Journal of Food Engineering 116 (2013) 674–680

Contents lists available at SciVerse ScienceDirect

Journal of Food Engineering

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

High hydrostatic pressure protection of a pectinase cocktail against thermal inactivation

B.D. Tomlin, S.E. Jones, J.I. Reyes-De-Corcuera *

University of Florida, Citrus Research and Education Center, Lake Alfred, FL 33850, United States

ARTICLE INFO

Article history:
Received 17 April 2012
Received in revised form 23 December 2012
Accepted 18 January 2013
Available online 27 January 2013

Keywords:
Pectinase
High hydrostatic pressure
Thermal inactivation
Enzyme stabilization

ABSTRACT

Pectinase cocktails, containing pectinases, hemicellulases, and cellulases are used in the production of commercial apple juice to reduce juice viscosity, increase yield, and to clarify the final product. The kinetics of inactivation of a commercial pectinase formulation was studied at 0.1–400 MPa and 55.0–85.0 °C. High hydrostatic pressure slowed the rate of inactivation of the pectinase cocktail treated at inactivating temperature conditions by up to 19-fold at 77.0 °C, 350 MPa compared to inactivation at atmospheric pressure at the same temperature. Apparent activation energies of enzyme inactivation at 200–400 MPa were lower (107.3–154.4 kJ mol^{-1}) than at 0.1 MPa (195.6 kJ mol^{-1}).

1. Introduction

Consumers continue to demand more fresh-like products that are minimally processed and that contain little to no artificial stabilizers (Deliza et al., 2005). Fruit juice consumption in the US has decreased in the past several years (Pollack and Perez, 2008). However, in the US apple juice consumption has experienced a slow but sustained increase. In 2007–2008, over 535,000 metric tons of concentrated apple juice were consumed (USFAS, 2008). Americans drank an average of 8.34 L of apple juice (single strength equivalent) per capita in 2006/07, while consuming only 3.79 L of all other non-citrus juices such as grape, pineapple, cranberry and prune juice (Pollack and Perez, 2008). Consistent high quality products are more likely to see increased popularity among juice drinkers and greater economic profits over competitors. Improved processing techniques leading to a fresher tasting product can help boost consumers' perception of processed fruit juice.

Juice clarification is a current area of study due to its importance in the juice processing. Juice clarification reduces viscosity and cloudy haze for ease of downstream processing and visual desirability. However, the clarification step is a time-consuming phase with the added operating costs associated to heating and the addition of enzymes. Current research focuses on improving clarification by decreasing clarification times and reducing processing costs.

Polysaccharides (pectins, starches, and gums), proteins, polyphenols, polyvalent cations, and lipids cause haze formation and increase juice viscosity. Pectin is the leading cause of the juice haze and it is specific to the middle lamella of fruit, the section between cells walls (Jayani et al., 2005). Apple pectins are large multifaceted molecules that are highly methoxylated (Lea, 1990). Though pectin is not a uniform substance, the core chain of molecules is made up of anhydrogalacturonic acid (Jayani et al., 2005).

Juice clarification is performed after the apples are pressed and before filtration, centrifugation, fining, and evaporation. The haze-causing particles are commonly removed by addition of enzymes that break down pectin and suspended insoluble particles allowing the particles to settle. Apple juice contains highly methylated pectin, which must be taken into account when selecting a suitable blend of enzymes for clarification. Enzymes are used during fruit mashing and clarification to decrease viscosity and improve juice yield (Binning and Possmann, 1993). There are several classes of enzymes used as blends in the apple juice clarification process, including pectinases, lyases or trans-eliminases, protopectinases, cellulases and proteases among others. Pectinases and their catalytic activities have been described in detail (Alkorta et al., 1998; Jayani et al., 2005; Pedrolli et al., 2009). Table 1 summarizes the optimal conditions of pectinases found in formulations for juice clarification. Polygalacturonase and PL from Pectinex 3XL®, a commercial formulation, had optimal pH of 4.7 and 5.0–6.5 respectively and optimal temperature of 50 °C and 35 °C respectively (Ortega et al., 2004a,b).

Industrial clarification is carried out at around 15 °C or at 55.0 °C (Lea, 1990). Clarification at low temperature minimizes microbial growth but requires long processing times of at least 8 h and, sometimes, large amounts of enzymes. Clarification at 55.0 °C is shorter (2 h) but requires heat and some of the enzymes

* Corresponding author.
E-mail address: jireyes@ufl.edu (J.I. Reyes-De-Corcuera).

0260-8774/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jfoodeng.2013.01.018

Table 1
Optimal catalytic conditions for pectinases used in formulations for juice clarification.

Enzyme	Reaction	Optimal pH	Optimal temp. (°C)
Pectinmethylesterase (PME) EC 3.1.1.11	Demethoxylation of pectin	4–8	40–50
Polygalacturonase (PG) endo-PG, EC 3.2.1.15 exo-PG, EC 3.2.1.67	Hydrolysis of α-1-4-glycosidic bond of pectin	3.5–5.5	30–50
Pectin lyase (PL) endo-PL, EC 4.2.2.2 exo-PL, EC 4.2.2.9	Cleave the α-1-4-glycosidic bond by trans-elimination	5–6.5	35–70

in the cocktail denature at that temperature. Therefore, if clarification is to be done under denaturing conditions, thermally labile enzymes need to be added in excess.

High pressure processing as a non-thermal alternative to juice pasteurization or as a pressure-assisted thermal treatment offers improved benefits to thermal processing by increasing the retention of juice nutrients, antioxidants, color, and clarity after pasteurization. Although much of the HHP research has focused on inactivation of microorganisms and enzymes such as PME (Basak and Ramaswamy, 1996, 2001; Basak et al., 2001), some reported stabilization and activation effects on enzymes (Eisenmenger and Reyes-De-Corcuera, 2009; Eisenmenger and Reyes-De-Corcuera, 2010; Vila-Real et al., 2010). Tomato PME was activated at 300 MPa, and carrot PME was stabilized at around 500 MPa (Hsu, 2008; Ly-Nguyen et al., 2003). Optima at 200 MPa and 45 °C or at 300 MPa and 50 °C were reported for *Aspergillus aculeatus* PME activity at pH 4.5. At 0.1 MPa, the activity decreased with the increase in temperature above the optimal 45 °C (Fraeye et al., 2007). However, inactivation kinetics varied for enzyme source. Apple, tomato, and banana PME followed first order, while strawberry and carrot PME demonstrated a biphasic thermal inactivation (Duvetter et al., 2005). Despite the increasing efforts in characterizing the stabilization and activation effects of HHP on enzymes (Eisenmenger and Reyes-De-Corcuera, 2009) to the best of our knowledge there is no industrial HHP-assisted enzymatic process. The economic viability of using HHP depends on the extent of stabilization and activation of the enzymes, and, hence, on the capital and operating costs associated with the implementation of this technology.

The central hypothesis of this research is that HHP stabilizes pectinases in a commercial formulation allowing for the increase in temperature and the rate of juice clarification, which results in shorter processing times and more fresh-like product quality, or lower enzyme addition. The objective of this study was to assess the effects of HHP on the stability of a commercial pectinase formulation at temperatures above levels customarily used for clarification as a first step towards determining the viability of combined thermal and HHP processing for fruit juice clarification.

2. Materials and methods

2.1. Materials

A pectinase formulation (Pectinex 3XL®, Novozymes, Napa, CA) from *Aspergillus niger* was purchased from Sigma–Aldrich (Product No. P2736, St. Louis, MO, USA). The commercial cocktail of enzymes contains pectintranseliminase, PG, PME, and smaller portions of PL, cellulases and hemicellulases. Sodium Citrate and Citric Acid were both obtained from Fisher Scientific (Pittsburg, PA, USA). Pectin (Product No. P-9135) from citrus fruit was obtained from Sigma–Aldrich (St. Louis, MO, USA) and dissolved in ultra-filtered water to make a 1.5% (w/v) stock solution.

2.2. Equipment

All high pressure equipment was from Unipress Equipment (Warsaw, Poland). Samples were submerged and treated in a high hydrostatic pressure cell model U111 with silicone oil serving as the pressure medium. The high pressure cell was pressurized with a micropump model MP5 and controller. The high pressure chamber was jacketed to control temperature with alternating Isotemp 3016D water baths, from Fisher Scientific (Pittsburg, PA, USA) and pinch valves described previously (Eisenmenger and Reyes-De-Corcuera, 2009). One water bath was set at 10 °C and the other at the desired process temperature. A computer program written in LabVIEW 8.5 and data acquisition board model DAQ Card 6062E from National Instruments (Austin, TX, USA) were used to control and record pressure, temperatures and processing time. 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 enzymatic viscosity reduction of pectin solutions at 45 °C. The jacketed cup was temperature controlled with a water bath model Isotemp 3016D from Fisher Scientific.

2.3. Methods

2.3.1. Sample preparation and HHP processing

The pectinase solution was diluted in citrate buffer to 0.5% w/v (15 unit mL^{-1}) and aliquots were placed in 1-mL plastic pouches, heat sealed, and placed on ice until treated. Enzyme solutions were made with 0.1 M citrate buffer at pH 3.5. Buffers and the enzyme cocktail were held separately at 4 °C before use, and placed on ice when enzymes were added to citrate buffer. For treatment, a pectinase aliquot was placed in the high pressure cell, held at 10 °C, and the pressure cell was closed. Pressure was raised to the process set point. Then, temperature was raised to the incubation set point. When 90% of the change in temperature was reached, processing time started. After processing, the pressure cell was cooled back to 13 °C, depressurized, and then the sample was placed on ice. To determine 100% residual activity for a processing

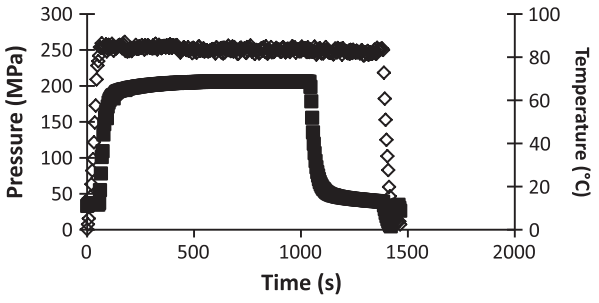

Fig. 1. Pressure (◇) and temperature (■) profile in high pressure cell for sample treated at 69.3 °C, 250 MPa, 15 min.