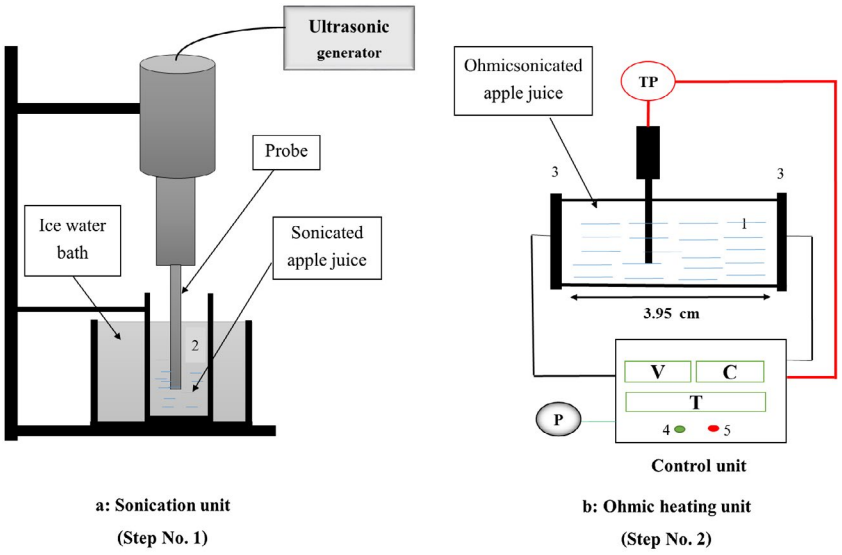

FIGURE 1 Diagram of the experimental lab scale ohmicsonication set—a: sonication unit; b: ohmic heating unit (P- power supply (0–230 V, 60 Hz), 1- Ohmic heating chamber; 2- sonication chamber; 3- Titanium electrodes, TP-Thermocouple probe (K-type), Control unit (contaning V:Volt meter, C:Current meter, and T:Thermocouple meter; 4- power on; 5- power off))

solution (0.25 g/L DCPIP in distilled water) as described previously by Abedelmaksoud et al. (2018b). The pink endpoint color should last for at least 15 s. Results were obtained as mg/100 ml.

2.5.4 | Total phenolic content

Total phenolic content of each apple juice samples was calorimetrically measured by Folin–Ciocalteu reagent according to the method described by Singleton and Rossi (1965) and modifications for microplate reader as in Abedelmaksoud et al. (2018b). The obtained results were expressed as mg of gallic acid/100 ml apple juice.

2.5.5 | Total carotenoids

Total carotenoids content was assessed on apple juice samples using the Lee and Castle (2001) method with some modifications. Briefly, 5 ml of each sample was mixed with 10 ml hexane solution (hexane/methanol/acetone, 50/25/25, v/v/v with 0.1% BHT) followed by centrifugation at 4°C for 10 min at 4,000 rpm. Absorbance at 450 nm were used for calculation of the total carotenoid contents based on an extinction coefficient of β-carotene (μg/g), $E^{1\%}$ = 2,505 according to Ritter and Purcell (1981).

2.5.6 | Total flavonoids content

The colorimetric determination of total flavonoids content in different apple juice samples was based on an assay developed by (Kim, Jeong, & Lee, 2003) at 510 nm. Total flavonoids expressed as mg catechin equivalents/100 g of apple juice were given.

2.5.7 | Polyphenoloxidase

The activity of PPO in various samples was determined according to the method described by Trejo-Gonzalez and Soto-Valdez (1991) with some modification for juice by Abedelmaksoud et al. (2018b).

2.6 | Microbiological load

Total plate count (TPC) and psychrotrophic bacteria (PB) were determined using the plate count agar medium (pour plate), while mold and yeast (M/Y) were determined using potato dextrose agar medium. Plates were incubated for TPC: at 35 °C for 48 ± 2 hr; for PB refrigerated at 5 °C for 10 days; for M/Y: in the dark at 25°C for 5 days (Andrews, 1992).

2.7 | Experimental design and statistical methods

The effects of sonication time and OH temperature on the activity of PPO were conducted using RSM. The factorial design (3^2) was used for two factors (OH temperature and sonication time) with three levels (−1, 0, +1), which are corresponding to 55, 65, and 75°C and 2, 5, and 8 min, respectively. The second-order polynomial model (Equation 1) was used to describe the effect of temperature and time.

$$Y = a_o + a_1x_1 + a_2x_2 + a_{12}x_1x_2 + a_{11}x_1^2 + a_{22}1x_2^2 \quad (1)$$

where, Y is the activity of PPO, x_1 is the OH temperature and x_2 is the sonication time, a_o, a_1, a_2, a_{11}, a_{22}, and a_{12} are regression coefficients for intercept, the linear, the quadratic, and interaction term, respectively. The analysis of variance (ANOVA) for the response (activity of PPO) was used to find the significant terms in the models (see details in Table 2) and for the analysis, Design Expert Version 10.0.6 software was used. The optimization of OS parameters for PPO inactivation was carried out using desirability function method according to Derringer and Suich (1980). The objective function was to maximize the PPO inactivation using desirability function as described by Abedelmaksoud et al. (2018b).

All presented data in Tables 3 and 4 were statistically analyzed with one-way ANOVA, using SPSS 13 software (SPSS Inc., Chicago IL, USA) with the Duncan test to evaluate differences between treatments at levels of significance ($p \leq 0.05$).

3 | RESULTS AND DISCUSSION

3.1 | Effect of OS parameters on PPO activity of NFC apple juice

Optimization of OS parameters was determined by pre-experiments. For selecting the suitable sonication time and OH temperatures some pre-experiments were conducted (the range of OH temperature were of 55–75°C and sonication time were of 2–8 min, where more than 8 min resulted in adverse color and ascorbic acid changes of apple juice). However, increasing the temperature (>80°C) caused dark color as well as an increase in juice bubbling that lead to loss of the juice. The voltage gradient of each OS treatments was 40 V/cm according to optimization of OH conditions by Abedelmaksoud et al. (2018b). However, the OH temperature and sonication time range were selected as 55–75°C and 2–8 min for RSM.

The measured experimental data (on PPO activity) is shown in Table 1. OS parameters were optimized using the equation (Equation 1) of 2nd order polynomial. Using multiple regression analysis, the regression coefficients of independent variables were obtained.

The data in Table 2 illustrates the effect of OH temperature and sonication time on PPO activity. The experimental values of PPO activity were high significant and good fit with the model, in addition, these values were less variation around the mean. The lack of fit was insignificant ($p > 0.05$). The R^2 and adj-R^2 values were 0.991 and 0.984, respectively, these indicate no significant difference between the model and the experimental data, and thus, the model was suitable for predicting the PPO activity for the factors within tested ranges.

TABLE 1 Three level factorial with experimental values of response variable (PPO activity)

Run order	OH temperature (°C), χ_1	Sonication time (min), χ_2	% inhibition of PPO
1	65 (0)	5 (0)	86.91
2	75 (+1)	2 (−1)	93.12
3	55 (−1)	5 (0)	69.20
4	55 (−1)	8 (+1)	74.79
5	75 (+1)	8 (+1)	98.06
6	65 (0)	5 (0)	86.41
7	75 (+1)	5 (0)	95.36
8	65 (0)	2 (−1)	82.52
9	55 (−1)	2 (−1)	61.19
10	65 (0)	8 (+1)	87.38

Note: In the second and third column: the coded values of the test parameters are in parenthesis and the real (un-coded) values are outside the parenthesis; Data are means ± standard deviation (n = 3); PPO: polyphenoloxidase.

The positive linear of the effect of factors (OH temperature (x_1) and sonication time (x_2)) on the PPO activity (response) were significant. The effect of interaction between OH temperature and sonication time (x_1x_2) and the quadratic effect of OH temperature (x_1^2) on PPO activity were significant. Nevertheless, the quadratic term of time (x_2^2) was insignificant ($p > 0.05$), after removal of the insignificant terms, the following equation (Equation 2) was obtained:

$$\text{PPO} = +85.80 + 13.56x_1 + 3.90x_2 - 2.17x_1x_2 - 3.85x_1^2 \quad (2)$$

where x_1: OH temperature (°C) and x_2: sonication time (min).

Then, the obtained second-order polynomial model (Equation 2) was used to determine the optimum parameters for the inactivation of PPO. From Figure 2, with increasing OH temperature and sonication time the activity of PPO was decreased. The optimum OS process conditions were obtained and found to be 75°C and 8 min for NFC apple that resulted in 97.24% PPO inactivation.

3.2 | Effect of OS and other treatments on PPO activity, microbial load, and phytochemical contents of apple juice

The presented data in Table 3 shows the effects of all treatments (S, CH, TS, OH, and OS) on the activity of PPO, phytochemical, and microbiological quality parameters. The PPO activity was significantly reduced after CH, TS, OH, and OS treatments. The highest reduction in the PPO activity was obtained by OS treatment (98%) followed by OH (97%), TS (93%), and CH (91%). Our results confirmed that S (reduction 31%) alone is not sufficient to reduce the PPO activity in the apple juice and this agreed with Anaya-Esparza et al. (2017).

Generally, the temperature range from 70 to 90°C is suitable for decrease the catalytic activity of PPO (Queiroz, Mendes Lopes, Fialho, & Valente-Mesquita, 2008). Therefore, the inactivation of PPO by the TS and CH is attributed to the thermal effect that cause denaturation of the enzyme protein. Besides the effect of heating (temperature effect), in the OH treatment, the increase of the PPO inhibition compared to TS and CH is due to an electric field that might remove the prosthetic groups (metallic) existing in the PPO increasing the loss of enzyme activity (which decrease the D value of PPO) (Icier, Yildiz, & Baysal, 2008). In this regards, Guida et al. (2013) reported that the inactivation of PPO in artichoke heads treated by OH was higher than CH. The same findings were found by Moreno et al. (2013) in apples and in watermelon juice by Makroo, Saxena, Rastogi, and Srivastava (2017). Therefore, the increasing the inhibition of PPO in the OS compared to the other treatments is due to a combination of the cavitation effect during sonication, the effect of heating (temperature) and electric field during OH treatment.

TABLE 2 Analysis variance (ANOVA) and significant coefficient for PPO activity

Source	Coefficient estimate	Sum of squares	*df*	*p*-value
Model	85.80	1,248.86	4	<0.0001
χ_1	13.56	1,103.24	1	<0.0001
χ_2	3.90	91.26	1	0.0012
$\chi_1\chi_2$	−2.17	18.75	1	0.0307
χ_1^2	−3.85	35.60	1	0.0093
Residual		10.54	5	
Lack of fit		10.42	4	0.1627
DF = 9				
R^2	0.991			
Adj-R^2	0.984			
Pred-R^2	0.951			
C.V. %	1.74			
PRESS	61.30			

Note: p-value is significant at $p < 0.05$; x_1 is coded OH temperature and x_2 is coded Sonication time; *df*: degrees of freedom; CV: coefficient of variation; PRESS: predicted residual sum of squares

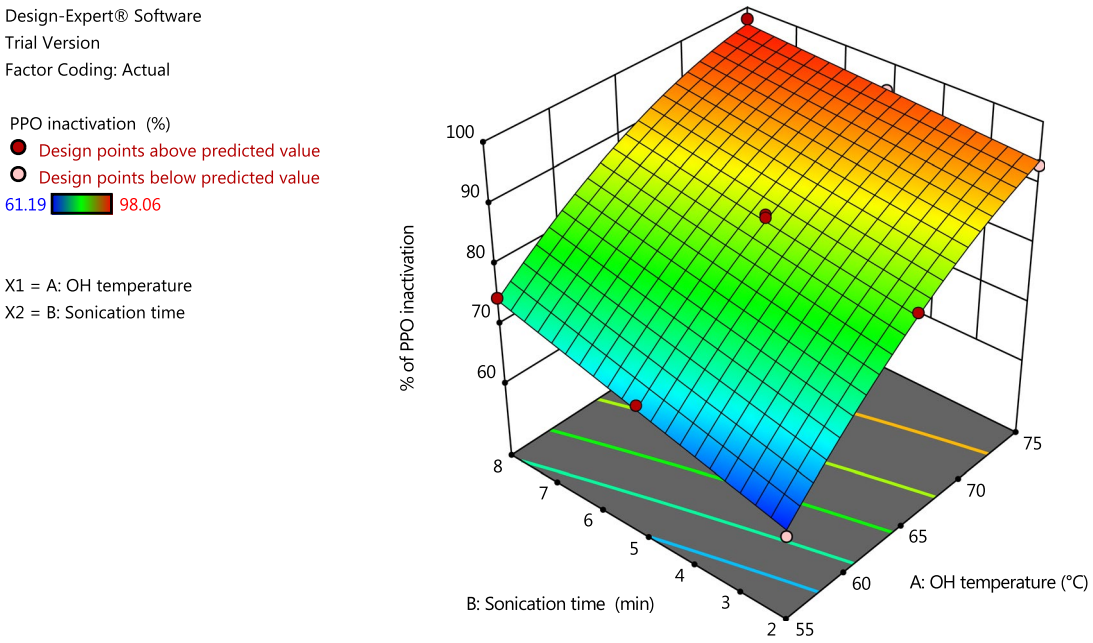

FIGURE 2 Effect of Ohmicsonication (OS) parameters (OH temperatures and sonication time) on the PPO activity of apple juice (U/ml/min)—response surface

TABLE 3 Polyphenoloxidase (PPO) activity, phytochemical contents, and microbial load of NFC apple juice

Quality parameters	Fresh apple juice	Sonication (S)	Conventional heating (CH)	Thermosonication (TS)	Ohmic heating (OH)	Ohmicsonication (OS)
PPO (U/ml/min)	21.74 ± 0.4^{a}	14.99 ± 0.5^{b}	1.90 ± 0.2^{c}	1.48 ± 0.3^{cd}	0.63 ± 0.2^{de}	0.42 ± 0.2^{e}
PPO (% inhibition)	0	31.07	91.26	93.20	97.12	98.06
Total plate count (log cfu/ml)	3.14 ± 0.2	2.58 ± 0.05	nd	nd	nd	nd
PB (log cfu/ml)	2.27 ± 0.5	nd	nd	nd	nd	nd
Mold and Yeast (log cfu/ml)	2.97 ± 0.2	2.49 ± 0.4	nd	nd	nd	nd
Total phenolic (mg/100 ml)	29.84 ± 0.26^{c}	33.08 ± 0.41^{a}	30.77 ± 0.09^{bc}	32.38 ± 0.35^{ab}	31.06 ± 1.15^{bc}	32.33 ± 0.54^{ab}
Total flavonoids (mg/100 ml)	14.46 ± 0.31^{a}	15.64 ± 0.91^{a}	14.48 ± 0.12^{a}	15.39 ± 0.36^{a}	15.12 ± 0.54^{a}	15.59 ± 0.20^{a}
Ascorbic acid (mg/100 ml)	4.84 ± 0.08^{a}	4.52 ± 0.06^{b}	0.96 ± 0.04^{e}	2.08 ± 0.04^{d}	2.40 ± 0.03^{c}	2.48 ± 0.04^{c}
Total carotenoids (µg/100 g)	85.16 ± 0.69^{b}	87.80 ± 1.19^{a}	55.70 ± 0.00^{e}	77.18 ± 0.71^{c}	72.55 ± 0.00^{d}	78.29 ± 0.00^{c}
HMF(mg/L)	nd	nd	5.07 ± 0.22^{b}	8.71 ± 0.02^{a}	4.50 ± 0.05^{c}	2.51 ± 0.28^{d}

Note: Different letters ($^{a, b, c, d, e}$) mean statistical significant difference ($p < 0.05$); the results represent the mean ± standard deviation; HMF: hydroxymethylfurfural; PB: psychrophilic bacteria; nd: not detected.

Table 3 also presents the effects of all treatments on microbial load (total plate count, psychrophilic bacteria, mold and yeast) of apple juice. After treatment of apple juice sample by CH, TS, OH, and OS no microbial growth neither total plate count, psychrophilic bacteria nor mold and yeast were detected. Regarding S apple juice sample, total plate count, and mold and yeast were 2.58 log cfu/ml and 2.49 log cfu/ml, respectively. Therefore, sonication alone was found to be insufficient to achieve a microbiological safe product as well as to extend the shelf life of apple juice. The reduction in the microbial count was due to thermal effect in the CH, the combined effect of thermal heating with electric field in OH, thermal heating with cavitation as in TS (Sun et al., 2008) and combined cavitation with thermal heating and electric field in OS. In addition, Abid, Jabbar, Hu, et al. (2014b) reported that the total plate counts and the yeast and mold load of apple juice were very sensitive to the temperature. They reported that a full inactivation of microorganisms was seen at 60°C sonication treatment. In agreement with our results, Abid et al. (2013) also found a microbial load reduction for

TABLE 4 Physical characteristics and sugar compounds of NFC apple juice

Quality parameters	Fresh apple juice	Sonication (S)	Conventional heating (CH)	Thermosonication (TS)	Ohmic Heating (OH)	Ohmicsonication (OS)
EC (S/m)	0.173 ± 0.002^{c}	0.175 ± 0.001^{c}	0.176 ± 0.002^{c}	0.178 ± 0.003^{bc}	0.183 ± 0.004^{ab}	0.185 ± 0.003^{a}
L^*	37.76 ± 0.02^{d}	38.54 ± 0.05^{c}	38.32 ± 0.08^{c}	41.32 ± 0.18^{a}	38.47 ± 0.03^{c}	39.61 ± 0.08^{b}
a^*	−2.43 ± 0.10^{c}	−1.56 ± 0.04^{a}	−2.31 ± 0.06^{c}	−1.49 ± 0.02^{a}	−1.84 ± 0.08^{b}	−1.51 ± 0.05^{a}
b^*	0.45 ± 0.02^{d}	0.71 ± 0.03^{b}	0.55 ± 0.02^{cd}	0.87 ± 0.06^{a}	0.62 ± 0.07^{bc}	0.74 ± 0.03^{ab}
ΔE	0	1.19 ± 0.07^{c}	0.58 ± 0.06^{d}	3.68 ± 0.18^{a}	0.94 ± 0.07^{c}	2.08 ± 0.12^{b}
Viscosity (cP)	2.4 ± 0.1^{ab}	2.6 ± 0.1^{a}	2.2 ± 0.1^{b}	2.2 ± 0.1^{b}	2.2 ± 0.1^{b}	2.2 ± 0.1^{b}
Cloud value (A)	0.071 ± 0.002^{f}	0.094 ± 0.002^{e}	0.247 ± 0.003^{d}	0.307 ± 0.007^{c}	0.352 ± 0.003^{b}	0.370 ± 0.001^{a}
ADS (μm)	0.691 ± 0.016^{a}	0.338 ± 0.011^{c}	0.468 ± 0.012^{b}	0.331 ± 0.017^{c}	0.458 ± 0.010^{b}	0.353 ± 0.013^{c}
Glucose (g/100 ml)	2.19 ± 0.01^{b}	2.23 ± 0.03^{ab}	2.22 ± 0.00^{ab}	2.27 ± 0.03^{a}	2.10 ± 0.02^{c}	2.21 ± 0.01^{ab}
Fructose(g/100 ml)	6.34 ± 0.02^{b}	6.42 ± 0.08^{ab}	6.32 ± 0.04^{b}	6.57 ± 0.10^{a}	6.39 ± 0.03^{ab}	6.39 ± 0.03^{ab}
Sucrose(g/100 ml)	1.88 ± 0.01^{ab}	1.90 ± 0.03^{ab}	1.83 ± 0.01^{b}	1.94 ± 0.04^{a}	1.89 ± 0.01^{ab}	1.93 ± 0.04^{b}
Total sugar(g/100 ml)	10.40 ± 0.04^{b}	10.55 ± 0.11^{ab}	10.37 ± 0.03^{b}	10.78 ± 0.16^{a}	10.39 ± 0.06^{b}	10.53 ± 0.02^{ab}

Note: Different letters ($^{a, b, c, d, e, f}$) mean statistical significant difference ($p < 0.05$); the results represent the mean ± standard deviation; ADS: Average droplet sizes (μm); EC: electric conductivity.

apple juice only treated with S but this reduction is insufficient to be safe for human consuming.

The total phenolic content was significantly increased in all sonicated treatments (S, TS, and OS), while a slightly increase (but not significant) was found for non-sonicated (CH and OH) treatments in compared to fresh apple juice (29.84 mg/100 ml) (Table 3). The total phenolic content in fresh apple juice agreed with the range reported by other researches (from 10 to 300 mg/100 ml) for European apple juices (Gasperi et al., 2009; Marszałek et al., 2018; Will et al., 2008). The increased release of total phenolic compounds during sonication treatments is due to the cavitation phenomenon resulting in breakdown of the cell wall based on liquid pressure changes during sonication treatment, thus increasing the availability of phenols in the juice Abid et al. (2013). While during CH and OH treatments, this increase due to improve the extraction of phenolic compounds as antioxidant compounds from tissue to intercellular juice during heat treatment (Gerard & Roberts, 2004; Marszałek, Mitek, & Skąpska, 2015). Regarding total flavonoids contents, there were no significant changes in all treatments compared to fresh apple juice (14.46 mg/100 ml).

The ascorbic acid content decreases significantly for all the treatments compared to untreated sample (4.84 mg/100 ml) (Table 3). The highest value of ascorbic acid content was obtained with the OS treatment (2.48 mg/100 ml) compared to all other treatments except Sonication. Sonication alone is not possible due to its insufficient inactivation of PPO activity and the load of microorganisms in the apple juice (see Table 3). The decreased concentration of ascorbic acid in the treated samples (OS, OH, TS, and CH) may induce a higher rate of chemical decomposition of ascorbic acid due to the effect of heating and processing time. Demirdöven and Baysal (2014) also noticed a decreased ascorbic acid content in the OH and CH of orange juice compared with their control.

The total carotenoids in the fresh apple juice was 85.16 μg/100 g (Table 3). The total carotenoids was significantly ($p \leq 0.05$) decreased and a reduction values of 8, 9.4, 14.8, and 34.5% were obtained in the OS, TS, OH, and CH treatments, respectively. This decrease is due to carotenoids sensitivity toward heat as reported for the carotenoids (namely lycopene) of watermelon juice (Rawson et al., 2011). However, Sonication resulted in 3.1% increase in the carotenoids content and such increase was due to the cavitation phenomena acting on more extraction of carotenoids in the apple juice.

OS treated juice sample contained the lowest value of HMF (2.51 mg/L) followed by OH (4.50 mg/L) and then CH (5.07 mg/L) and TS treated juice sample contained the highest concentration of HMF (8.71 mg/L). The presence of HMF in the juices is due to excessive heat treatment of carbohydrates and amino acids with higher temperature and long time for TS. As expected, the levels for HMF values were within the range from no detection up to 25 mg/L recommended by the international federation of fruit juice processors (IFFJP) in the fruit juices (Kalábová & Večerek, 2006; Matić et al., 2009). In addition, Matić et al. (2009) determined the 5-HMF in 20 samples of apple juice and found that in 17 samples the HMF concentration was less than 20 mg/kg.

3.3 | Effect of OS and other treatments on physical contents and sugar compounds of apple juice

Table 4 presents the impact of S, CH, TS, OH, and OS on physical contents and sugar compounds of apple juice. The electrical conductivity (EC) significantly increased for OS (0.185 S/m) and OH (0.183 S/m) compared to TS fresh apple juice (0.173 S/m), while insignificantly increased for TS (0.178 S/m), CH (0.176 S/m), and S (0.175 S/m) treatments ($p > 0.05$). The increase in EC of juice may be attributed due to temperature, electric current, and sonication leading to an enhanced release of vitamins, minerals, and bioactive components and an increase in the ionic mobility (Zou & Jiang, 2016).

The effects of different treatments on color values (lightness (L^*), redness (a^*), yellowness (b^*), and the total color difference (ΔE)) of apple juice are illustrated in Table 4. The L^* values were significantly increased for TS and OS, while it was not significantly increased for S, OH and CH compared to fresh apple juice. The a^* values were not significantly different for TS, OS, and S treatments, however, their values were significantly increased compared to fresh apple juice. Nevertheless, a^* value for OH was significantly increased however it was not significantly increased for CH compared to fresh apple juice. The b^* values were significantly increased for TS>OS>S>OH treatments, however the b^* value was not significantly increased for CH treated apple. The ΔE values were significantly increased for all treatment (TS>OS>S> OH>CH). The increase in the color values (L^*, a^*, and b^*) might be due to: (1) moisture migration and chemical changes by thermal treatments, (2) increase in the cloud value of the juice by sonication improving homogenization of the juice or (3) inhibition of PPO as the color change indicate more condensation for phenolics components (Tiwari, Muthukumarappan, O'Donnell, & Cullen, 2008). Park, Ha, and Kang (2017) found that the L^*, a^*, and b^* apple juice values increased during OH process and Abid, Jabbar, Hu, et al. (2014a) also found that the L^*, a^*, and b^* values in the apple juice increased significantly by TS process.

The viscosity (cP) decreases slightly in all treatments except for S (i.e., viscosity slightly increases for S) compared to fresh apple juice (2.4 cP) (Table 4), which may be attributed to the effect of thermal treatment that cause acid hydrolysis of pectin (Diaz, Anthon, & Barrett, 2007). On the other hand, the rise of viscosity in the sonicated juice sample is due to the sonication causing extraction of bound macromolecules with the resulting increase in the colloidal system concentration, which leads to higher viscosity (Suárez-Jacobo et al., 2011).

The cloud value increases significantly in all treatments compared to fresh apple juice (0.071 A) (Table 4). The highest cloud value was obtained for OS (0.370 A) treatment which indicates the highest percentage of cloud stability and inhibition of enzymes. The increase in cloud value by OS might be due to the conjoint effects of thermal, voltage gradient, and cavitation on enzyme activity, which act on decreasing the separation of layers, thus keeping a cloud stability of the juice. At the same time, cavitation by sonication, are acting on the breakdown and release of particles in the juice (Brujan & Vogel, 2006).

Average droplet size (ADS) of fresh apple juice was 0.691 μm. The result showed that the lowest average ADS was obtained in the sonicated samples (OS, S, and TS) compared to non-sonicated samples (fresh, CH, and OH) (Table 4). This means that OS, S, and TS treatments resulted in more homogeneous juices compared to fresh CH and OH-treated juices. This is due to the result of cavitational collapse sonication on particle size reduction (Franco, Pérez-Maqueda, & Pérez-Rodrıguez, 2004).

Table 4 also presents the sugar content (total sugar content, glucose, fructose, and sucrose) of all treatments of apple juice. The total sugar content was significantly increased for TS, S, and OS treated apple juices, while insignificant changes was observed in the CH and OH treated apple juices. The slight increase in total sugar contents are associated to sonication treatment, which might be attributed to the mechanical effects breaking the cell, exerted by sonication produced shear forces. Our results agree with Abid, Jabbar, Wu, et al. (2014c) who also observed significant increases in the glucose, fructose, and sucrose levels in the sonicated apple juice samples (S, OS, and TS) compared to the fresh.

4 | CONCLUSION

We concluded that the use of OS as a novel combined technology for apple juice pasteurization result in better juice quality compared to OH, TS, CH, and sonication (S) treatments. OS treatment resulted in the highest inactivation of PPO activity with no detected for microbial growth as well as the lowest losses of ascorbic acid, carotenoids, phenolics, and flavonoids compared to the other treatments (OH, TS, S, and CH). In addition, OS treatment resulted in increased electric conductivity, cloud value and color values. Sonication treatment alone was not sufficient for inactivation of neither PPO nor microbial load at lower temperature. Therefore, OS treatment led to improve juice quality (physical and chemical characteristics), reduce PPO activity, and microbial load and prolong the shelf life of apple juice. Overall, OS improved the overall quality apple juice in laboratory scale compared to other treatment and can be a potential technology for pasteurization of juice. An application in a pilot plant or large scale could be interesting and needs to be considered for further studies.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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