Journal of Food Engineering 129 (2014) 47–52

Contents lists available at ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Kinetics of viscosity reduction of pectin solutions using a pectinase formulation at high hydrostatic pressure

B.D. Tomlin [a], S.E. Jones [a], A.A. Teixeira [b], M.J. Correll [b], J.I. Reyes-De-Corcuera [a,*]

[a] University of Florida, Food Science and Human Nutrition, Citrus Research and Education Center, Lake Alfred, FL 33850, United States
[b] University of Florida, Agricultural and Biological Engineering Department, Gainesville, FL 32611, United States

ARTICLE INFO

Article history:
Received 30 May 2013
Received in revised form 30 September 2013
Accepted 3 January 2014
Available online 11 January 2014

Keywords:
Pectinase
High hydrostatic pressure
Enzyme stabilization
Enzyme activation

ABSTRACT

Pectinase formulations from *Aspergillus niger*, are commonly used in fruit juice clarification. The effect of moderate temperature (42.4–62.4 °C) at pressures that stabilize a pectinase formulation (200 and 300 MPa) was studied. The rate of viscosity reduction of pectin solutions increased with temperature with a maximum of 0.0960 ± 0.0108 Pa^{-1} s^{-2} at 62.4 °C and 300 MPa which represents a 2.6-fold increase in rate of viscosity reduction relative to the recommended conditions of 45 °C and 0.1 MPa. Negative apparent activation volumes of −0.22 ± 0.18 to −5.21 ± 0.39 cm^3 mol^{-1} indicate that pressure favored the increase in the rate of viscosity reduction with pressure having the greatest effect at 57.1 °C. Apparent activation energies of 27.7 ± 0.7 to 42.4 ± 11.3 kJ mol^{-1} K^{-1} indicate that temperature had greater effects at high pressure (200–300 MPa) than at atmospheric pressure (15.0 ± 4.4 kJ mol^{-1} K^{-1}).

1. Introduction

Minimally processed products that retain fresh-like quality (i.e. nutrients, antioxidants, color, etc.) are in high demand by some consumers (Deliza et al., 2005). Non-thermal processing such as high hydrostatic pressure (HHP) or mild thermal processes assisted by HHP meet part of the demand for such products. (Barba et al., 2012; Vervoort et al., 2012).

The first commercially HHP processed foods (jams) were produced in Japan in the early 1990s (Demazeau and Rivalain, 2011). Other foods pasteurized by HHP available worldwide now include squid and rice cakes, fruit juices, and oysters and guacamole (Rastogi et al., 2007). Available pressure-treated juices include orange, grapefruit, and apple juice (Patterson et al., 2007).

Most of the work on the effects of HHP on enzyme activity in food processing has focused on inactivating enzymes that are deleterious to food quality such as polyphenoloxidase, lipoxygenase, or pectinmethylesterase. Conversely, for some industrial food processes such as in fruit juice clarification, enzymes are added and maximal activity and stability are desired. A few studies document the stabilization and activation effects that high pressure can have on enzymes. Tomato pectinmethylesterase (PME) was activated at 300 MPa, and carrot PME was stabilized around 500 MPa (Hsu, 2008; Ly-Nguyen et al., 2003). Reports on the stabilization of PMEs and polygalacturonase (PG) with HHP processing are prevalent in the literature due to the improved texture results seen in whole fruit and vegetables with this method. Optimal activity of PME from *Aspergillus aculeatus* PME at moderate HHP and activity retention have been reported (Fraeye et al., 2007).

Ortega et al. (2004) investigated inactivation kinetics at atmospheric pressure for the commercial blends of pectinases. For temperatures of 40–60 °C inactivation of commercial cocktails Pectinex 3XL® (Novozyme), Pectinase CCM (Biocon), and Rapidase® C80 (Gist-Brocades) did not follow first order kinetics. Of the three commercial products studied, Rapidase® C80 was the most heat tolerant. These studies indicate that maximal activity is achieved under thermal inactivating conditions. In a recent study we reported the stabilization of a pectinase formulation (Pectinex 3XL®) by HHP (Tomlin et al., 2013). Although the effects of HHP and temperature on the activity of individual plant pectinases and in some individual microbial pectinases have been reported, to the best of our knowledge, the combined effect of HHP and temperature on the rate of enzyme catalysis of industrial pectinase formulations has not been characterized. In our previous study (Tomlin et al., 2013) we determined the kinetics of inactivation of the pectinase cocktail at selected time–temperature–pressure combinations by measuring residual enzyme activity *ex situ* after depressurization. However, the kinetics of pectinase-catalyzed viscosity reduction at HHP has not been determined for a commercial formulation. Based on our findings with lipase (Eisenmenger and Reyes-De-Corcuera, 2009; Eisenmenger and Reyes-De-Corcuera, 2010), we hypothesize that raising the temperature during high pressure treatments between 200 and 300 MPa, i.e. the pressure

* Corresponding author. Current Address: Department of Food Science and Technology, Food Science Building, 100 Cedar St., Athens, GA 30602-3610, United States. Tel.:+1 706 542 5136; fax: +1 706 542 1050.
E-mail address: jireyes@uga.edu (J.I. Reyes-De-Corcuera).

0260-8774/$ - see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jfoodeng.2014.01.005

range at which the proposed pectinase formulation was the most stable, would result in increased rate of depectination with little loss of activity, thus requiring smaller amounts of enzymes.

Therefore, the objective of this study was to maximize the activity of a commercial pectinase formulation at HHP for optimal depectination while minimizing thermal degradation of the enzyme formulation.

2. Materials and methods

2.1. Materials and equipment

A pectinase formulation (Pectinex 3XL®, Novozymes, Napa, CA) from *Aspergillus niger* purchased from Sigma–Aldrich (Product No. P2736, St. Louis, MO, USA), pectin from citrus fruit and all other reagents were as described in our previous report (Tomlin et al., 2013). The high pressure system was also the same as that detailed in that report with the addition of a third Isotemp 3016D water bath from Fisher Scientific (Pittsburg, PA, USA). One of the three water baths was set at 4 °C for cooling, the second for the selected processing temperature, and the third at 95 °C for enzyme inactivation. A temperature-controlled cone and plate viscometer–rheometer, with a Wells-Brookfield Cone and Plate and CP-40 cone spindle, model LVDV-II+Pro and Rheocalc software from Brookfield Engineering Laboratories, Inc. (Middleboro, MA USA) were used to record viscosity of pectin solutions at 45 °C. The jacketed cup was temperature controlled with a water bath model Isotemp 3016D from Fisher Scientific.

2.2. Methods

2.2.1. Sample preparation and HHP processing

Pectin was diluted to 1.5% (w/v) solution in 0.2 M acetate buffer pH 4.5, and ionic strength of 0.1 M. The pectinase solution was diluted in 0.5 M citrate buffer (pH 4 and ionic strength of 0.844 M) to 0.05% (1.5 unit mL^{-1}) and placed on ice before treatments. Of the untreated enzyme sample, 132 μL were added to 1,800 μL of pectin solution and stirred for 40 s with a miniature magnetic bar and stirrer in an ice bath. The pectinase and pectin solution were placed in 1-mL plastic pouches, heat sealed and placed in the high pressure cell held at 4 °C to minimize enzyme activity prior to setting the desired processing conditions. For each treatment, the HHP cell was closed and a computer program written in LabVIEW 8.5 (National Instruments, Austin, TX) was started exactly 2 min and 30 s after the pectinase had been added to the pectin solution. The constant hold time prior to pressurization and heating was to minimize variation in viscosity reduction that occurred before treatment.

Pressure was raised to the process set point. Then, temperature was raised to the incubation set point and when 90% of the change in temperature was reached, processing time started. After processing, the pressure cell was heated to over 89 °C for 3 min while the cell was depressurized to completely inactivate the enzymes. After the inactivation period, the cell was cooled to 8 °C and the sample was placed on ice before viscosity determination.

2.2.2. Processing conditions

Samples were treated at 0.1 MPa (control) or 200 to 300 MPa at 25 MPa increments, at 42.4, 47.1, 52.1, 57.1, or 62.4 °C and for a processing time of 0–30 min with 7.5 min increments. Temperature increments were chosen for even distribution of T^{-1} for the calculation of activation energy using Arrhenius equation. Come-up and come-down times were accounted for as follows. Ramp-up time included pressurizing and heating the cell to 90% of set point temperature. Ramp-down time included inactivation, depressurization, and cooling of the cell. Fig. 1 shows the pressure and temperature profile for a sample treated at 62.4 °C and 250 MPa for 15 min. Treatments were done in duplicate. The study was performed in a randomized block design, with temperature blocks. Pressure and process times were applied in random order.

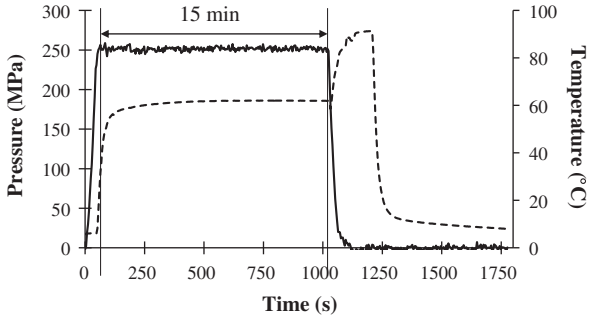

Fig. 1. Pressure (——) and temperature (- - -) profiles in high pressure cell for a 2-mL aliquot of a 1.5% (w/v) pectin, 0.05% (v/v) pectinase solution in 0.2 M acetate buffer pH 4.5 treated at 62.4 °C, 250 MPa, 15 min.

2.2.3. Activity measurements

To assess the activity of the enzyme cocktail, after treatment, each pouch with pectinase formulation and pectin solution were placed in a water bath to raise the sample temperature to 20 °C. An aliquot of 0.5 mL was pipetted into the rheometer cell and viscosity was recorded every 1.2 s for 5 min with a maximal viscometer rotational speed of 20 rpm. The average of the last 50 viscosity measurements was recorded. The viscosity of samples that contained pectin only or pectin with only citrate buffer was determined for 10 and 5 min respectively as controls. The extent of viscosity reduction at HHP was determined after processing at selected temperatures followed by enzyme inactivation. Full enzyme inactivation was verified by a constant viscosity of the reaction mixture after treatment. The rate of viscosity reduction at high pressure was determined as a percent viscosity reduction with respect to the viscosity at time 0; that is, accounting for come-up and come-down times. Rate constants were determined using a pseudo-second order rate as reported earlier (Tomlin et al., 2013). Error in the determination of the rate constant was reported using the standard error of the slope from the linear regression from the plot of the reciprocal of percent viscosity reduction *vs.* process time for each of the selected pressures and temperatures. Viscosity reduction has also been used to characterize total pectinase activity of commercial products Pectinex®Clear (PC) and Pectinex®BE Colour (PB) and extracts from *A. niger* and *Aspergillus oryzae* for the clarification of apple, butia palm, grape and blueberry juice (Sandri et al., 2011). Viscosity reduction was used for total pectinase activity with one unit of activity stated the necessary amount of enzyme required to reduce the viscosity by 50% (Sandri et al., 2011). A lower optimal temperature of 40 °C for commercial products was observed than the stated manufacturer optima of 50 °C for PC and 54 °C for PB (though manufacturer's processing conditions were not known to researchers). Arrhenius and Eyring equations (Eqs. (1) and (2) respectively) were used to determine the apparent activation energies and activation volumes of viscosity reduction respectively.

$$\ln(k) = -\frac{E_a}{R}\frac{1}{T} + \ln(k_{T_0}) \tag{1}$$

where k is the rate constant, E_a is the activation energy, T is the absolute temperature, R is the ideal gas constant, and k_{T_0} is the rate constant at a reference absolute temperature T_0.

$$\ln(k) = -\frac{\Delta V^{\neq}}{RT}\cdot P + \ln(k_{P_0}) \tag{2}$$