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# Temperature model for process impact non-uniformity in genipin recovery by high pressure processing

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ABSTRACT

A model for the process impact temperature non-uniformity during high pressure processing (HPP) of genipap fruit purees was found during genipin recovery. Purees were subjected to HPP (130–530 MPa) under quasi-isobaric non-isothermal conditions (15 min; 0, 4.6 and 9.3 mg pectinases/g fruit). Genipin and protein concentration was determined, and pH was measured. Polygalacturonase activity was quantified indirectly by protein content (mg/g fruit). First order kinetics described temperature changes (0–4 min). Polygalacturonase was activated at 130 MPa, inactivated reversibly at 330 MPa and activated again at 530 MPa. Enzyme reaction rate constant ($k$) was placed in the 0–4 min model and temperature from 2 to 15 min was described. Protein content and pH characterization in terms of decimal reduction time improved highly the 2–15 min model. Since temperature changes were modeled, more insight of its behavior in an HPP reactor was obtained, avoiding uniformity assumptions, making easier the industrial scale HPP implementation.



## 1. Introduction

*Genipa americana* L. is a plant native to northern South America and the Caribbean, southern Mexico (Fernandes & Rodrigues, 2012; Ueda, Iwahashi, & Iokuda, 1991). The fruit is called genipap, but it is also known as jagua, chipara, guayatil, maluco, caruto and huito (Fernandes & Rodrigues, 2012). Genipin is an iridoid cross-linking compound that constitutes *G. americana* (1–3 g/100 g) (Djerassi, Gray, & Kincl, 1960; Ramos-de-la-Peña, 2014). Genipin is able to react spontaneously with primary amine groups of amino acids, peptides or proteins to form dark blue pigments and it can be absorbed in the intestine to act as a genuine choleretic (Akao, Kobashi, & Aburada, 1994). Ramos-de-la-Peña (2014) demonstrated that the recovery of genipin using emerging technologies such as ultrasound combined with enzymatic treatment and HPP enhance the genipin recovery. During HPP, pressure acts uniformly whereas temperature heterogeneities occur due to differences in compression heating and heat transfer (Grauwet, 2010). A high concern in temperature behavior inside an HPP reactor exists, due to its effect on the impact distribution if the kinetics of change of the target attributes are sensitive to non-uniform temperature. To date, there are more than 500 known studies about modeling the enzyme and micro-organisms inactivation kinetics in fruits, vegetables, meats, seafood, dairy and egg products during HPP, but these process models should work at pilot and industrial scale. The insufficient insight of the temperature distribution in an HPP reactor (Knoerzer, Juliano, Gladman, Versteeg, & Fryer, 2007) and the assumption of uniform temperature for the proposed models as iso-thermal conditions that are used in the experiments should be avoided.

Until now, no reports on the development of a first-order model for temperature during HPP have been found. In this paper, the potential of a first-order model for temperature in the pressure transmitting medium during HPP was explored, taking into account the parameters obtained during genipin recovery from genipap fruit purees.

## 2. Materials and methods

### 2.1. Materials

Genipap fruits were obtained from Guayacan Export (Bello Horizonte, Managua, Nicaragua). Fruits were collected from wild trees (soil conditions were not controlled) in the South of Nicaragua (Buena Vista, Municipio del Castillo, Departamento de

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Río San Juan) and were stored at room temperature until use. Pectinex Ultra SP-L®, a commercial preparation from *Aspergillus aculeatus* was purchased from Novozymes (Krogshøjvej, Bagsværd, Denmark). 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). All other reagents were analytical grade.

### 2.2. Genipin recovery by high pressure processing combined with enzymatic treatment

As described by (Ramos-de-la-Peña, Renard, Montañez, Reyes-Vega, & Contreras-Esquivel, 2015), genipap fruit was cut with a stainless steel knife in two parts and the seeds were separated from the fruit, which 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; 0.0, 4.6 and 9.3 mg of Pectinex Ultra SP-L/g of genipap fruit was added to the purees before the plastic bags were sealed. The bags were vacuum-packed (Multivac A300/16, Wolfertschwenden, Germany) and were pressurized to 130, 330 and 530 MPa at a rate of 500 MPa/min through indirect compression. A pilot scale vertically oriented HP equipment was used (Engineered Pressure Systems International, Temse, Belgium). Two sample bags were placed into the pressure vessel. The vessel volume is 0.5 L, the inner vessel diameter is 5 cm and the chamber length is 30 cm. A propylene–glycol mixture (60% Dowcal, Dow Chemical Co., Horgen, Switzerland) was used as a pressure transmitting medium that was injected at the reactor bottom. The pressure was kept constant for 15 min. The temperature of the vessel was controlled by a cryostat and the initial temperature inside the vessel was 10 °C. Temperature was not constant during the pressure treatment due to adiabatic heating. The vessel was decompressed after preset holding time and samples were removed after pressure release. The 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. Experiments were performed in quadruplicate. Liquid phase was separated from the solid phase and it was centrifuged at 27,200*g* with JA-20 rotor (Beckman J2-HS Centrifuge, Beckman Coulter, Brea, CA, USA) at 12 °C for 25 min. Liquid phase was separated from the pellet and was stored in polypropylene tubes at −40 °C until further analysis. pH was determined after each HPP treatment at room temperature using a pH meter with a glass electrode (MeterLab PHM210, Radiometer Analytical, Lyon, France). Genipin was quantified according to Ramos-de-la-Peña et al. (2014) and protein content was determined (Bradford, 1976). Analysis of variance was carried out using Minitab, Inc. version 14 (State College, Pennsylvania, USA) software.

### 2.3. Kinetic modeling and parameter estimation

A kinetic study was developed in the pressure range of 130–530 MPa at initial temperature of 10 °C under quasi – isobaric non-isothermal conditions. Changes in temperature were registered for at least three runs under high pressure processing for 15 min. These temperature values were averaged, and the mean was entered into the modeling procedure. Temperature changes could be modeled by first-order reaction kinetics taking into account the initial part of the graph (0–4 min). Kinetic parameters were performed using a two-step regression approach. The time-dependent change of temperature was modeled. The reaction rate constant $k$ at a given temperature and pressure was determined by plotting the temperature as a function of time.

$$T = T_0 \exp(-kt) \tag{1}$$

where $T$ is the temperature at treatment time $t$ (min) and $T_0$ the value of the temperature after the cut (time in which the desired pressure is reached), and $k$ the reaction rate constant (1/min). As a measure of the ability of a model to fit all experimental data, a visual inspection of the residuals plot was performed, the corrected $R^2$ (Eq. (2)) and the model standard deviation (Eq. (3)) were calculated.

$$R^2_{\text{adj}} = 1 - \frac{\left(1 - \frac{\text{SSQ}_{\text{regression}}}{\text{SSQ}_{\text{total}}}\right)(x-1)}{(x-p'-1)} \tag{2}$$

$$\text{RMSE} = \sqrt{\frac{\text{MSQ}_{\text{residuals}}}{x-p'}} \tag{3}$$

pH and protein concentration were characterized in terms of the decimal reduction time $D$ value (decimal reduction time required to reduce the initial activity or concentration by a factor of ten at constant pressure (Tucker, 2001) in min (Eq. (4)) and $z$ (°C) value (Eq. (5)), which is the temperature change required at constant pressure to achieve a tenfold change in $D$ value, by the use of the Thermal Death Time model (Bigelow, 1986).

$$D = \frac{\ln(10)}{k} \tag{4}$$

$$D = D_{\text{ref}} 10^{\frac{T_{\text{ref}} - T}{z}} \tag{5}$$

A first-order model was developed to describe the temperature changes during 2–15 min of pressure treatment. Protein content was found to be an indirect indicator of the enzymatic activity of the main enzyme in Pectinex Ultra SP-L, as protein content showed to be pressure–temperature dependent. The rate constants obtained for protein content (the enzymatic activation rate constants) were manually inserted in the first-order model except for 330 MPa, due to the model was based on enzymatic activation, and enzyme was inactivated at this pressure. As pH has demonstrated to affect $D$-values on the thermal destruction of spores of *Clostridium sporogenes* (Cameron, Leonard, & Barrett, 1980), and has shown to be highly graphic, a correlation from $D$-values was found to obtain a factor which was used to be subtracted from the predicted value from the first-order model. The developed model for temperature was found to be according to Eq. (6), where $T$ represents the temperature at treatment time (min) and $T_0$ the temperature after the cut, respectively, and $k_{\text{enz}}$ the enzyme activation rate constant (1/min). $D_{\text{refpH}}$ and $D_{\text{obspH}}$ are the decimal-reduction time at a constant pressure (min), including the reference and observed values obtained for pH.

$$T = (T_0 \exp(-k_{\text{enz}} t)) - \left(\frac{D_{\text{ref pH}}}{D_{\text{obs pH}}}\right) \tag{6}$$

## 3. Results and discussion

### 3.1. Effect of HPP on genipin recovery

Genipin concentration in extracts subjected to HPP was higher as temperature was lower. When temperature was raised, genipin concentration decreased and no further increase was observed. The yield of genipin after HPP was increased by a factor of two when compared to atmospheric pressure treatment (Table 1). The recovery of genipin by HPP could be considered as split-stream processing, which focuses on a specific end-product functionality (Houben et al., 2014), for example, the avoiding of the blue color formation of genipin during its recovery process. Immediately after HPP,