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Research note

Recovery of genipin from genipap fruit by high pressure processing

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ABSTRACT

High hydrostatic pressure (130–530 MPa) under quasi isobaric non-isothermal conditions was used for genipin recovery. Treatments were carried out on fresh made genipap fruit purees for 15 min with pectinases (0, 4.6 and 9.3 mg enzymes/g genipap). Liquid phase was subjected to pH and volume measurements, and wet weight of the solid phase was recorded. Genipin yield and protein concentration were determined. The highest genipin recovery (34.0 ± 1.5 mg/g) in the extract was found after treatment at 130 MPa without pectinases. Liberated liquid volume, wet solid weight and pH results were in line with reported data on the influence of pressure on pectinesterase and polygalacturonase.

1. Introduction

Genipa americana L. is a plant native to tropical Central and South America (Ueda, Iwahashi, & Iokuda, 1991). Genipap is used in beverage production, and the flesh is added as a substitute for commercial pectin (Fernandes & Rodrigues, 2012). Genipin is an iridoid that can be found in genipap fruit (Ono, Ueno, Masuoka, Ikeda, & Nohara, 2005) and it has been used as a traditional herbal medicine and as a natural blue colorant in food and fabric industries via its reaction with primary amino groups of natural or synthetic molecules (Park, Lee, Kim, Hahn, & Paik, 2002). Genipin has been used as a cross-linking agent of biopolymers such as proteins or chitosan (Muzzarelli, 2009). Genipin has been obtained from genipap fruit via organic solvent extraction (Djerassi, Gray, & Kincl, 1960), including rather complex purification steps. There is a need to use green techniques for genipin recovery, which avoid the solvent residue and the use of polluting solvents and reduce the recovery time. The recovery of genipin from genipap fruit in aqueous solution is a critical technological step because extracts simultaneously release proteins leading to the cross-linking of genipin prior to recovery, resulting in the undesired blue color formation during the extraction process (Ramos-de-la-Peña et al., 2014). In the search of new alternatives for its recovery, genipin has been obtained by mechanical, enzymatic and ultrasound assisted processes (Ramos-de-la-Peña et al., 2014). Non-thermal technologies such as high pressure processing (HPP) are currently studied to preserve foods, while maintaining food texture and flavor or influencing enzymatic reactions. HPP has been used for extraction purposes, and retention and stability of anthocyanins and flavonols of berry fruits have been found (Altuner & Tokuşoğlu, 2013). Polysaccharides extraction from *Lentinus edodes* stems has shown higher yields and shorter extraction time after HPP treatments when compared to conventional thermal methods (Wang, Yu, He, Zhu, & Li, 2012). Until now, there are no reports on the recovery of genipin by HPP nor in combination with enzymatic treatment. In this study, the recovery of genipin by HPP and the effect of pectinases during this process was explored.

2. Materials and methods

2.1. Materials

Fresh genipap fruits were obtained from Guayacan Export (Bello Horizonte, Managua, Nicaragua). Genipap fruits were collected

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from wild trees in Nicaragua (Buena Vista, Municipio del Castillo, Departamento de Río San Juan) and were stored at room temperature. Pectinex Ultra SP-L ®, a commercial pectinases preparation from *Aspergillus aculeatus* was purchased from Novozymes (Krogshøjvej, Bagsværd, Denmark). D-glucosamine was obtained from Cargill (Indianapolis, Minnesota, USA). Genipin was procured from Chengdu King-tiger, Pharm-chem, Tech. Co., Ltd. (Dayi, Chengdu, China). Propylene glycol was procured from Dow Chemical Company (Horgen, Switzerland). Albumin from bovine serum ≥98% and Bradford reagent were procured from Sigma Aldrich (Diegem, Belgium).

2.2. Genipin liberation by HPP combined with enzymatic treatment

Genipap fruit was cut in two parts with a stainless steel knife, and the seeds were separated from the fruit. The fruit was cut into cubes of 2 × 2 × 2 cm and 25 g was mixed with 100 mL of distilled water at 10°C for 1 min in a kitchen mixer. The purees were packed into polyethylene plastic bags of 9 × 16 cm and 4.6 and 9.3 mg pectinases/g fruit was added to the purees before the bags were sealed. A reference sample without enzymes was included. Bags were vacuum-packed (Multivac A300/16, Wolfertschwenden, Germany) and pressurized at 130, 330 and 530 MPa (500 MPa/min through indirect compression). A pilot-scale vertically oriented HP equipment was used (Engineered Pressure Systems International, Temse, Belgium). Two double-bagged samples were placed into the pressure vessel. The vessel volume is 0.5 L, the inner diameter is 5 cm and the chamber length is 30 cm. A propylene–glycol mixture (60 ml/100 mL Dowcal, Dow Chemical Co., Horgen, Switzerland) was used as pressure-transmitting medium. Pressure was kept constant for 15 min and temperature was controlled by a cryostat. Temperature inside the vessel was 10 ± 0.1 °C before treatments and 9.3 ± 0.5, 10.4 ± 0.8 and 11.8 ± 0.8 °C after pressurizing at 130, 330 and 530 MPa, respectively. Temperature was not constant during HPP due to adiabatic heating. The vessel was decompressed after preset holding time and samples were removed after pressure release. Controls were treated at 0.1 MPa at 10 C for 15 min in a temperature-controlled water bath (Thermo Scientific, Antilles, The Netherlands). Samples were stored in ice immediately after processing. Ultrasonic assisted extraction of genipin reported the highest yield after 15 min at 10 C (Ramos-de-la-Peña et al., 2014) and these conditions were selected for HPP. Pressure between 130 and 530 MPa was included in our experiments because polysaccharides extraction from *Phellinus linteus and* anthocyanins and flavonols from berries by HPP was enhanced in the range of 200–600 MPa (Altuner & Tokuşoğlu, 2013; Kim & Iwahashi, 2015). Studies about pectinases for pectin hydrolysis during juice recovery from fruits (Braddock, 1981; Pinelo, Zeuner, & Meyer, 2010) suggested the pectinases dosage used in our study. Treatments were performed in quadruplicate. Analysis of variance was carried out using Minitab, Inc. version 14 (State College, Pennsylvania, USA) software.

2.3. Genipin recovery

Liquid phase was separated from the solid phase and volume and wet weight were determined. The solid was weighed and dried at 37 C in an incubator (Jouan EB 53, Jouan GmbH, Unterhaching, Germany) for 3 days. The liquid was centrifuged at 27200 × *g* with JA-20 rotor (Beckman J2-HS Centrifuge, Beckman Coulter, CA, USA) at 12°C for 25 min pH was determined at room temperature using a pH meter with a glass electrode (MeterLab PHM210, Radiometer Analytical, Lyon, France). Supernatant was separated from the pellet and was stored in polypropylene tubes at −40 °C until further analysis. Protein content was determined (Bradford, 1976).

2.4. Genipin assay

2.4.1. Cross-linked genipin

Frozen genipap fruit extracts were thawed and a calibration curve of genipin was prepared (0–500 mg/L). 500 μL of 1 g/L D-glucosamine was added to the calibration curve. Aliquots of 500 μL (dilution1:20) of extracts were placed in test tubes, which were heated in an oil bath (Memmert, WBU 45, Germany) (100°C; 1 h). After incubation, samples were cooled at room temperature (25 C) for 15 min and absorbance at 589 nm was measured using a spectrophotometer UV–Vis Ultrospec 2100 pro (GE Healthcare, Diegem, Belgium) (Ramos-de-la-Peña et al., 2014).

2.4.2. Non-cross-linked genipin

Non cross-linked genipin with proteins was determined using the same method for cross-linked genipin, but in this case, 500 μL of 1 g/L D-glucosamine was added to all test tubes (calibration curve and samples).

3. Results and discussion

3.1. Liquid liberated volume, wet solid weight and pH changes

Liberated liquid volume is shown in Fig. 1 (a). Volume from purees without pectinases somewhat decreased after pressurizing at 130 MPa and increased slightly after treatments at 350 and 530 MPa; no statistical difference $P < 0.05$ was observed in these treatments. Volume from purees after HPP with enzymes was higher than those without pectinases. Volume increased with the addition of enzymes and this was observed at all pressure levels. This might indicate a decrease in water absorption due to the activity of exogenous pectinases resulting in a polymer network breakdown (Van der Plancken et al., 2012). Further experiments should be carried out to confirm this hypothesis.

Fig. 1 (b) shows wet solid weight after processing of genipap purees. In line with liberated liquid volume, wet solid weight depends on the addition of pectic enzymes. At any given pressure level, except 530 MPa, the solid wet weight of samples with enzymes was significantly lower than without enzymes. This could indicate a pectin polymer (and network) breakdown, leading to a decrease in water retention. At a given enzyme level, wet solid weight seems to increase at lower pressure levels and to decrease again at higher pressure levels, being the maximum between 130 and 330 MPa. This in line with existing data on the effect of pressure on plant polygalacturonase, showing a decrease of enzyme activity with pressure (Shook, Shellhammer, & Schwartz, 2001; Verlent, Van Loey, Smout, Duvetter, & Hendrickx, 2004).

pH of extracts after HPP and 0.1 MPa treatments, with and without enzymes is shown in Fig. 1 (c). There is a distinct difference in pH for samples with and without pectinases, whereby the pH of samples without enzymes is significantly higher than the samples with enzymes. As a mixture of pectinases was used, this change most probably is related to pectinesterase activity enhancement, causing demethoxylation of pectin, resulting in free carboxyl groups, which caused the pH decrease. pH changes with the pressure level applied (at a given level of enzyme concentration) seem negligible. Data on liberated liquid volume, solid wet weight and pH changes are in line with suggested pectinases activities.

3.2. Protein content

Protein concentration was between 0.0 and 1.5 mg/g (wet basis) and data are shown in Table 1. Only supernatants after 130 MPa treatment without enzymes showed statistical difference ($P < 0.05$). After enzymes addition, protein content decreased after