Enzymatic inactivation of apple juice using HPCD (High Pressure Carbon Dioxide) technology and its effect on the quality parameters of the juice

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ABSTRACT

The quality of cloudy apple juice seems to be better than the clarified juice due to the loss of phenolic compounds during processing. Enzymatic browning and cloud stability are the main problems in processing and storage of the juice. In this work, HPCD is proposed as an alternative to the traditional heat treatments that can degrade the quality of the juice. Polyphenol oxidase (PPO) and pectin methylesterase (PME) inactivation by HPCD were determined for both enzymes. The effect of stirring speed on PPO inactivation was studied; obtaining that at higher speed inactivation was also higher. Other quality parameters in the juice were also determined after HPCD treatment, such as particle size distribution, colour, turbidity and total phenolic compounds.

INTRODUCTION

Cloudy apple juice can be more beneficial to human health than the consumption of clear apple juice, due to its higher nutrient content, which is lost in juice processing [1]. The main problem with cloudy apple juice during production and storage is the color and cloud stability. The discoloring of cloudy apple juice is a result of enzymatic browning, which involves the action of polyphenol oxidase (PPO) catalyzing oxidation of phenolic compounds. The mechanism of cloud stability in juices is not yet understood, but it is closely related to the pectin methylesterase (PME) activity. Among the different mechanisms, electrostatic repulsion by negative charges present in the partly demethylated pectin due to galacturonic residues could be responsible of cloud stability avoiding aggregation [2]. This way, the activity of pectinmethylesterase (PME) causes pectin demethylation and formation of insoluble calcium pectate gels that could precipitate causing clarification and the loss of turbidity.

Traditionally, thermal treatments are used to inactivate the enzymes but cause undesirable effects in the juice quality attributes such as flavor and loss of nutritional components [3]. As an alternative, high pressure carbon dioxide (HPCD) has been proposed as a non-thermal technique that can inactivate certain enzymes under mild operation conditions. This technology is growing attention as a cold pasteurization method through the effect of CO_2 held under pressures below 50 MPa and temperatures lower than thermal treatments (below 50ºC). The mechanism of pressurized CO_2 on enzyme inactivation is not yet fully understood. In the literature different approaches can be found such as pH lowering due to dissolved CO_2, conformational changes or formation of different complex with CO_2 molecules.

In this work, HPCD technology has been applied to cloudy apple juice. The influence of agitation on enzyme inactivation under high-pressure environment is a key factor in the design of reactor volumes of industrial scale [4]. Therefore, the effect of stirring speed on PPO inactivation was studied. Sampling procedure and the number of successive pressurization/depressurization cycles was also studied.
Inactivation kinetics of PPO and PME in cloudy apple juice were determined in the pressure range from 100 to 200 bar and temperature range from 35 to 45 ºC. Kinetic data have been successfully correlated by the Weibull model.
Some quality parameters of cloudy apple juice, such as color, pH, ºBrix, turbidity, antioxidant capacity and particle size distribution (PSD) were also determined right after HPCD treatment.

MATERIALS AND METHODS

Juice preparation: *Golden deliciuos* apples were peeled and cut in cubes and then were added to a 0.3 % of L-ascorbic acid solution to avoid enzymatic browning during processing. Apple cubes were wiped and immediately squeezed with a screw juice extractor and the liqueur was filtered with 2 layers of cheesecloth. The pH of the juice obtained was 3.89 ± 0.01 and the soluble solid was 12.5 ± 0.1 °Brix. The juice was stored frozen at −18 °C until further use. No significant change of PPO and PME activity was noticed during the storage of the frozen samples.

HPCD equipment and processing: The HPCD cell was made of stainless steel (SS-116) and had an internal volume of 100 mL with a maximum operating pressure and temperature of 30 MPa and 80ºC. For each experiment, approximately 40 mL of apple juice was charged into the high pressure cell and it was immersed in a thermostatic water bath at the operating temperature. Magnetic stirrer was connected. The system was then pressurized by using a syringe pump with a pressure controller (ISCO 260 D) up to the desired pressure. Samples were collected periodically at different treatment times up to 120 minutes to follow the inactivation kinetics of the enzyme. Experiments were carried out in a temperature (T) range from 35 to 45 ºC, pressure (p) from 100 to 200 bar and stirrer speed from 200 to 600 rpm. The effect of sampling procedure and the number of depressurization cycles on enzyme activity was also analyzed.
To compare the effect of HPCD treatment on enzyme activity under mild heating treatment, cloudy apple juice was subjected in the same temperature range (from 35 to 45ºC) but at atmospheric pressure.

Determination of PPO activity: Activity of PPO was determined spectrophotometrically by using a 0.05 M catechol solution prepared in a 0.1 M phosphate buffer (pH 6,5) as substrate. Oxidation of catechol was determined by the increase in absorbance at 420 nm at 30 ºC during 3 minutes of reaction after mixing 100 µL of juice with 2900 µL of the catechol solution. A *Jasco V-750* Spectrophotometer was used. PPO activity was taken as the very first linear part of the reaction curve.

Determination of PME activity: PME activity was determined by using an automatic titrator system (Metrohm ® Titrando) by using a 1 % of pectin solution (Alfa Aesar ® pectin citrus) prepared in NaCl 0.3 M as substrate. 50 mL of pectin solution was mixed with 1 mL of cloudy apple juice and pH was adjusted to 7.5 with NaOH 0.02 N. During pectin hydrolysis at 30ºC, pH was maintained at 7.5 by adding NaOH 0.02 N. The amount of NaOH added for 15 minutes was recorded. One PME activity unit (UPE) is defined as the micromoles of carboxylic groups produced per minute and mL of juice at pH 7.5 and 30 ºC [3].

Physico-chemical analysis: Some other important quality attributes of cloudy apple juice were also determined before and after HPCD treatment. Particle size distribution was determined by laser diffraction with a Mastersizer 2000 (Malvern® Inst., MA). Total phenolic compounds were determined by using the Folin-Ciocalteau reagent, and antioxidant capacity was measured by the ABTS method. Turbidity was measured with a turbidimeter (Eutech Instruments, TN-100) and expressed as nephelometric turbidity units (NTU). pH of apple juice was determined with a pH-meter (Crison® pH & Ion-Meter GLP 22). Finally, color was measured using a colorimeter an L*, a* and b* values were obtained representing brightness, red to green color and yellow to blue color, respectively.

Kynetic data analysis: In this work, the inactivation kinetic data at different operating temperatures and pressures were correlated by the Weibull model.

$$log\frac{A}{A_o} = -bt^n \quad [1]$$

where b and n are the scale and shape parameters, respectively. Z_T or Z_P (temperature or pressure increase needed for a 90% reduction of the D value was evaluated as the negative reciprocal slope of the regression of log D as function of T or p respectively:

$$log\left[\frac{D_1}{D_2}\right] = \frac{T_2 - T_1}{Z_T} \quad [2]$$

$$ln\ D = \ln D_{atm} - \frac{1}{Z_p}[p - p_{atm}] \quad [3]$$

Statistical analysis: Analyses were conducted using software Statgraphics X64. The results are presented as a mean ± standard deviation of at least three replicates. The significance of the differences was determined based on an analysis of the variance with the Fisher's least significant (LSD) method at p-value ≤ 0.05.

RESULTS

Stirring speed: The effect of agitation on the inactivation of PPO from cloudy apple juice was evaluated at 20 MPa at three different stirring speeds, 200, 400 and 600 rpm (Figure 1). When working at higher stirrer speeds, the magnetic stirrer did not work properly in our experimental equipment; therefore stirrer speeds higher than 600 rpm are not included. At any of the stirring speeds, the inactivation degree increased with increasing time. From Figure 1, it can be also observed that an increase in the stirrer speed led to a higher inactivation degree.

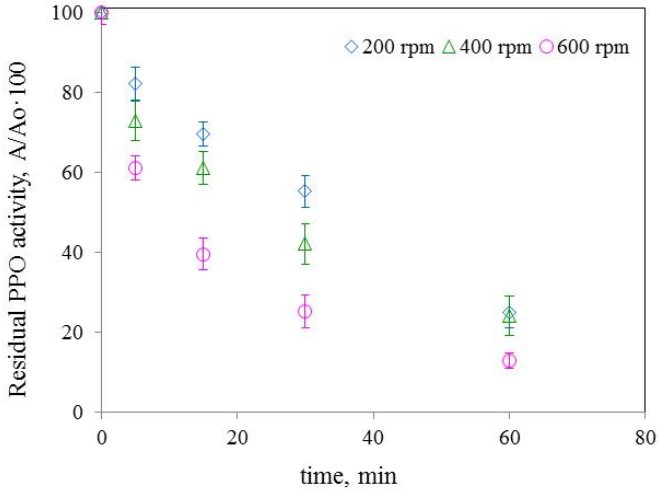

Figure 1. Effect of stirrer speed on PPO inactivation from cloudy apple juice by HPCD at 20 MPa and 45ºC (◇ 200 rpm, Δ 400 rpm, □600 rpm).

Sampling procedure: PPO activity after HPCD treatment was compared when sample was obtained after system depressurization or removed while the system was under pressure for one single cycle pressurization. HPCD treatment was carried out at 45ºC and 20 MPa at two different treatment times, 15 and 30 min. No significant differences have been determined among sample means of different sampling procedure when applying the Fisher's least significant (LSD) method at p-value ≤ 0.05.

The effect of a single cycle has been compared with a triple cycle for 15 and 30 minutes of total treatment time, that is 5 and 10 min of pressure-treatment each cycle, respectively. For PPO inactivation same effectiveness for a single pressurization cycle as for a three cycle pressurization can be determined when applying the Fisher's least significant (LSD) method at p-value ≤ 0.05.

Inactivation kinetics for PPO and PME Inactivation kinetics for PPO of cloudy apple juice have been determined at 20 MPa in the temperature range from 35 to 45ºC. By increasing temperature, enzyme inactivation rate also increased (Figure 2a). Figure 2b shows the inactivation kinetic at 45 ºC in the pressure range from 10 MPa to 20 MPa. Higher operating pressure led to faster inactivation rates, but the fraction of the resistant form of the enzyme is nearly independent of the operating pressure, being around 6 %. Inactivation kinetic data by HPCD were correlated by the Weibull model. D values were calculated and it can be observed that D values decreased with an increase in pressure and temperature (Table 1).

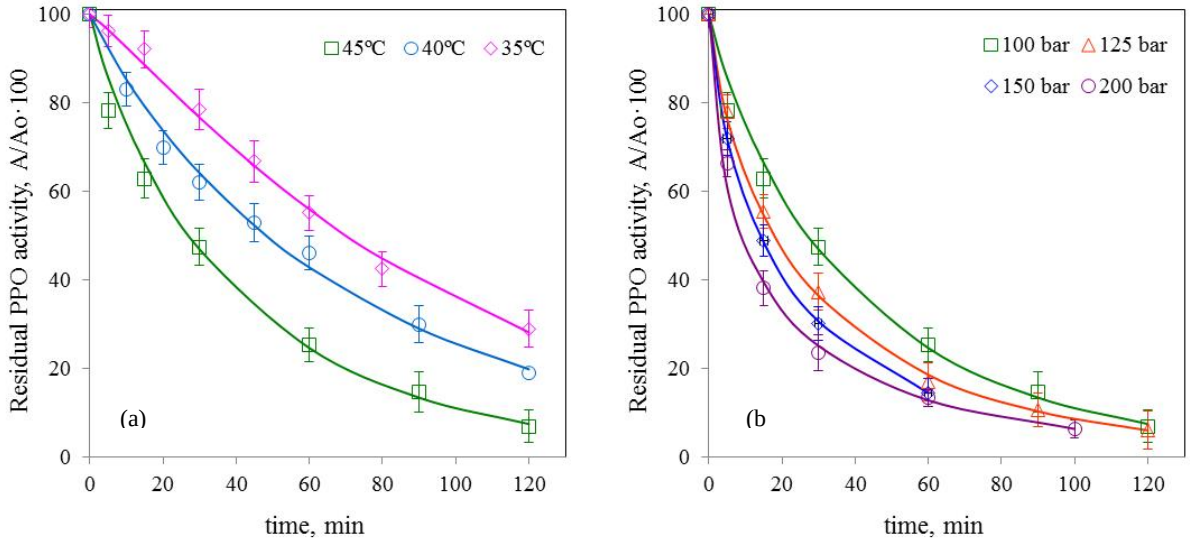

Figure 2. Inactivation of PPO in cloudy apple juice by HPCD (a) 20 MPa and different temperatures (b) 45ºC and different pressures The continuous lines represent the Weibull model.

To compare the different HPCD resistance of PME and PPO, PME kinetics inactivation were performed at 45ºC and 100 bar and 200 bar. PME inactivation was also determined in cloudy apple juice for mild heated treatment at the same temperature. At 45ºC and atmospheric pressure residual PME activity was still 90% after 120 minutes. Using HPCD treatment, higher inactivation rates can be observed than for mild heat treatment, however, at 20 MPa and 45ºC residual activity was still around 60 %. These results prove that PME from cloudy apple juice is more resistant to HPCD than PPO in the pressure range covered in this work.

Inactivation kinetic data for PME were successfully correlated by the Weibull model (Table 1). Compared to PPO inactivation, lower b and n values have been obtained and therefore higher D values.

Table 1. Kinetic parameter for the Weibull model for PPO and PME inactivation by HPCD and mild thermal heating at 45ºC and 0.1 MPa

p, MPa	T, ºC	b	n	D, min		R^2
PPO inactivation kinetic parameters						
10	35	0.0025±0.0006	1.12±0.05	204	Z_T = 35±10 ºC	0.995
10	40	0.008±0.002	0.93±0.04	175		0.994
10	45	0.0160±0.003	0.89±0.04	105		0.991
12.5	45	0.0357±0.004	0.74±0.02	92	Z_P = 22 ± 5 MPa	0.998
15	45	0.046±0.001	0.710±0.006	77		0.999
20	45	0.086±0.009	0.57±0.02	73		0.997
0.1	45	0.0019±0.0004	1.00±0.04	519		
PME inactivation kinetic parameters						
0.1	45	0.0011±0.0009	0.8±0.2	7385	Z_P = 65 ± 9 ºC	0.956
10	45	0.011±0.004	0.55±0.09	3568		0.959
20	45	0.031±0.004	0.45±0.04	2397		0.994

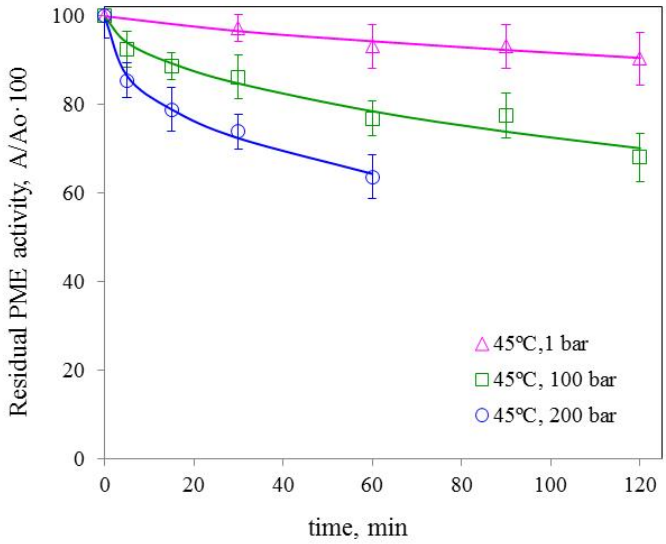

Figure 3. Inactivation of PME in cloudy apple juice at 45ºC and atmospheric pressure and by HPCD. The continuous lines represent the Weibull model.

Effect of HPCD on quality parameters of the juice: Initial pH of the juice was 3.89 ± 0.01 and immediately after HPCD treatment pH decreased to 3.74 ± 0.01, although after 2 hours after depressurization pH returned to its initial value.

The color of the juice did not visually change, and that was proved by the color measurement. After HPCD treatment, lightness (L*) presented slightly lower values. It only changed from 41.36 ± 0.03 to 41.01 ± 0.01. The same occurred with the a* value, where no significant differences were observed. The value of b* was increased after the treatment, indicating more yellow components.

No significant differences were found in total polyphenols compounds content and in the antioxidant capacity in the juice before and after the treatment.

Turbidity increased significantly in the juice after being treated from 105 ± 2 to 168 ± 3, reaching a better value for a cloudy apple juice, although it is not enough because in naturally cloudy juices, turbidity should be in a range between 250 and 300 NTU [5].

Two opposite effects on PSD by HPCD have been reported in the literature either protein coagulation or homogenization effect [6]. When studying the particle size a big change was observed in the distribution of the particles. The maximum peaks move to smaller particle diameter suggesting that HPCD treatment induced a coagulation effect.

CONCLUSION

In conclusion, HPCD treatment is a valid alternative for inactivating PPO in cloudy Golden delicious apple juice, nearly reaching its total inactivation. Better results were obtained in comparison with the mild heating inactivation, and not antioxidant activity was lost. PME was more resistant to inactivation. Stirring speed is an important factor to take into account for enzyme inactivation. HPCD also affected significantly some quality parameters of the juice, such as particle size distribution and turbidity.

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REFERENCES

[1] MARKOWSKI, J., BARON, A., PLOCHARSKI, W. LWT-Food Science and Technology, Vol. 62, **2015**, p.813.

[2] LIU, X., GAO, Y.X., PENG, X.T., YANG, B., XU, H.G., ZHAO, J. Innovative Food Science and Emerging Technologies, Vol. 9, **2008**, p. 24.

[3] BRIONGOS, H., ILLERA, A.E., SANZ, M.T., MELGOSA, R., BELTRAN, S., SOLAESA, A.G. LWT-Food Science and Technology, Vol. 74, **2016**, p. 411.

[4] WANG, S.S., LAI, J.T., HUANG, M.S., TAI, C.Y., LIU, H.S. Bioprocess and Biosystems Engineering, Vol. 33, **2010**, p. 1007.

[5] TELESZKO, M., NOWICKA, P., WOJDYTO, A. Agricultural and Food Science, Vol. 25, **2016**, p. 34.

[6] ZHOU, L., ZHANG, Y., HU, X., LIAO, X., HE, J. Food Chemistry, Vol. 115, **2009**, p. 449.