



Effect of high pressure carbon dioxide on the properties of water soluble pectin in peach juice



Linyan Zhou, Yongtao Wang, Fengxia Liu, Xiufang Bi, Xiaojun Liao*

College of Food Science and Nutritional Engineering, China Agricultural University, China Engineering Research Center for Fruit & Vegetable Processing, Beijing, 100083, China

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ABSTRACT

To better understand the pectin and its role in precipitation formation in peach juice induced by high pressure carbon dioxide (HPCD) in a previous study, demethoxylation, molecular weight distribution (MWD), ζ -potential, rheological properties and nanostructure of water soluble pectin (WSP) from peach juice treated by combining HPCD with pectin methylesterase (PME) were studied. The MWD, ζ -potential and nanostructure of WSP were not changed, whereas demethoxylation reaction was stimulated by HPCD. Significant decreases in degree of methoxylation (DM) and apparent viscosity of WSP were obtained with PME action after moderate heat treatment (MHT) at 55 °C, and the nanostructure image of WSP exhibited more aggregation than control and HPCD alone. PME in buffer system was susceptible to HPCD, whereas it in juice was difficult to be inactivated by HPCD. It was deduced that the properties and structure of pectin could be significantly changed with PME action in peach juice after HPCD. The apparent viscosity of WSP in peach juice could be decreased due to demethoxylation reaction catalyzed by PME in HPCD-treated juice, resulting in the aggregation of WSP, and finally contributing to accelerate the formation of precipitation in peach juice.

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1. Introduction

In the juice industry, thermal treatment is the most common and least expensive technology for pasteurization or sterilization (de Assis, Lima, & deF Oliveira, 2001), unfortunately this easily leads to degradation of some product qualities as well. Moreover, as consumers are demanding minimally processed and fresh-like 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; Liao, Hu, Liao, Chen, & Wu, 2007). HPCD was applied to cloudy peach juice processing in our previous study, and an acceleration of the precipitation formation was observed (Zhou, Zhang, Leng, Liao, & Hu, 2010). In the cloudy juice products, the cloudy mass related to the flavor, turbidity and color of the juice provides a significant quality attribute in processing. This mass can be caused by a colloidal suspension where the continuous medium refers to a solution of pectin, sugars and

organic acids etc, and the dispersed matter is mainly formed by the cellular tissue comminuted during fruit processing (Fillipi, Genovese, & Lozano, 2008). Soluble pectin dispersed in juice carries negative charges, play a crucial role to keep juice with a high ζ -potential for strong electrostatic force. Mostly, in view of structure and functional properties, pectin is regarded to be the most interesting cell wall polymer because of its abundance, solubility and sensitivity to chemical reactions (Roeck, Sila, Duvvetter, Loey, & Hendrickx, 2008).

Pectic polysaccharides, a group of complex structural polymers of the plant cell wall, are critical in many quality-related aspects of fruits/vegetables and other plant-based foods (Sila et al., 2009). One of the main structural components of pectin is homogalacturonan, a linear chain of α (1, 4)-linked galacturonic acid residues which can be methoxylated (Roeck et al., 2008). Pectin tends to subject to enzymatic and non-enzymatic changes during ripening, storage and processing (Sila et al., 2009). During thermal processing, pectin was subject to depolymerization reactions (Roeck et al., 2008). Depending on pH and degree of methoxylation (DM), β -elimination and/or acid hydrolysis may occur. Chemical demethoxylation can occur at a wide range of pH, generating pectin with a lower DM. In addition, a wide range of endogenous and exogenous enzymes can synergistically modify and degrade pectin smooth and hairy

* Corresponding author. Fax: +86 10 62737434.

E-mail address: liaoxtjun@hotmail.com (X. Liao).

regions. Pectin methyltransferase (PME) hydrolyzed methyl esters from O6 of GalA in HG releasing methanol and H_3O^+ , creating contiguous carboxylic acid groups that are dependent on type and source of PME and conditions of de-esterification (Kim & Wicker, 2009), which can cross-link with the polyvalent cations, such as Ca^{2+} , to form insoluble pectate precipitates (Guiavarc'h, Segovia, Hendrickx, & Van Loey, 2005). When combining high temperature with high pressure, β -elimination was retarded or even stopped, whereas demethoxylation was stimulated (Roeck et al., 2009). Although no change of water soluble pectin (WSP) content was found in the HPCD treated peach juice in previous study, the properties and structure alterations of pectin may change and contribute to the precipitation formation (Zhou et al., 2010). About 30 mg/L of calcium content and high PME activity in peach juice were determined, and it could be deduced that demethoxylation reaction may occur (Zhou et al., 2010). Therefore, to better understand the WSP and its role in precipitation formation induced by HPCD, the focus of the present research was to investigate the change of WSP properties and structures during HPCD.

2. Materials and methods

2.1. Materials

Peaches (Cultivar No. 24 Beijing) were purchased from Beijing Guangyuan Yanwei Agricultural Science and Technology Co., Ltd., belonging to the practice base of China Agricultural University. Apple pectin (DE 70–75%) was obtained from Andre Co. (Shandong, China). Alcohol oxidase from *P. pastoris*, 2, 4-Pentanedione, cyanoacetamide, D-(+)-Galacturonic acid, polygalacturonic acid, monogalacturonic acid, and 3-Hydroxybiphenyl were obtained from Sigma–Aldrich Chemical Co. (Beijing, China). All other chemicals were of analytical grade.

2.2. Isolation of water soluble pectin (WSP) from peach

Alcohol-insoluble residue (AIR) of peach was obtained as described by Roeck et al. (2008) with some modification. 25 g of peach was completely homogenized in 120 mL of 95% ethanol using a mixer (Sanyang mixer, China). After boiling for 20 min, the insoluble solids were collected on a Buchner funnel. The final extract was sequentially washed with the boiling 95% ethanol, a mixture of chloroform and methanol (V:V = 1:1), and acetone, followed by drying overnight under vacuum at 35 °C.

The AIR (0.5 g) was suspended in 60 mL of distilled water and stirred at 40 °C for 30 min (Roeck et al., 2008). After centrifugation at 15,000 × g for 25 min the cake was resuspended in distilled water (60 mL), extracted at 40 °C for 1 h under stirring and centrifuged again. The combined supernatants were dialyzed exhaustively against distilled water for 24 h. The WSP extract was then freeze-dried.

2.3. PME Preparation

The extraction of PME from peach was performed according to the method proposed by Ly Nguyen with a little modification (Ly Nguyen et al., 2003). The pomace of peach juice was obtained according to the method of Zhou et al. (2010), then it 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). The mixture was filtrated by using two layers of cheese cloth and the filtrate was partially purified by ammonium sulfate precipitation at 30%–80% saturation. The precipitate containing PME was dissolved in a minimum volume of 20 mM Tris-chloride buffer (pH 7.5) and dialyzed against the same buffer overnight with three changes of buffer. The crude PME were quickly

frozen with liquid nitrogen and stored at –18 °C until used. All procedures were conducted at 4 °C.

2.4. PME Assay

PME activity was determined by measuring the release of acid as a function of time at pH 7.5 and 30 °C according to the method described by Sampedro, Rodrigo, and Hendrickx (2008) with some modifications. The reaction mixture consisted of 0.5 mL peach PME solution and 60 mL 1% apple pectin solution (DE 75%) containing 0.1 M NaCl. Before injection, the pectin solution was adjusted to pH 7.5. During hydrolysis at 30 °C, pH was maintained at 7.5 by adding 0.01 N NaOH using an automatic pH-stat titrator (Metrohm 842, Switzerland). The consumption of NaOH was recorded for a 15 min reaction period. The PME activity unit (U) was defined as the micromoles of acid produced per minute at pH 7.5 and 30 °C.

2.5. Peach pectin solution preparation

Two pectin solution A and B were prepared for experiment. 0.5% of pectin solution was prepared by dissolving pectin powder into deionized water with stirring overnight at room temperature. Pectin solution A was composed of 1.5 mL 0.5% peach WSP solution and 50 μL 20 mM Tris-chloride buffer as a blank; Pectin solution B was composed of 1.5 mL 0.5% peach WSP solution and 50 μL PME solution containing approximately 0.25 units of PME activity. PME solution and enzyme buffer were added to WSP solution just before HPCD and heat treatment.

2.6. HPCD Process system

The diagram of the HPCD system was described by Liao et al. (2007). The stainless steel pressure vessel with a volume of 850 mL was designed to withstand a pressure of 50 MPa. Commercially available CO_2 of 99.5% purity was purchased from Beijing Jingcheng Co. (Beijing, China), and was passed through an active carbon filter before entering the pressure vessel.

2.7. Processing of peach pectin solution using HPCD system

Pectin solution was filled in a 15 mL plastic tube (Beijing Bomex Company, Beijing, China) without cap and then was enclosed in the HPCD vessel already equilibrated at experimental temperature. For each experiment, the HPCD vessel containing two pectin solution A and two pectin solution B was pressurized by the plunger pump to a required level. The treatment was performed by HPCD, combined a moderate temperature of 55 °C for varied times. After pressure release, pectin solution samples were immediately placed in boiling water for 5 min in order to inactivate PME and cooled in an ice bath. For each experiment, tubes containing pectin solution and enzyme buffer (blanks) underwent the same treatment simultaneously, in order to investigate chemical, and non-enzymatic de-esterification of pectin.

The treatment parameters applied in this study were as follows: the pressure was 30 MPa, the temperature was 55 °C, and the treatment time was 15, 30, 45, and 60 min. The parameters of pressure and time were selected based on Liao et al. (2007) study, were effective in inactivating microbes.

2.8. Moderate heat treatment (MHT)

Pectin solution was filled in a 15 mL plastic tube (Beijing Bomex Company, Beijing, China) without cap and then was enclosed in the HPCD vessel already equilibrated at experimental temperature 55 °C (MHT). For each experiment, two pectin solution B were

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