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Effects of high pressure processing on activity and structure of soluble acid invertase in mango pulp, crude extract, purified form and model systems

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ABSTRACT

The effects of high pressure processing (HPP) on the activity of soluble acid invertase (SAI) in mango pulp, crude extract, purified SAI and purified SAI in model systems (pectin, bovine serum albumin (BSA), sugars and pH 3–7) were investigated. The activity of SAI in mango pulp was increased after HPP, and that in crude extract stayed unchanged. The activity of purified SAI was decreased after HPP at 45 and 50 °C. Pectin exhibited a concentration-dependent protection for purified SAI against HPP at 50 °C/600 MPa for 30 min. Pectin that had an esterification degree (DE) of 85% exhibited a greater protection than pectin that had a DE of 20–34%. BSA, acidic pH (3–6) and sucrose also exhibited protection for purified SAI against HPP. HPP at 50 °C/600 MPa for 30 min disrupted the secondary structure and tertiary structure of purified SAI, but no aggregation of purified SAI was observed after HPP.

1. Introduction

High pressure processing (HPP) is a non-thermal food processing technology. HPP at pressures from 400 to 600 MPa can effectively kill the cells of microorganisms. However, enzymes in foods, such as polyphenol oxidase (PPO), pectin methylesterase (PME) and acid invertase, exhibit high pressure resistance (Liu, Li, Wang, Bi, & Liao, 2014; Liu et al., 2013; Weemaes, Ludikhuyze, Van Den Broeck, & Hendrickx, 1998). The activity of PPO in strawberry puree was reduced by 6% after treatment at 690 MPa/57 °C for 10 min (Terefe, Yang, Knoerzer, Buckow, & Versteeg, 2010). The activity of pepper PME was not decreased after 15 min at 500 MPa/25–60 °C (Castro, Van Loey, Saraiva, Smout, & Hendrickx, 2005).

The pressure stability of an enzyme/protein is strongly dependent on the type of enzyme/protein, pH, medium composition and temperature, among other conditions. Mushroom PPO at pH 6.5 has a higher pressure stability at 750–800 MPa/25 °C than at pH 4 (Weemaes et al., 1997). Tangwongchai, Ledward, and Ames (2000) observed that purified PME from potato was partially inactivated at pressures above 200 MPa, but PME in the whole cherry tomatoes showed no significant inactivation after treatment at 600 MPa. It was also reported that carrageenan, dextran sulphate, polyols and sucrose could reduce the pressure-induced denaturation and aggregation of proteins (Dumay, Kalichevsky, & Cheftel, 1994; Galazka, Dickinson, & Ledward, 1999). However, there are few reports on the influences of pectin, protein and sugars on the pressure stability of enzymes.

Invertase is an enzyme that is widely distributed among plants and microorganisms and that catalyses the hydrolysis of disaccharide sucrose into glucose and fructose. Plants possess three types of invertase isoenzymes. Based on their solubility, subcellular localization, pH-optima and isoelectric point, three different types of invertase isoenzymes can be distinguished: vacuolar, cell wall bound and neutral invertases (Pan, Zou, Peng, Wang, & Zhang, 2005). Vacuolar invertase is also labelled as soluble acidic invertase (SAI), and localized in the vacuole. Cell wall invertase (CWI) is bound to the cell wall via positive charges. Vacuolar and cell wall bound invertases are characterized by an acidic pH optimum (pH 4.5–5.0), and neutral invertase is also known as an alkaline or

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cytoplasmic invertase because of its optimal pH 6.8–8.0 and subcellular localization (Sturm, 1999).

SAI in mango fruit is highly resistant to high pressure; it was not inactivated after HPP at 600 MPa for 1 min, and its residual activity induced the degradation of sucrose in mango nectar (pH 3.95) (Liu et al., 2014). However, there are few reports on the effects of high pressure on the activity and structure of purified SAI from plants in different mediums (Cavaille & Combes, 1995).

Ripe mango fruit is rich in pectin (around 0.8%), and its content of sugars and protein is approximatively 12% and 0.6%, respectively (Liu et al., 2013). The pH value of ripe mango fruit is 3.40–4.80 (Grahn, 1984). These factors may contribute to the pressure resistance of SAI. In a preliminary study, SAI in the crude extract after ammonium sulphate fractionation precipitation and dialysis also exhibited high pressure resistance. Protein in the crude extract may protect SAI against HPP inactivation. In this study, the activities of the SAI in mango pulp, in the crude extract and in the purified form after HPP at 50–600 MPa and 20–50 °C for 10 min were compared. Effects of HPP (600 MPa/50 °C/30 min) on the activity of SAI in the model systems containing pectin, bovine serum albumin (BSA), sugars (sucrose and trehalose) and with acidic pH values were also investigated to explore the protective effect of different factors for SAI. The secondary structure, tertiary structure of the SAI and its particle size distribution (PSD) after HPP were also examined in this study.

2. Materials and methods

2.1. Chemicals and Materials

Mango fruits (Tainong No.1) were harvested at mature-green from the orchard at Sanya, Hainan province in southern China. Ripeness was also ascertained by conventional indices such as the ripening period in terms of days after harvesting (7–15 days). The total soluble solids, pH and titratable acidity were 15.9 °Brix, 3.95 and 0.76%, respectively (Liu et al., 2013). Further characterization of the mango fruits, including their aroma, color, firmness, and enzyme profiles, was previously reported by Liu et al. (2013). Ripe mangoes were washed and peeled, and the mango flesh was sliced and mashed with a blender (Joyong Electric Appliance Co., Shandong, China) to yield pulp. The pulp was frozen immediately after mashing, and was stored at −20 °C for further use. DEAE-Sepharose Fast Flow (GE healthcare, USA) was purchased from Beijing Biodee Biotechnology Co., Ltd (Beijing, China). Tris base, low esterified degree pectin (20–34%, P9311), high esterified degree pectin (≥85%, P9561), and bovine serum albumin (BSA, A2153) were purchased from Sigma–Aldrich Co. (Shanghai, China). Other chemicals were obtained from China National Pharmaceutical Group Corporation (Beijing, China). All chemicals were of guaranteed reagents. The water used in this study was pure water (18.2 MΩ·cm) produced by the PURELAB® Ultra water system (Elga, UK).

2.2. Purification of soluble acid invertase

The purification of SAI from mango was carried out at 4 °C, according to the method of (Pan et al., 2005) with modifications. One kilogram of mashed mango pulp and ten grams of polyvinylpyrrolidone were mixed in 2 L of buffer A (pH 7.1, 50 mM Tris-HCl, containing 2 mM EDTA-Na_2, 1 mM benzamidine-HCl, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride), and were homogenized with a high speed homogenizer (JHBE-50T, Jinding Co., Henan, China) at 30,000 r/min for 10 min. The mixing and homogenizing were carried out in an ice bath. After that, the homogenates stood at 4 °C for 2 h. Then, they were centrifuged at 13,000*g* for 20 min at 4 °C in a CR21GIII centrifuge (Hitachi Koki Co., Tokyo, Japan) and the supernatant was collected as an enzyme solution. Ammonium sulphate was gradually added (in 4 h) to the enzyme solution to 85% saturation (fixed concentration), and the protein was allowed to precipitate overnight at 0 °C. Protein precipitate was harvested by centrifugation at 13,000*g* for 20 min at 4 °C and was dissolved in 400 mL of buffer A. The protein solution was dialysed against 5 L of buffer A (1 change) for 24 h with four changes at 4 °C, and it was used as the crude extract. After dialysis, it was loaded onto a DEAE Sepharose Fast Flow column (3.5 cm × 28 cm, pre-equilibrated with buffer A) and was purified, using the LP protein purification system (Bio-Rad, USA). After loading, the column was washed with 10 column volumes of buffer A at a flow rate of 4 mL/min to elute the unbound proteins, and the wash was followed by elution of the SAI with 20 column volumes of buffer A with a linear gradient NaCl 0–0.27 mol/L at the same flow rate. Fractions of 18 mL with high activity of the SAI were collected and concentrated using a centrifugal filter device 50 kDa (Amicon Ultra-15, Millipore, USA). After concentration, the samples of enzyme solution were immediately frozen in liquid nitrogen and stored at −80 °C.

2.3. Preparation of samples

Before HPP treatment, the mango pulp was homogenized with a high-speed homogenizer (FJ150, Biaoma, shanghai) at 30,000 r/min for 3 min. After that, 5 g of homogenized mango pulp was packed in a polyethylene bag (5 cm × 5 cm) with a vacuum-packing machine (Rishang Co., Ltd., Beijing, China).

The crude extract and purified SAI in solution (500 μL) was packed respectively in small polyethylene bags (1 cm × 5 cm) with the vacuum-packing machine. To avoid the influence of the heat generated by the heat sealing process, the polyethylene bag (5 cm in length and 1 cm in width) was submerged in ice water throughout the whole sealing procedure. Samples were kept in ice water and were treated by HPP within 2 h.

The concentration of pectin, protein and sugars in the mango pulp was approximately 0.8, 0.6 and 12%, respectively (Liu et al., 2013). The pH value of the mango fruits used in this study was 3.95 (Liu et al., 2013). Therefore, the influence of pectin, protein, and sugars on the inactivation of the activity of SAI by HPP were carried out with pectin (0.005–0.8%), BSA (0.006–0.6%) and sugars (0.6–12%), respectively. To study the effects of HPP on the activity of purified SAI at specific pH values, the enzyme solution was dialysed for 3 times in citrate phosphate buffers (100 mM with pH 3, 4, 6 and 7) using a 50 kDa centrifugal filter device (Amicon Ultra-15, Millipore, USA).

For the samples of circular dichroism (CD) and fluorescence spectroscopy analysis, buffer A in the samples was replaced with phosphate-buffer (pH 7, 10 mM, K_2HPO_4-KH_2PO_4, using O_2 excluded pure water) by dialysis using a centrifugal filter device 50 kDa (Amicon Ultra-15, Millipore, USA) for 3 times (Greenfield, 2007). After that, pectin was added at different levels. For the samples with pH 6 and 7, buffer A was replaced with phosphate-buffers (pH 6 and 7, 10 mM, K_2HPO_4-KH_2PO_4, using O_2 excluded pure water). For samples at pH 4, buffer A was replaced with phosphate-buffer (pH 4, 10 mM, H_3PO_4-KH_2PO_4, using O_2 excluded pure water).

2.4. HPP treatments

HPP treatments were conducted using a 5.0 L HPP unit (HHP 700, Baotou Kefa Co., Ltd., Inner Mongolia, China) according to (Liu et al., 2013). Distilled water was used as the pressure-transmitting fluid. The pressurization rate was approximately 200 MPa/min and the depressurization time was less than 3 s. The high-pressure vessel was wrapped with electrically heating