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Comparison of the inactivation kinetics of pectin methylesterases from carrot and peach by high-pressure carbon dioxide

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ABSTRACT

The inactivation of pectin methylesterases (PMEs) from carrot and peach in buffer by high-pressure carbon dioxide (HPCD) at 55 °C was investigated. The two PMEs were effectively inactivated by HPCD, their residual activity (*RA*) decreasing with increasing pressures. The *RA* of the two PMEs exhibited a fast decrease firstly and reached a constant after a prolonged treatment time; their inactivation kinetics was adequately modelled by a fractional-conversion model. The non-zero $RA(A_\infty)$of the two PMEs was 6–7%, with increasing pressures the kinetic rate constant, *k*, increased and the decimal reduction time, *D*, decreased for the HPCD-labile fraction of the two PMEs. The labile fraction of carrot PME was more susceptible to HPCD than that of peach PME; the activation volume, V_a, and Z_P (the temperature increase needed for a 90% reduction of *D*) was −1079.37 cm^3/mol and 5.80 MPa for carrot PME, and −130.51 cm^3/mol and 48.31 MPa for peach PME.

1. Introduction

In the juice industry, stabilisation of cloudiness is a key problem of cloudy juices during storage (Denès, Baron, & Drilleau, 2000). This undesired defect is induced by demethylation of pectin by endogenous pectin methylesterase (PME, EC 3.1.1.11) yielding acidic low methoxy pectin, which can cross-link with polyvalent cations, such as Ca^{2+}, to form insoluble pectate precipitates (Guiavarc'h, Segovia, Hendrickx, & Van Loey, 2005) or becomes a target for pectin-degrading polygalacturonases (PG) (Cameron, Niedz, & Grohmann, 1994; Ly Nguyen et al., 2003a). Thus, the control of PME activity is crucial for the cloud stability of juices (Assis, Lima, & Oliveira, 2001). This enzyme is widely distributed in plants, and the data published in the past few years have established that plants contain multiple forms of this enzyme (Rillo et al., 1992). PME is a cell-wall bound enzyme, commonly existing in plants as a complex with pectin, through electrostatic interaction (Basak & Ramaswamy, 1996). The inactivation of PME is generally used as an indicator of the adequacy of pasteurisation because it is known to be more heat-resistant than the common microorganisms (Basak & Ramaswamy, 1996). Thermal treatment (e.g. 90 °C for 1 min in citrus juices) is the most common and least expensive technology that has been used to solve the problem (Assis et al., 2001); unfortunately this leads to degradation of some product qualities as well.

As consumers are demanding minimally processed and fresh-tasting food products, the application of non-thermal technologies is gaining popularity. In the past decades, a noticeable inactivation effect of high-pressure carbon dioxide (HPCD) on microorganisms in liquid food has been shown as a non-thermal technology (Enomoto, Nakamura, Hakoda, & Amaya, 1997; Hong & Pyun, 2001; Liao, Hu, Liao, Chen, & Wu, 2007). However, few data on the inactivation of enzymes by HPCD have been provided. Recently, interest in HPCD inactivation of enzymes has increased (Balaban et al., 1991; Gui et al., 2006; Gui et al., 2007; Liu et al., 2008; Zhi, Zhang, Hu, Wu, & Liao, 2008). Enzyme inactivation by HPCD could be due to many causes, such as pH lowering, conformational changes of the enzyme, and the inhibitory effect of molecular CO_2 on enzyme activity (Damar & Balaban, 2006). Alterations in the secondary and tertiary conformations of HPCD-treated enzymes have been observed (Gui et al., 2007; Liao, Zhang, Bei, Hu, & Wu, 2009). PMEs from different sources have different characteristics, and multiple isoenzymes exist in the same source with different molecular weights, isoelectric points and/or kinetic properties (Cameron et al., 1994; Guiavarc'h et al., 2005; Ly Nguyen et al., 2003a). The complexity of modelling the inactivation kinetics of HPCD-treated enzymes is worth investigating.

The inactivation of carrot PME and peach PME by HPCD has not been reported until now. The purpose of this work was to investigate and compare the effect of HPCD on the inactivation of carrot

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PME and peach PME, and to model the inactivation kinetics of the two PMEs.

2. Materials and methods

2.1. Materials

Peaches (*Cultivar* No. 24 Beijing) were purchased from a local supermarket, carrots (*Cultivar* No. 1 orange-red) were harvested from Beijing Vegetable Experimental Station of the Chinese Academy of Agriculture Science. Peaches and carrots were stored at 0 ± 1 °C in a cold warehouse until processing. Apple pectin (DE 70–75%) was obtained from Andre Co. (Shangdong, China). All other chemicals were of analytical grade.

2.2. PME extraction

The extraction of PME from peach and carrot was performed according to the method proposed by Ly Nguyen with a little modification (Ly Nguyen et al., 2003b). The halves of peaches were pitted and sliced by hand, juiced with a screw juice extractor (GT6G7, Zhejiang Light Industry Machinery Plant, Zhejiang, China), and filtered with four layers of cheesecloth. As an antibrowning agent, 0.10% (w/w) of L-ascorbic acid was added to the slices before juicing. The pomace was mixed overnight at 4 °C in a 0.2 M Tris–chloride buffer containing 1 M NaCl, pH 8.0 (1:2 w/w). In the case of carrot, a similar PME extraction process was performed, but juiced with another juice extractor (ZHJ-308A1, Fushan Ouke Electric Appliance Co., Guangdong, China). The resulting juices were kept frozen in glass bottles at −18 °C until used.

The mixture was filtered by using two layers of cheesecloth and the filtrate was partially purified by ammonium sulphate precipitation at 30% saturation. The mixture was stirred for 20 min and centrifuged (13 000*g*, 20 min) after standing for 1 h. The supernatants were precipitated again by ammonium sulphate up to 80% saturation, stirred for 20 min, and centrifuged (13 000*g*, 20 min) after standing for 2 h. The precipitate containing PME was dissolved in a minimum volume of 20 mM Tris–chloride buffer (pH 7.5) and dialysed against the same buffer overnight with three changes of buffer. The crude PME was quickly frozen with liquid nitrogen and stored at −18 °C until used. All procedures were conducted at 4 °C.

2.3. PME assay

PME activity was measured by titrating the free carboxyl groups at pH 7.50 and 30 °C, according to the method described by Yeom, Zhang, and Chism (2002). A 0.20 ml aliquot of crude PME or 2.0 ml of peach juice or 5.0 ml of carrot juice were added to 20 ml of 1% pectin solution containing 0.1 N NaCl, which was initially adjusted to pH 7.0 with 2.0 N NaOH at 30 °C. The pH of solution was then readjusted to 7.5 with 0.05 N NaOH. After the pH reached 7.5, 0.05 ml of 0.05 N NaOH was added. The time required for the pH of the solution to return to 7.50 was measured. PME activity (A) expressed in pectin methylesterase units (PMEU) was calculated by the following equation:

$$A = \frac{[\mathrm{NaOH}] \times V_{\mathrm{NaOH}}}{V_{\mathrm{sample}} \times t'} \quad (1)$$

where [NaOH] is the NaOH concentration (0.05 N), V_{NaOH} is the volume of NaOH used (0.05 ml), V_{sample} is the volume of sample used (0.20 ml PME or 2.0 ml of peach juice or 0.5 ml of carrot juice), and t' is the time (in minutes) needed for the pH to return to 7.50 after the addition of NaOH.

Percentage of PME residual activity (RA) was defined as indicated by

$$RA = 100 \times \frac{A_t}{A_0} \quad (2)$$

where A_t is the enzyme activity in the samples after HPCD treatment, and A_0 is the initial enzyme activity in the samples to be treated.

2.4. HPCD Process system

The HPCD system used was described by Liao et al. (2007). A stainless steel pressure vessel with a volume of 850 ml was designed to withstand a pressure of 50 MPa. The vessel temperature was maintained by a THYS-15 thermostatic bath (Ningbo Tianheng Instrument Factory, Zhejiang, China). An XMTA-7512 temperature controller (Yuyao Temperature Meter Factory, Zhejiang, China) was used to monitor the temperature with two thermocouples. One thermocouple was fixed in the vessel lid to monitor the CO_2 temperature in the upper part of the vessel and the other was placed at the middle wall of the vessel to monitor the temperature of the middle part of the vessel, which was used as process temperature. A 2TD plunger pump (Huaan Supercritical Fluids Extraction Co. Ltd., Jiangsu, China) with a maximum pressure of 50 MPa and a maximum flow rate of 50 lL/h was used to pressurise the vessel. A DBY-300 pressure transducer (Shanxi Qingming Electronic Group Corporation, Shanxi, China) was fixed in the vessel lid to monitor the vessel pressure. All the data for temperature and pressure were displayed on a control panel. All parts of the system exposed to high pressure were made of stainless steel. The vessel had gas-tight connections to the gas inlet and outlet, and the fluid sample inlet and outlet. The vessel lid could be sealed by screws during HPCD processing. A 2XZ-4 vacuum pump (Huangyan Qiujing Vacuum Pump Factory, Zhejiang, China) was connected to the vessel for evacuating the air in the vessel. Commercially-available CO_2 of 99.5% or 99.9% purity was purchased from Beijing JingCheng Co. (Beijing, China), and was passed through an active carbon filter before entering the pressure vessel.

2.5. Inactivation of PME in buffer by HPCD

Crude PME sample (1.5 ml) was transferred into a 15 ml plastic tube (Beijing Bomex Company, Beijing, China) without cap and then was enclosed in the HPCD vessel already equilibrated to a certain temperature. For each experiment, the HPCD vessel containing two enzyme samples was pressurised by the plunger pump to a required level. After a preset time interval, the vessel was decompressed and the decompressed time was closely related to the applied treatment pressures. The treatment was performed by HPCD, at a moderate temperature of 55 °C for various times. After pressure release, PME samples were immediately cooled in an ice bath, then frozen with liquid nitrogen and stored at −18 °C until the activity determination. After HPCD, the pH of the two PME solutions in buffer dropped to around 5.0.

2.6. Inactivation of PME in buffer by mild heat alone or/and lowering pH

Crude PME sample (1.5 ml) was transferred into a 15 ml plastic tube (Beijing Bomex Company) without cap and then was enclosed in the HPCD vessel already equilibrated to a certain temperature. For each experiment, two tubes filled with sample were subjected to mild heat alone at 55 °C and/or lowering pH to 5.0 (using hydrochloric acid adjustment, because the pH of PME buffer solution was reduced to around 5.0) under atmospheric pressure. The remaining procedures were in accord with that of HPCD inactivation experiments without pressurising.

2.7. Inactivation of PME in juice

Peach juice (2 ml) or carrot juice (5 ml) were transferred into a 15 ml plastic tube (Beijing Bomex Company) without cap and then was enclosed in the HPCD vessel already equilibrated to a certain temperature. For each experiment, three tubes filled with sample were subjected to HPCD, mild heat at 55 °C or lowering pH to 5.0 under atmospheric pressure or both. The HPCD vessel was pressurised by the plunger pump to a required level. After a preset time interval, the vessel was decompressed and the decompressed time was closely related to the applied treatment pressures. The treatment was performed by HPCD at 30 MPa or 35 MPa, combined with a moderate temperature of 55 °C. Samples were immediately cooled in an ice bath, and then stored in −18 °C until the activity determination.

2.8. Kinetic data analysis

Inactivation of enzymes can often be described by a first-order kinetic model, which is given by

$$\ln\frac{A}{A_0} = -kt \tag{3}$$

where A_0 is the initial activity, A is the remaining activity at time t, and k (min^{-1}) is the inactivation rate constant. Eq. (3) is valid under most isothermal and isothermal–isobaric conditions, whereby the inactivation rate constant, k, can be determined from a linear regression analysis of $\ln A/A_0$ *versus* time.

A special case of first-order model is a fractional-conversion model (Ly Nguyen et al., 2002a). Fractional conversion, f, takes into account the non-zero activity after prolonged heating and/or pressure (A_∞) and can be expressed mathematically as:

$$f = \frac{(A_0 - A_t)}{(A_0 - A_\infty)} \tag{4}$$

For most irreversible first-order reactions, A_∞ approaches zero, and Eq. (4) can be reduced to

$$f = \frac{(A_0 - A_t)}{A_0} \tag{5}$$

A plot of the logarithm of $(1 - f)$ *versus* time yields a straight line with a rate constant expressed by the negative slope value:

$$\ln\left(\frac{A_t}{A_0}\right) = \ln(l - f) = -kt \tag{6}$$

So, it is clear that Eq. (6) is identical to Eq. (3) when A_∞ approaches to zero.

To account for the non-zero activity after prolonged heating and/or pressurising, we should use fractional conversion in the following form:

$$\ln(1 - f) = \ln\left[\frac{(A_t - A_\infty)}{(A_0 - A_\infty)}\right] = -kt \tag{7}$$

Rearranging Eq. (7) yields Eq. (8). By plotting A_t *versus* inactivation time at constant pressure and/or temperature conditions, the inactivation rate constant, k, and remaining activity, A_∞ can be estimated using nonlinear regression analysis:

$$A = A_\infty + (A_0 - A_\infty)\exp(-kt) \tag{8}$$

It should be stressed that a fractional-conversion model is usually applied when a fraction is inactivated and another fraction remains constant and A_∞, a non-zero residual activity after prolonged thermal/pressure treatment, is observed (Sampedro, Rodrigo, & Hendrickx, 2008).

The decimal reduction time (D value) is the time needed for a 10-fold reduction of the initial activity at a given temperature:

$$D = \frac{\ln 10}{k} \tag{9}$$

The pressure sensitivity parameter, Z_P (MPa), is the pressure range between which the D value changes 10-fold.

$$\log\left[\frac{D_1}{D_2}\right] = \frac{P_2 - P_1}{Z_P} \tag{10}$$

The pressure (at constant temperature) dependence of the inactivation rate constants can be calculated by the activation volume using the linearised Eyring equation (Gui et al., 2007):

$$\ln\left(\frac{k_1}{k_2}\right) = \frac{V_a}{RT}(P_2 - P_1) \tag{11}$$

where P_2 and P_1 are pressures corresponding to the decimal reduction times D_1 and D_2 or constants k_1 and k_2, respectively. R is the gas constant; T is the absolute temperature (K). The value of Z_P is obtained as the negative reciprocal slope of the regression line representing the log D *versus* P relationship. V_a is estimated from the linear regression of $\ln k$ *versus* P.

2.9. Statistics analysis

Analyses of variance (ANOVA) were carried out by using the software Microcal Origin 7.5 (Microcal Software Inc., Northampton). ANOVA tests were performed for all experiments run, to determine the significance at 95% confidence. All experiments were performed in triplicate. All data and kinetic parameters were presented as means and standard deviations.

3. Results and discussion

3.1. Effect of HPCD on carrot PME and peach PME in buffer solution

As shown in Figs. 1 and 2, carrot PME and peach PME in buffer was effectively inactivated by HPCD at 55 °C, the *RA* of the two PMEs decreased with increasing pressures of HPCD. Obviously, it was seen that the *RA* of the two PMEs rapidly decreased firstly and then seemed to reach a constant after a prolonged HPCD treatment time. This indicated that HPCD-labile and HPCD-stable fractions possibly occurred in the two PMEs. The presence of labile and stable fractions of PME exposed to different treatments (such

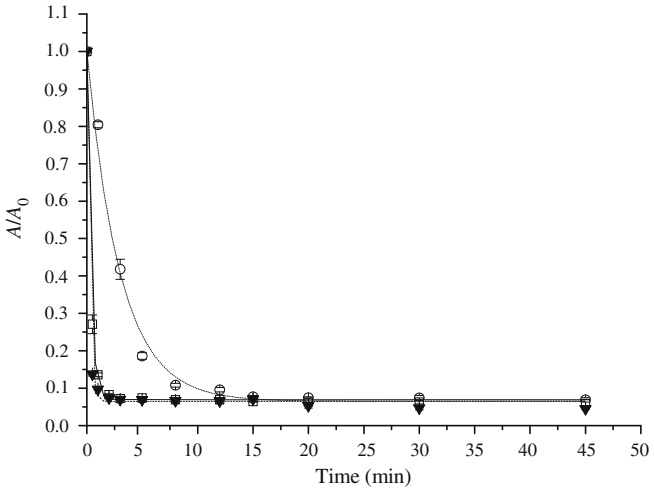

Fig. 1. HPCD inactivation of carrot PME dissolved in 20 mM Tris–HCl buffer at pH 7.5 and 55 °C, modelled using a fractional-conversion inactivation model. 8 MPa (○); 12 MPa (□); 15 MPa (▼). All data are the means ± SD, $n = 3$. Data points without error bars have a smaller standard deviation than the data symbol.

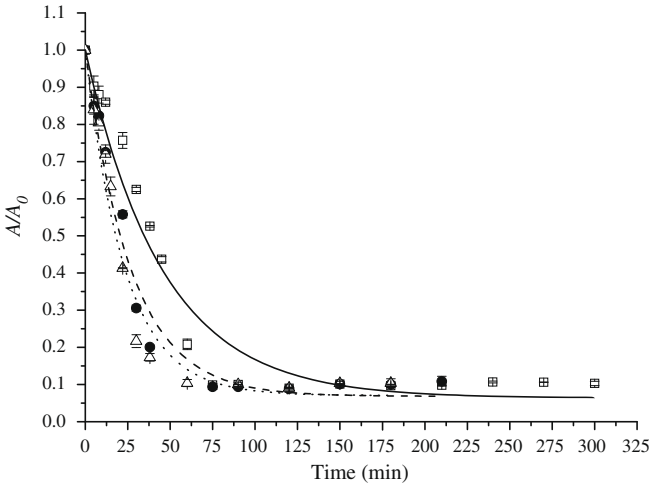

Fig. 2. HPCD inactivation of peach PME dissolved in 20 mM Tris–HCl buffer at pH 7.5 and 55 °C, modelled using a fractional-conversion inactivation model. 8 MPa (□); 15 MPa (●); 22 MPa (△). All data are the means ± SD, $n = 3$. Data points without error bars have a smaller standard deviation than the data symbol.

as heat or high hydrostatic pressure) has been widely reported in PME from several fruits and vegetables, such as plum (Nunes et al., 2006), carrot (Ly Nguyen et al., 2003b), banana (Ly Nguyen, Van Loey, Fachin, Verlent, & Hendrickx, 2002b), and grapefruit (Guiavarc'h et al., 2005). Visually, the *RA* of carrot PME showed a steeper decrease than that of peach PME, and the time to reach a constant after a prolonged HPCD treatment for carrot PME was far shorter than for peach PME (Figs. 1 and 2). The *RA* of carrot PME was reduced to a constant within 3 min at 12 or 15 MPa, while in peach it became constant after 100 min using 15 or 22 MPa, indicating that the HPCD-labile fractions of carrot and peach PMEs exhibited different pressure stability to HPCD. It was concluded that carrot PME was more susceptible to HPCD than peach PME. The difference of the two PMEs in susceptibility to HPCD possibly resulted from their plant sources. Previously, it was proposed that the heat stability of PME not only depended on the cultivars, pH, isoenzyme forms of PME, total solids level, and extraction method (Yeom et al., 2002), but also largely depended on its origin (Espachs-Barroso, Van Loey, Hendrickx, & Martín-Belloso, 2006). Specifically in this study, the original carrot itself had a neutral pH of around 6.5 and the original peach an acidic pH of around 4.2, which probably caused a higher susceptibility of carrot PME in buffer due to lowering of pH to about 5.0 after HPCD.

For better understanding of the contribution of the temperature and/or pH lowering to inactivation of PME exposed to HPCD, the *RA* values of two PMEs in buffer by mild heat alone at pH 7.5 and/or lowering pH to 5.0 in buffer is shown in Table 1. The *RA* of peach PME in buffer still remained at 91% after mild heat alone at 55 °C for 300 min. Javeri and Wicker (1991) also found only a 2% loss of crude peach PME activity (0.1 M KCl, potassium phosphate buffer, 50 mM, pH 7.0) after heating for 5 min at 55 °C. The *RA* of carrot PME was 84.2% at 55 °C for 45 min. These data indicated that the two PMEs were less inactivated by the mild heat alone at 55 °C. Similar findings were shown in previous studies. Viar-Vera, Salazar-Montoya, Calva-Calva, and Ramos-Ramírez (2007) reported that hawthorn PME still exhibited about 80% of its *RA* after 40 min at 50 °C. Nunes et al. (2006) found plum PME (Tris buffer, 20 mM, pH 7.5) maintained at least 80% of the *RA* at a temperature below 55 °C for 5 min. Espachs-Barroso et al. (2006) found a fraction of banana PME in 20 mM Tris buffer (pH 7.0) was not inactivated at low temperature (<70 °C) after long treatment times. These findings were possibly due to the optimum temperature of

Table 1
RA of two PMEs in buffer after different treatments.

PME source	*RA* (%)			
	L[A]	M[B]	L + M[C]	HPCD[D]
Carrot	$82.7^{c} \pm 2.3$	$84.2^{c} \pm 1.3$	$54.6^{b} \pm 1.3$	$4.5^{a} \pm 0.1$
Peach	$88.4^{b} \pm 2.5$	$91.3^{b} \pm 2.5$	$85.8^{b} \pm 1.2$	$10.3^{a} \pm 0.6$

All data are means ± SD, $n = 3$. The treatment time for carrot PME was 45 min and for peach PME 300 min.
[a,b,c] Values in the same row with different subscripts are different ($p < 0.05$).
[A] L: Lowering of pH to 5.0 alone.
[B] M: Mild heat at 55 °C alone.
[C] L + M: Combination of lowering pH to 5.0 and mild heat at 55 °C
[D] HPCD: Inactivation of carrot PME by HPCD at 15 MPa and 55 °C, and of peach PME at 22 MPa and 55 °C.

most plant PMEs ranging from 55 °C to 65 °C (Javeri et al., 1991; Denès et al., 2000; Ly Nguyen et al., 2002b; Nunes et al., 2006; Viar-Vera et al., 2007). Moreover, the data also confirmed that the two PMEs were more susceptible to the temperature by HPCD than under atmospheric conditions. Balaban et al. (1991) found that HPCD could inactivate certain enzymes at temperatures where thermal inactivation was not effective. Zhi et al. (2008) also reported that HPCD significantly enhanced the susceptibility of apple PME to temperature. This increase in the susceptibility of PME to temperature was possibly attributed to an interaction of pressure and pH lowering of HPCD (Zhi et al., 2008). Similar to mild heat alone in Table 1, lowering pH alone or a combination with the mild heat was still incomparable to HPCD for the inactivation effect. Thus, the inactivation of two PMEs by HPCD was probably a complex interaction amongst temperature, pH lowering and pressure; this interaction of HPCD could alter the secondary and tertiary conformation of treated enzymes (Gui et al., 2007; Liao et al., 2009).

The different effects for inactivating PMEs in various matrices has been found previously upon thermal or high hydrostatic pressure treatments. Balogh, Smout, Ly Nguyen, Van Loey, and Hendrickx (2004) reported that PME in carrot pieces was the most thermo-stable and pressure stable and PME in carrot juice was more stable than PME in purified form. Sampedro et al. (2008) found higher stability of PME in an orange juice-milk beverage than purified PME. Thus, the effect of HPCD on the inactivation of the original PMEs in the juices was also investigated. As shown in Fig. 3, the *RA* of PME in peach juice was significantly greater than that in buffer, indicating that the original PME in the juice was less inactivated by HPCD, even if using more intensive HPCD conditions to treat the juices. For instance, the *RA* of PME in carrot juice was

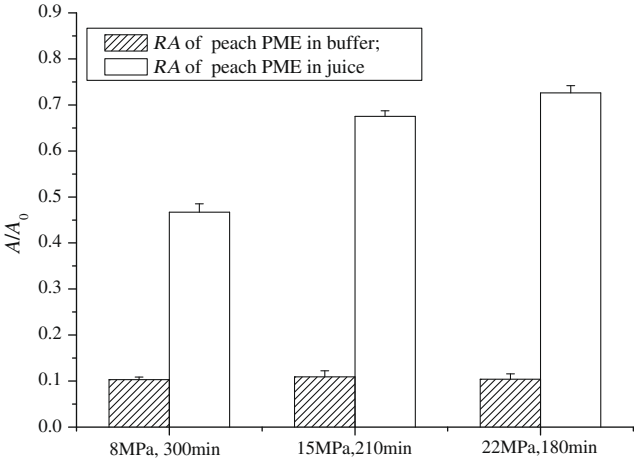

Fig. 3. Comparison of *RA* of peach PME in juice and buffer treated by HPCD.

Table 2
Estimation of inactivation kinetic parameters of carrot PME and peach PME by HPCD at 55 °C using a fractional-conversion model.

PME source	Pressure (MPa)	k (min^{-1})	D (min)	A_∞ (%)	r^2 ($p < 0.05$)
Carrot	8	0.334 ± 0.025	6.89	6.53 ± 1.51	0.992
	12	2.972 ± 0.083	0.78	6.95 ± 0.30	0.999
	15	4.982 ± 0.354	0.46	6.48 ± 0.42	0.998
Peach	8	0.022 ± 0.002	104.68	6.37 ± 3.01	0.963
	15	0.037 ± 0.003	61.74	6.83 ± 2.40	0.980
	22	0.043 ± 0.005	53.56	7.06 ± 3.14	0.969

52.6% at 30 MPa and 55 °C for 60 min, and in peach juice it was more than 90% at 35 MPa and 55 °C for 120 min. These results indicated that the inactivation of two PMEs in buffer was significantly enhanced. This enhancement of inactivating two PMEs in buffer by HPCD could be possibly attributed to the fact that PMEs in the juices were bound to the cell wall and/or to the presence of original stabilizing factors present in plants. As a matter of fact, it was more important to inactivate the original PMEs in juices than in buffer for the juice industry, so the inactivation of the original PMEs in juices exposed to HPCD was in need of further investigation.

3.2. Modelling the inactivation kinetics of PMEs using a fractional-conversion model

The inactivation kinetics for two PMEs by HPCD in 20 mM Tris–HCl buffer at pH 7.5 and 55 °C was well fitted to a fractional-conversion model ($r^2 \geqslant 0.963$), as shown in Figs. 1 and 2, indicating first-order inactivation of an HPCD-labile PME fraction and the occurrence of an HPCD-stable PME fraction; this confirmed the above-mentioned observation in this study. The fractional-conversion model has been used to adequately describe the inactivation kinetics of plant PMEs upon different treatments in previous investigations. The thermal inactivation alone (48–60 °C) and the combination of thermal and high-pressure (10–65 °C, 100–825 MPa) inactivation of purified carrot PME (20 mM Tris–HCl buffer, pH 7.0) both followed the fractional-conversion model (Ly Nguyen et al., 2003b). Polydera, Galanou, Stoforos, and Taoukis (2004) showed that the inactivation of PME in orange juice by high hydrostatic pressure from 100 to 800 MPa at 50 °C could be adequately modelled by the fractional-conversion model. However, a first-order model also well described the inactivation of some enzymes by HPCD. Balaban et al. (1991) showed the inactivation of PME in orange juice by HPCD using a first-order model. The inactivation of polyphenol oxidase in cloudy apple juice exposed to HPCD followed a first-order model (Gui et al., 2007). The inactivation of peroxidase and polyphenol oxidase in red beet extract by HPCD also conformed to a first-order model (Liu et al., 2008). The inactivation kinetics of apple PME by HPCD was adequately described by a two fraction model (Zhi et al., 2008). Therefore, the modelling of the inactivation kinetics of various enzymes by HPCD is very complex, and possibly depends on their origins, enzyme properties, inactivation technologies, and so on.

The kinetic inactivation parameters were estimated using the fraction conversion model and shown in Table 2. The non-zero residual activity, A_∞, obtained from Eq. (8) was 6–7% for the two PMEs after HPCD treatment at 55 °C. Similar results were shown in earlier investigations of PME from different sources and inactivation conditions. An A_∞ of 5–6% for a thermo-stable PME fraction occurred in purified carrot PME exposed to thermal and high-pressure treatments (Ly Nguyen et al., 2002a). Ly Nguyen et al. (2002b) also found the pressure-stable fraction A_∞ of banana PME (Tris buffer, 20 mM, pH 7.0) contributed to 8% of the total activity after high-pressure (600–700 MPa) at 10 °C. Sampedro et al. (2008) reported 7% of the PME initial activity was estimated as the pressure-stable fraction in an orange juice-milk based beverage. Tajchakavit and Ramaswamy (1997) found only a small percentage of activity (A_∞ = 8%) in orange juice was due to the more heat-resistant fraction. These investigations showed that A_∞ of PMEs from different origins and treatments seemed to be less than 10%.

The k, D, Z_P and V_a values were estimated using nonlinear regression analysis (Eqs. (8),(9),(10),(11), respectively). With increasing pressures the k value increased and the D value decreased for the HPCD-labile fractions of the two PMEs. The k and D values of carrot PME were far greater and less, respectively, than those of peach PME, confirming that the HPCD-labile fraction of carrot PME was more susceptible than that of peach PME, which was in agreement with the previous observation in this study.

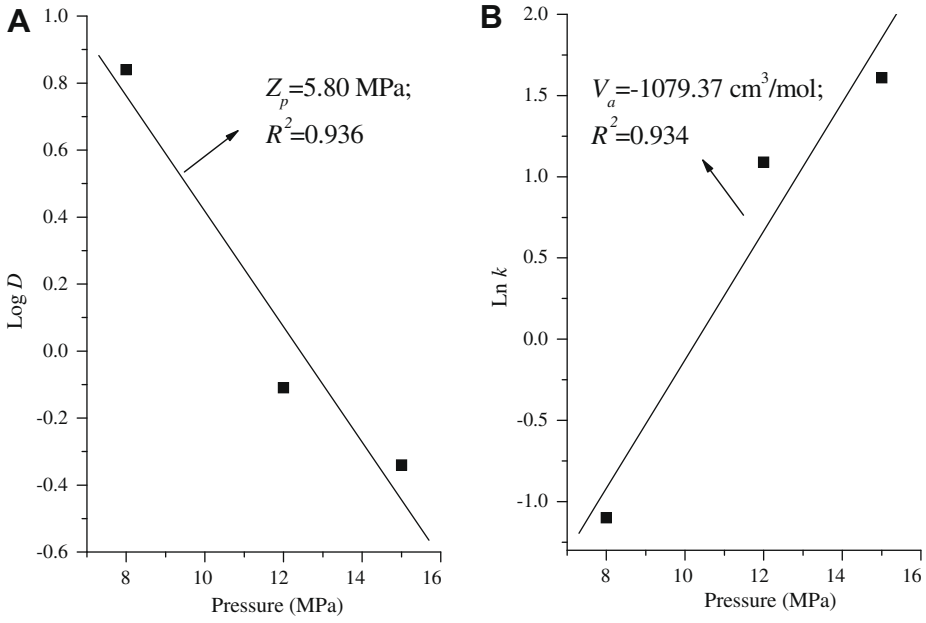

Fig. 4. Z_P value and V_a value for HPCD inactivation of carrot PME at 55 °C.

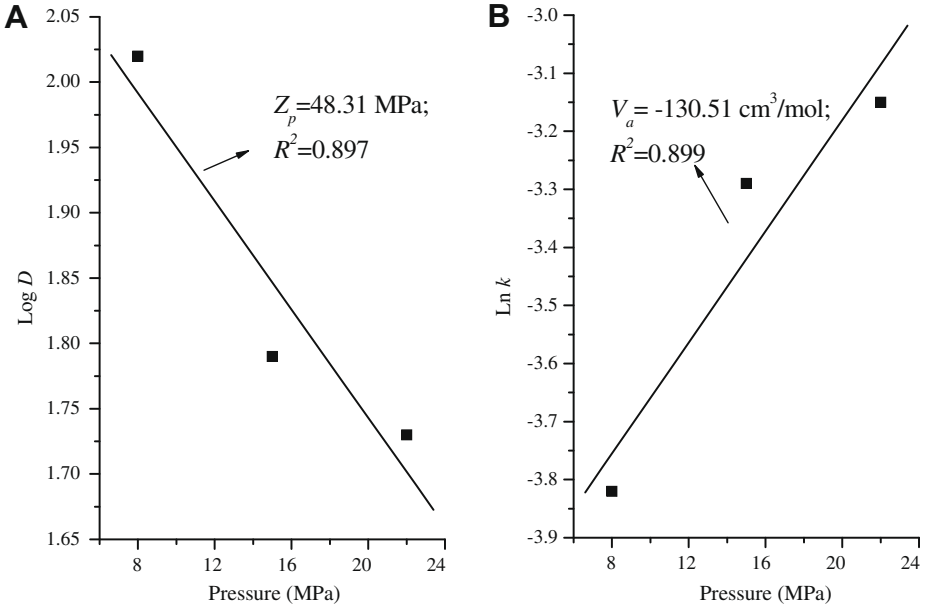

Fig. 5. Z_P value and V_a value for HPCD inactivation of peach PME in at 55 °C.

As shown in Figs. 4 and 5, the V_a and Z_P values of the HPCD-labile fraction were −1079 cm^3/mol and 5.80 MPa for carrot PME, and −131 cm^3/mol and 48.31 MPa for peach PME in buffer upon HPCD treatment, respectively. Zhi et al. (2008) reported the V_a and Z_P of a HPCD-labile fraction of apple PME in buffer at 55 °C were −288.38 cm^3/mol and 21.75 MPa. Liu et al. (2008) showed that the V_a and Z_P values were −35.45 cm^3/mol and 178.57 MPa for peroxidase, and −50.18 cm^3/mol and 125.00 MPa for polyphenol oxidase in red beet extract, following HPCD treatment at 7.5 MPa and 55 °C. Gui et al. (2007) observed that the V_a and Z_P of polyphenol oxidase in cloudy apple juice at 55 °C were −94.3 cm^3/mol and 66.7 MPa.

More data for the V_a and Z_P values of enzymes have been obtained at high hydrostatic pressure. A V_a value of −5.73 cm^3/mol was reported after high-pressure (0.1–500 MPa) treatment of carrot PME in 20 mM Bis–Tris buffer (pH 6.5) at 55 °C (Sila et al., 2007). A V_a value of −25.1 cm^3/mol for PME in orange juice by high hydrostatic pressure from 100 to 800 MPa at 50 °C was obtained using the fractional-conversion model (Polydera et al., 2004). Purified labile grapefruit PME in 20 mM Tris buffer (pH 7) had V_a value of −29.7 cm^3/mol using high-pressure treatment from 500 to 750 MPa at 54 °C (Guiavarc'h et al., 2005). A V_a value for labile fraction of PME in an orange juice-milk based beverage was −59.081 cm^3/mol using 550–700 MPa treatment at 55 °C (Sampedro et al., 2008). The high-pressure inactivation of carrot PME (citrate buffer, 0.1 M, pH 6.0) followed first-order kinetics in the study of Balogh et al. (2004), and a Z_P of 121.33 MPa after 650–800 MPa at 25 °C was obtained. Basak and Ramaswamy (1996) calculated the Z_P values for high-pressure inactivation of orange PME of 200 MPa and 530 MPa at pH 3.7 and 3.2, respectively. The data obtained by HPCD in this study were difficult to compare with those data by high hydrostatic pressure, this was possibly due to the inactivation technologies, the inactivation mechanism and enzyme properties.

4. Conclusions

Carrot PME and peach PME in buffer was effectively inactivated by HPCD, the *RA* values of the two PMEs exhibited a fast decrease and then reached a constant after a prolonged HPCD treatment time, the inactivation kinetics following a fractional-conversion model. As compared to the original PMEs in their juices, the inactivation of the two PMEs in buffer was enhanced under identical HPCD conditions. Thus, the intensification of inactivation of the original PMEs in juices exposed to HPCD is in need of further investigation.

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