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SHORT COMMUNICATION

Impact of ohmicsonication treatment on pectinmethylesterase in not-from-concentrate orange juice

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Abstract The present study investigates the application of ohmicsonication (OS) as a new hurdle technology for pasteurization of Not-from-concentrate orange juice (NFCOJ). OS process parameters to inactivate pectinmethylesterase (PME) activity in NFCOJ were optimized using response surface methodology. The influence of Sonication (S), Thermosonication (TS), Ohmic heating (OH) and OS on inactivation of PME were compared to conventional heat (CH) treatment. Their effects on physical, chemical and microbiological contents were included. In comparison to fresh orange juice, the inactivation of PME was 96%, 95%, 89%, 90% and 29% for OS, OH, TS, CH and S treatments, respectively. Highest cloud value was obtained for OS (1.240 A) treatment. OS treatment gave a lower vitamin C loss compared to TS, OH and CH treatments. A significant increase in the total phenolic content were obtained in the following order OS > TS > OH > CH. OS treated juice also contained the lowest value of hydroxymethyl furfural (0.90 mg/L) compared to OH (0.95 mg/L), TS (1.37 mg/L) and CH (2.72 mg/L) treated samples. Overall, the results indicated that OS can be integrated as a substitute to pasteurization of NFCOJ.

Keywords Ohmic heating · Ohmic-ultrasonic heating · Pectinmethylesterase · Phenolic content

Introduction

Orange juice (OJ) is a good source of bioactive compounds (i.e. vitamin C, phenolics and carotenoids) (Galaverna and Dall'Asta 2014). During storage, OJ can be subject to deteriorative reactions including enzymatic activities, microbial spoilage, vitamin C degradation, cloud loss and changes in flavor and color, all reactions that lead to loss of product quality. Due to the perishable nature of juices, several technologies have been used to prolong the shelf life (Polydera et al. 2005). Today, recent technologies in food processing either thermal or non-thermal are designed to meet the consumer demands (Williams 1994). Not-From-Concentrate (NFC) juice is the fruit juice extract without concentration or dilution. Insoluble pulp, skin and seeds are removed before heat treatment of the juice for controlling the microbial load and enzymatic activities (Abedelmaksoud et al. 2018a).

The activity of pectinmethylesterase (PME) results in unwanted layer separation during processing. Conventional heat (CH) treatment reduce PME activity as well as microbial load, however, this treatment can cause loss of nutritional value and produce undesirable flavor in the juice product, especially at temperatures higher than 80 °C (Giner et al. 2013). Hence, the juice industry searches for mild thermal technologies, which are able to inactivate both enzymes and microorganisms with retention of nutritional value (Chemat and Khan 2011).

Hurdle technology is one of the promising technologies in food processing to improve the quality, safety, and stability of food products. Sonication (S) is one of the

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emerging green methods for food processing and preservation. S is defined as a potential technology acting on reduction of the microbial load in food by 5-log when S generates cavitation bubbles due to pressure changes (USFDA 2001). S as a substitute to the CH has the ability to decrease processing time, cost and energy as well as enhanced quality, shelf life and ensured safety of fruit juices (Chemat and Khan 2011). However, S alone has limited applications in juices due to its insufficient enzymes and microorganisms inactivation. Therefore, a combination of S with other technologies with improved efficiencies has been investigated and considered due to the possibility for the application at the industrial scale (Leistner 2000). Previous literature however lack the combination of ohmic heating (OH) with S as an alternative to the CH for production and evaluation of NFC orange juice (NFCOJ).

Therefore, the main objectives of this study was to optimize the OS parameters (OH temperature and S time) for inactivation of PME and evaluate the effects on the final NFCOJ. Effects on quality characteristics of the final OJ product at optimum OS conditions were compared to production by other technologies such as S, Thermosonication (TS), OH and CH.

Materials and methods

Chemicals

All chemicals in this study were purchased from (Sigma-Aldrich Chemical Co., Denmark).

Raw material

Orange fruits (*Citrus sinensis, cv. Navel*) purchased from a local supermarket in Copenhagen, Denmark were rinsed and cut into halves. The juice was extracted (Extractor, Krups Citrus Juicer, Spain) and filtrated using a double-layered muslin cloth. The extracted juice was divided into six groups and subjected to S, OS, TS, OH, CH or kept fresh and all groups were rapidly cooled to 4 °C and stored at − 18° C for further analysis.

Processing methods

Conventional heating (CH)

Orange juice (OJ) (150 mL) was heated at 95 °C for 60 s using a shaker water bath (Julabo, SW22, made in Germany) according to Abedelmaksoud et al. (2018b).

Sonication and Thermosonication (S and TS)

Samples of 150 mL OJ was treated with the S processor of 550 W at 20 kHz with a 0.5-inch probe (Sonifier SFX550 Model, Mexico). Treatments at 25 °C using 100% power (550 W) for 8 min at pulse durations of 5 s was used. TS was done at 60 °C at conditions similar to S.

Ohmic heating (OH)

The treatment of OJ (150 mL) by OH (at 42 V/cm, 69 °C and held for 60 s) was conducted according to Demirdöven and Baysal (2014).

Ohmicsonication (OS)

OS was done by a combination of S and OH treatments at the obtained optimum parameters, firstly treated by S for 8 min at 25 °C and then directly followed by OH at 40 V/cm, to 68 °C for a holding time of 60 s.

Experimental design and statistical methods

Response surface methodology (RSM) was used for optimization of OS parameters (OH temperature and S time). Two factors (OH temperature and S time) with three levels (− 1, 0, + 1), the factorial design (3^2) was used. The OH temperature and S time range were 60, 65, 70 °C and 2, 5, 8 min, respectively. To describe the effect of parameters, the second-order polynomial model was used (Eq. 1).

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

where Y is the % of PME inactivation, x_1 is OH temperature and x_2 is S time, ao,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 was used to find the significant terms in the models (Table S1, supplementary data). Design Expert Version 10.0.6 software was used for the analysis. To optimize the OS parameters, the desirability function method was used. The objective function was to maximize the PME inactivation using desirability function as in Abedelmaksoud et al. (2018b).

To evaluate the variances among different treatments at significance levels ($p \leq 0.05$), data in Tables 2 was statistically analyzed with one-way ANOVA (Duncan test), using SPSS 13 software (SPSS Inc., Chicago IL, USA).

Physical analysis

Electric conductivity (EC), cloud value and color values (L, a and b) were determined using methods described by

Abedelmaksoud et al. (2018a). Size distribution particles were detected with the Mastersizer (Model 2000, Malvern, UK).

Chemical analysis

Vitamin C content was determined using a 2,6-dichlorophenol indophenol (DCPIP) visual titration method (Ranganna1986).

Total phenolic content was calorimetrically measured applying Folin-Ciocalteu reagent as described by Abdullakasim et al. (2007) with modifications for microplate reader in Abedelmaksoud et al. (2018b). Results were given as mg of Gallic acid/100 mL OJ.

Total carotenoids was measured according to Lee and Castle, (2001) with some modifications based on 5 mL of OJ (Abedelmaksoud et al. 2018a). Absorbance at 450 nm of the final supernatant was measured and total carotenoid contents were calculated according to Ritter & Purcell (1981) using an extinction coefficient of β-carotene (μg/g), $E^1\% = 2505$.

Total flavonoids was measured based on an assay developed by Kim et al. (2003) and the results were expressed as mg catechin equivalents/100 g OJ.

Hydroxymethyl furfural (HMF) was determined according to a method of Vorlova et al. (2006) using a Vortex Genie II (Scientific Industries, Bohemia, USA) for mixing 1 mL methanol and 0.5 mL juice. The centrifuged and filtered (0.45 μm) extract was injected (20 μl) onto HPLC (Alliance, Waters Company) equipped with a Zorbax Eclipse XDB-C8, 4.6 × 150 mm, 5 μm column (Waters, Milford, USA) at 30 °C using a flow rate of 1 mL/min and an isocratic mobile phase (10% methanol in water). UV detection at 285 (2996 diode array detector) using external standard method for quantification of HMF (retention time 3.17 min) with a linearity concentration range of 0.01–200 mg/L based on Empower software (Waters).

Pectinmethylesterase activity was determined according to the method described by Rouse and Atkins (1955) and Ting and Rouseff (1986).

Microbial load

Total plate count and mold and yeast were determined as in Andrews (1992).

Results and discussion

Optimization of ohmicsonication (OS) conditions

Pre-experiments determined optimal OH temperatures in the range 60–70 °C and S time in the range of 2–8 min, where S time ≥ 8 min would result in adverse color and vitamin C changes for OJ (data not included). Also, increasing the temperature more than 80 °C caused deterioration of the color and increased juice bubbling leading to juice loss. The voltage gradient of each OH treatments was selected to be 42 V/cm according to optimzation of OH conditions by Demirdöven and Baysal (2014). RSM set up resulted in PME inhibitions (%) presented in Table 1.

Optimization of the conditions of OS by applying second order polynomial equation and multiple regression analysis were used to obtain the regression coefficients for independent variables (Eq. 1).

The effect of OH temperature and S time on PME activity at 95% confidence interval (Table S1, supplementary data). The experimental data was fitted and significant with the used model. Insignificant difference between adj-R^2 value (0.968) and R^2 for PME—this means high degree of correlation between the predicted and experimental values with insignificant lack-of-fit. The model was suitable for describing the % inactivation of PME within tested experimental ranges.

The positive linear effect of OH temperature (χ_1), S time (χ_2) were found to be significant for the response variable (*Y:* % inactivation of PME). Also, the interaction ($\chi_1\ \chi_2$) and the quadratic effect of OH temperature (χ_1^2) on PME were found to be significant. However, the quadratic of S time (χ_2^2) had an insignificant effect on the PME. The fitted second order polynomial equation are presented as (Eq. 2):

$$Y = +93.35 + 3.94\chi_1 + 1.76\chi_2 - 1.00\chi_1\chi_2 - 2.37\chi_1^2 \quad (2)$$

where χ_1: OH temperature (°C) and χ_2: S time (min). According to the obtained second order polynomial models, the optimum conditions for OS of NFCOJ was obtained at the maximum PME inactivation by applying desirability function. The obtained optimum parameters were OH at 68 °C for 60 s combined with S at 550 W for 8 min, with a PME inactivation of 96% in NFCOJ (Fig. 1).

Physical, chemical and microbiological contents of fresh and treated NFC orange juice

Table 2 shows the effects of OS, S, CH, TS and OH on physical, chemical and microbial load contents of NFCOJ. Compared to fresh orange juice (FOJ), an increase in the EC was observed for all treatments of OJ in the following

Table 1 Experimental design of ohmicsonication (OS) and PME activity in NFC orange juice

Run order	OH temperature (°C), χ1	Sonication time (min), χ2	% inhibition of PME
1	65 (0)	5 (0)	93.53
2	65 (0)	5 (0)	93.40
3	70 (+ 1)	5 (0)	94.93
4	70 (+ 1)	2 (− 1)	93.88
5	60 (− 1)	5 (0)	87.98
6	65 (0)	2 (− 1)	92.04
7	70 (+ 1)	8 (+ 1)	95.94
8	60 (− 1)	2 (− 1)	83.53
9	60 (− 1)	8 (+ 1)	89.61
10	65 (0)	8 (+ 1)	94.44

In the 2nd and 3rd column: the coded values of the test parameters are in parenthesis and the real (uncoded) values are outside the parenthesis, *PME* pectinmethylesterase

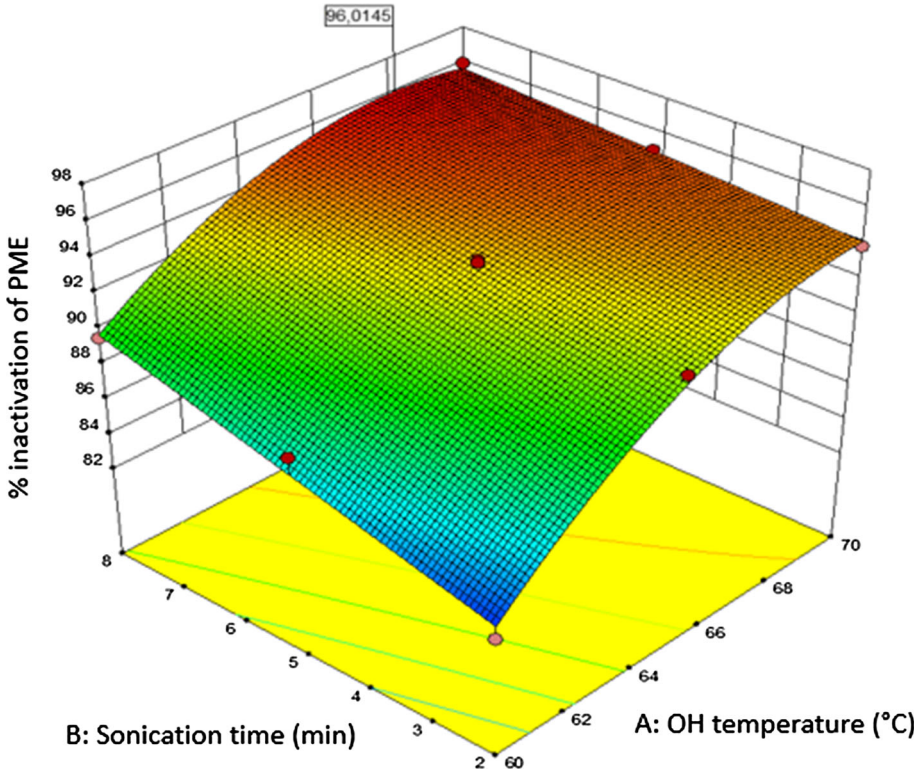

Fig. 1 Effect of Ohmicsonication (OS) parameters (OH temperature and Sonication time) on the PME activity of orange juice (U/mL/min)—response surface and contour plots

order: OS > TS > S > OH > CH. Increased EC of juice, especially for S (S, TS and OS) might be attributed to the agitated enhanced release of minerals and bioactive components as well as increased ionic mobility (Zou and Jiang 2016).

A lower ADS, indicating a more homogenous juice, was observed in the sonicated samples (OS, S, TS) compared to non-sonicated samples (FOJ, CH and OH) (Table 2). This is due to the effect of cavitational collapse by S and the resulting particle size reduction (Franco et al. 2004).

For color values (L,a and b) the L*value was increased by S, TS and OS,while insignificantly changed by CH and OH treatments. The a* and b* were significantly increased for all treatments (OS > TS > S > OH > CH) compared to FOJ, attributed to chemical changes by thermal treatments (Bhale 2004). Increased color values by S could be due to less enzymatic color changes and an increased phenolic and carotenoid condensation reducing their oxidation as explained by Tiwari et al. (2008).

Vitamin C were significantly decreased for all treatments when compared to FOJ (52.77 mg/100 mL) and followed the order S > OS > OH > TS > CH. S alone was not efficient in the inactivation of PME and microorganisms (Table 2) and therefore not accepted for

Table 2 Physical, chemical and microbiological contents of fresh and processed NFC orange juice during different treatments of NFC orange juice

Parameters	FOJ	S	CH	TS	OH	OS
EC (s/m)	0.272 ± 0.003c	0.293 ± 0.004ab	0.282 ± 0.004bc	0.297 ± 0.007ab	0.286 ± 0.004abc	0.303 ± 0.009a
Cloud value (A)	0.253 ± 0.07c	0.274 ± 0.003c	0.970 ± 0.005b	0.946 ± 0.008b	0.994 ± 0.003b	1.240 ± 0.04a
ADS (μm)	3.970 ± 0.04b	0.304 ± 0.01d	2.096 ± 0.02c	0.290 ± 0.01f	4.191 ± 0.02a	0.323 ± 0.01e
L*	42.57 ± 0.02d	44.71 ± 0.09c	42.63 ± 0.03d	46.03 ± 0.08b	42.77 ± 0.04d	46.95 ± 0.29a
a*	− 6.66 ± 0.02e	− 5.15 ± 0.04b	− 6.08 ± 0.02d	− 4.96 ± 0.03 cd	− 5.83 ± 0.02 cd	− 4.73 ± 0.01a
b*	9.65 ± 0.02e	10.36 ± 0.14c	9.95 ± 0.06f	11.33 ± 0.02a	10.15 ± 0.05d	10.76 ± 0.03b
ΔE	–	2.72 ± 0.27c	0.65 ± 0.02e	4.21 ± 0.04a	0.99 ± 0.03d	4.91 ± 0.28b
Vitamin C (mg/100 mL)	52.77 ± 0.14a	48.22 ± 0.22b	43.58 ± 0.25f	46.08 ± 0.16e	47.22 ± 0.18d	47.62 ± 0.19c
Total carotenoids (μg / 100 g)	1270 ± 13.5b	1308 ± 3.22a	982 ± 8.16f	1107 ± 7.62e	1126 ± 5.54d	1183 ± 8.73c
Total flavonoids (mg/ 100 mL)	20.01 ± 0.39c	21.80 ± 0.47b	20.32 ± 0.62c	22.27 ± 0.24a	20.58 ± 1.38c	22.83 ± 0.31a
Total phenolic (mg/ 100 mL)	37.47 ± 0.13e	42.06 ± 0.22a	37.61 ± 0.26e	39.64 ± 0.10c	38.22 ± 0.09d	40.73 ± 0.69b
HMF (mg/L)	nd	0.73 ± 0.02c	2.72 ± 0.02a	1.37 ± 0.18b	0.95 ± 0.21c	0.90 ± 0.08c
PME (U/mL/min)	47.57 ± 0.6a	33.55 ± 0.4b	5.38 ± 0.3c	4.62 ± 0.2d	2.21 ± 0.1e	1.93 ± 0.3f
Total plate count (log cfu/ mL)	2.27 ± 0.5	nd	nd	nd	nd	nd
Mold and yeast (log cfu/ mL)	1.92 ± 0.3	nd	nd	nd	nd	nd

Different letters (a, b, c) mean statistical significant difference ($p < 0.05$); the results represent the mean ± SD

FOJ fresh orange juice, *S* sonication, *CH* conventional heating, *TS* thermosonication, *OH* ohmic heating, *OS* ohmicsonication, *EC* electric conductivity, *ADS* average droplet sizes (μm), *HMF* hydroxymethylfurfural, *PME* pectinmethylesterase

processing of OJ. The vitamin C reduction was attributed to chemical decomposition due to both temperature and time of processing correlating with the findings by Demirdöven and Baysal (2014), who reported a decrease of vitamin C in the OH and CH treatments compared to the FOJ.

A significant increase in the total carotenoids of OJ for S (1308 μg/100 g) correlates with cell wall disruption causing more free carotenoids in the juice stated by Plaza et al. (2011). Also, Abid et al. (2014) observed increased carotenoid levels with S. Oxygen, light, metals and enzyme availability as well as heat application led to loss of carotenoids for OS, TS, OH and CH (Table 2), which is in agreement with previous reports on carotenoid stability (Rawson et al. 2011; Esteve et al. 2009).

Total phenolic content (TPC) of OJ were significantly increased in S, OS, TS and OH treatments (S > OS > TS > OH) compared to FOJ, while insignificant increase was found for CH treated sample (Table 2). Heating (during CH and OH treatments) might increase the extractability of TPC due to breakdown of the interaction between proteins and polyphenols (Girgin and El 2015). Previous reports explain TPC release during OH induced by the alternating current (Roy et al. 2009), while the increased release of TPC during S is due to the cavitation phenomenon resulting in breakdown of the cell wall based on liquid pressure changes during S treatment, thus increasing the availability of phenols in the juice (Abid et al. 2014).

OS treated juice contained the lowest value of HMF (0.90 mg/L) followed by OH (0.95 mg/L) and TS (1.37 mg/L) with highest values in CH treated juice (2.72 mg/L). The presence of HMF in foods (containing carbohydrates in an acidic environment) is a result of high heat treatment (T > 80 °C) and inappropriate and long-term storage. As expected, levels for HMF varies e.g. from not detected to 27 mg/L in fruit juices (Vorlova et al. 2006).

All treatments showed a significant increase in cloud value with the highest value indicating highest cloud stability and PME inhibition obtained for OS (Table 2). Increased parameters were attributed to the combined effects of heat and voltage gradient (which might remove the metallic prosthetic groups present in the PME) (Castro et al. 2004) and cavitation on enzyme activity, causing a decreased layer separation.

After treatment of OJ sample by S, CH, TS, OH and OS no microbial growth neither total plate count nor mold or yeast were detected (Table 2).

Conclusion

The use of OS result in an improved quality of OJ compared to other treatments. The highest inactivation of PME activity with no microbial load as well as highest retention of vitamin C, carotenoids, phenolics, and flavonoids were obtained with OS compared to the other treatments (OH, TS, S and CH). In addition, OS treatment resulted in increased EC, cloud value and color values. S treatment alone was not sufficient for inactivation of PME at lower temperature. Overall, OS improved the quality of OJ in laboratory scale compared to other treatments 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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