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High Pressure Carbon Dioxide Technology. Application to Orange Juice.

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ABSTRACT

High pressure carbon dioxide (HPCD) treatment has been applied to orange juice as alternative non-thermal treatment. Kinetics inactivation for pectin methyl esterase has been determined at different operating conditions. PME residual activity was correlated succesfully to the two-fraction model.
Some quality parameters such as colour, pH, calcium content, turbidity and particle size distribution (PSD) were also determined right after HPCD treatment. PSD shows that HPCD treatment results in a homogenization effect with a volume increase of small particles and a volume decrease of large particles regarding the non-treated orange juice.

INTRODUCTION

In the last years there is an increasing demand for the consumers for minimally processed foods with high quality parameters. Therefore the Food Industry has been focus on finding alternative food processing methods of preservation. Traditional preservation methods are based mainly on thermal treatments, however, some quality attributes are lost such as flavour. Non-thermal treatments have been studied to determine its potential as an alternative to thermal treatment to preserve foods. Among them, high pressure-carbon dioxide (HPCD) is growing interest due to the mild condition operations. In HPCD treatments, operating temperatures can range between 5 – 60ºC and pressures usually below 50 MPa.

HPCD treatment has been mainly applied to fruit or vegetable juices mainly focus on microorganism inactivation. However, it has been reported that HPCD is able to inactivate certain enzymes that causes deterioration on foods, such as pectin methyl esterase (PME) that it is believed to causes loss of cloud in some juices of polyphenol oxidase (PPO) that is responsible of enzymatic browning [1].

In this work, HPCD treatment has been applied to orange juice. According to the European Fruit Juice Association orange juice is one of the most consumed fruit juices. Pasteurization at 90ºC and 1 minute time is currently used to prevent microbial spoilage and to reach inactivation of some enzymes, such as PME. One of the most important quality parameters or orange juice is the cloud stability. Cloud particles are range from 0.4 to 5 μm and determine colour, flavour, aroma and texture of orange juice. One of the most accepted theories in cloud loss involves the action of PME that causes pectin demethylation and the formation of insoluble calcium pectate gels that precipitate and cause clarification of the juice. In any case, other components with negative charge in addition to pectin may be act as stabilizing agents of the cloud [2].

In this work, the effect of HPCD treatment on PME activity has been studied. In addition, other physical and chemical parameters of orange juice will be also determined after HPCD such as colour, pH, turbidity and particle size distribution. Calcium has been also determined due to its role in gel formation with low-methoxy pectin, by acting as a bridge between pairs of carboxyl groups of different pectin chains.

MATERIALS AND METHODS

HPCD processing. Valencia oranges were purchased from a local supplier and squeezed in an orange squeezer. In a typical HPCD experiment, orange juice was charged into the high pressure cell, which was then placed in the thermostatic water bath at the established temperature. Afterwards, the system was pressurized and maintained at constant temperature and pressure for a pre-established treatment time. The high pressure cells were magnetically stirred. Experiments were carried out in a temperature (T) range from 21 to 40 ºC, pressure (p) from 10 to 30 MPa and exposure time (t) from 3 to 60 min. After HPCD treatment, the high pressure cells were depressurized and the treated orange juice was analysed.

Physico-chemical analysis.

Calcium content. Calcium in orange juice was determined by atomic absorption spectrometry (Perkin Elmer 3300). The orange juice was firstly centrifuged and the precipitate was discharged. La_2O_3 (Merck®) was added to the supernatant to a final concentration of 0.5% of lanthanum to avoid the interference of phosphates in the calcium determination. HCl was also added (5% in the sample) to promote dissolution of both calcium and lanthanum in the medium [3]. Calcium content was obtained by previous calibration with different standard solutions of calcium (Merck *Certipur*®, 1 g/L).

Determination of pectin methylesterase activity. PME activity was determined by using an automatic titrator system (Metrohm® Titrando). A 1% of pectin solution (Alfa Aesar® Pectin Citrus) prepared in NaCl 0.3 M was used as substrate. 50 mL of pectin solution mixed with 5 mL of orange juice were adjusted to pH 7.5 with NaOH 0.02 N. During hydrolysis at room temperature, pH was maintained at 7.5 by adding NaOH 0.02 N. The amount of NaOH added for 30 minutes was recorded.

Determination of pH and colour. pH of orange juice was determined with a pH-meter (Crison® pH & Ion-Meter GLP 22).
Colour was evaluated by a Konica Minolta ® CM-2600d colorimeter. The L*, a* and b* values were obtained representing brightness, red to green color and yellow to blue color, respectively. Changes in colour were expressed as [4]:

$$\Delta E = \sqrt{\left(L^*_{before} - L^*_{after}\right)^2 + \left(a^*_{before} - a^*_{after}\right)^2 + \left(b^*_{before} - b^*_{after}\right)^2} \quad [1]$$

Determination of turbidity and particle size distribution. Cloud quality was determined by measuring the absorbance at 660 nm after centrifugation . Particle size distribution (PSD) was determined by laser diffraction with a Mastersizer 2000 (Malvern® Inst., MA). The system uses a laser light at 750 nm wavelength to size particles from 0.4 to 2000 µm by light diffraction. Particle size distribution was calculated by the Fraunhofer model.

Inactivation kinetic data. In this work, the inactivation kinetic data have been correlated by the two-fraction kinetic model. This model takes into account the existence of several isoenzymes of PME in orange juice, grouped into two fractions, a labile and a stable fraction. Both enzymes were considered to be inactivated according to first-order kinetics, but independently of each other:

$$A = A_L exp(-k_L t) + A_S exp(-k_S t) \qquad [2]$$

where A_L and A_S ($A_S = 1 - A_L$) are the activity of the labile and stable fractions respectively and k_L and k_S (min^{-1}) the inactivation rate constants of both the labile and stable fractions respectively.

RESULTS

PME inactivation kinetics. Figure 1 shows different kinetic inactivation for PME of freshly squeezed orange juice treated by HPCD. In all cases, a sharp decrease of PME activity is observed at the beginning of the process, while longer operation times do not involve further substantial enzyme inactivation. This behaviour may indicate that HPCD-labile and HPCD-stable PME fractions coexist in the Valence orange juice [5].

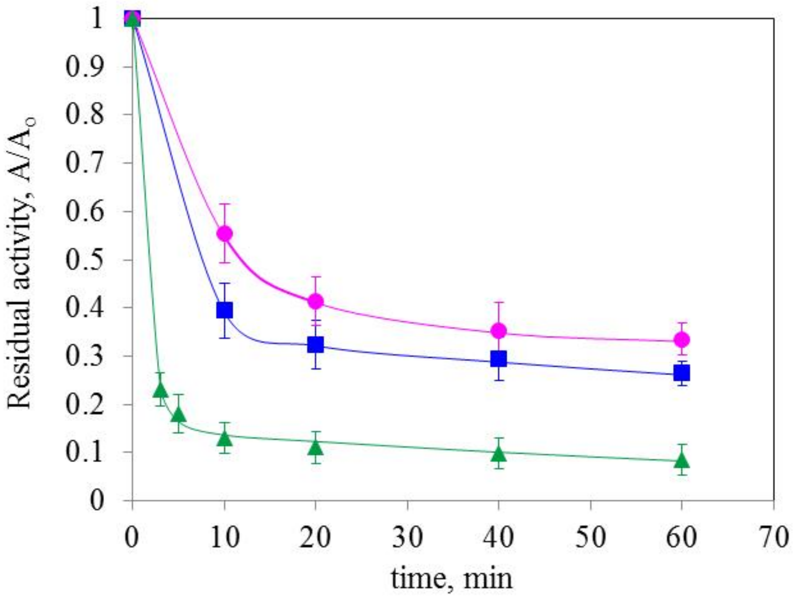

Figure 1 PME inactivation kinetics at different operating conditions (▲ 40ºC and 30 MPa, ■ 21ºC and 20 MPa, ● 21ºC and 10 MPa). Continuous lines correspond to the two fraction model.

The kinetic parameters for the two fraction model are listed in Table 1. This Table also includes the decimal reduction time (D value), defined as the treatment time needed for a 10-fold reduction of the initial enzyme activity at a given condition and the statistical parameters for the fit of the kinetic model, r^2. It can be observed that A_L was higher than A_S and k_L was 30-70 times higher than k_S indicating that there is a fast inactivation period followed by a decelerated decay. Therefore, the corresponding D_L and D_S followed the opposite trend. k_L and A_L from the two-fraction model increased with increasing pressure.

Table 1. Estimated kinetic parameters for the two fraction model for PME inactivation by HPCD.

T, ºC	p, MPa	k, min^{-1}	A	D value, min	r^2 ($p<0.05$)
40	30	$k_L = 0.701$	$A_L = 0.859$	$D_L = 3.3$	0.998
		$k_S = 0.010$	$A_S = 0.141$	$D_S = 240.3$	
21	20	$k_L = 0.224$	$A_L = 0.671$	$D_L = 10.3$	0.999
		$k_S = 0.004$	$A_S = 0.329$	$D_S = 583.2$	
21	10	$k_L = 0.153$	$A_L = 0.548$	$D_L = 15.1$	0.999
		$k_S = 0.005$	$A_S = 0.453$	$D_S = 462.8$	

Other quality parameters of orange juice. After HPCD treatment cloud was improved, increasing nearly a 30% compared to the freshly squeezed orange juice . Arreola et al. [6] found that cloud increased from 27% to 400%. These authors found that cloud improvement was less in orange juice drained after depressurization of the system compared to orange juice samples withdrawn while the system was under pressure. This could explain the values of cloud enhancement obtained in this work.

Taking into account these results, cloud seems to be stabilized after HPCD in a non-enzymatic way, since some PME is still active. Kincal et al. (2006) suggested that HPCD treatment could lead to precipitation of calcium ions present in the orange juice due to the formation of insoluble calcium carbonate. Table 2 shows the residual calcium content after HPCD treatment at different operating conditions in the fresh orange juice. Although calcium content presented slightly lower values after HPCD treatment, no significant differences have been determined among sample means of buffer and orange juices when applying the Tukey's HSD method.

Table 2. pH and Calcium content after HPCD treatment.

pH before HPCD treatment	pH after HPCD treatment	HPCD treatment			Residual Ca^{2+}, %
		p, MPa	T, ºC	t, min	
3.92	3.86	20	21	20	95 ± 2^a
4.18	4.18	10	21	20	91 ± 2^a
4.16	4.15	30	40	20	93 ± 4^a

Different letters indicate significant differences by the Tukey's HSD method at p-value ≤ 0.05.

PSD of orange juice before and after HPCD treatment has been represented in Figure 2 with two maximums around 0.8 µm and 850 µm. After HPCD treatment an increase of the volume peak of the smaller particles and a decrease of large particles can be observed. Taking into account this behaviour it can be concluded that HPCD treatment leads to an homogenization effect probably due to the explosive action and the bubbling of CO_2 during depressurization [3].

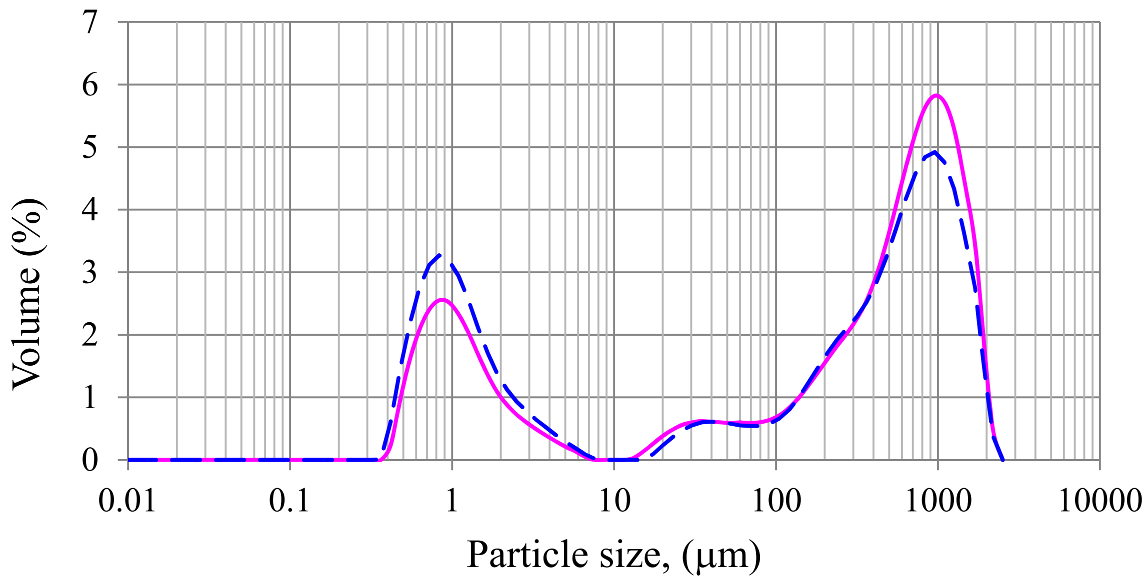

Figure 2. Particle Size Distribution (PSD) of orange juice freshly squeezed (—), immediately after treatment by HPCD at 30 MPa, 40ºC for 40 min (– – –).

Table 3 lists the L*, a*, b* parameters of orange juice before and after HPCD treatment. Lightness (L*) and yellowness (b*) significantly decreased indicating the darkening of the orange juice and less yellow and more blue colour after HPCD processing. On the contrary, redness (a*) was not significant different in the untreated and HPCD processed orange juice. The change in colour, ΔE (Eq 1) is also presented and visible differences in colour after HPCD treatment have been determined (ΔE ≈ 5).

Table 3. Changes in orange juice colour.

Orange juice	L	a	b	ΔE
Before treatment	31.62 ± 0.08^{a}	4.26 ± 0.07^{a}	19.9 ± 0.3^{a}	
After treatment	28.1 ± 0.2^{b}	4.1 ± 0.1^{a}	16.2 ± 0.2^{b}	5.1 ± 0.5

Different letters in a column indicate significant differences by the Tukey's HSF method at p-value ≤ 0.05.

CONCLUSION

PME in orange juice was effectively inactivated by HPCD showing a fast initial decrease that remained nearly constant after prolonged HPCD treatment. The inactivation degree increased with pressure and temperature. Residual PME activity data were correlate by the two-fraction model. PSD shows an increase of the volume peak of the smaller particles (0.3-5 µm) and a decrease of large particles after HPCD treatment, supporting the cloud enhancement observed. Calcium content does not change significantly after HPCD treatment, proving that insoluble calcium content was not formed.

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