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Kinetic modelling of polyphenol oxidase, peroxidase, pectin esterase, polygalacturonase, degradation of the main pigments and polyphenols in beetroot juice during high pressure carbon dioxide treatment

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ABSTRACT

Supercritical carbon dioxide (SCCD) was applied for the inactivation of tissue enzymes in beetroot juice. The SCCD process parameters had a significant effect on the inactivation kinetics of polyphenol oxidase (PPO), peroxidase (POD), pectin esterase (PE) and polygalacturonase (PG). The use of traditional pasteurization (TP) resulted in a higher level of inactivation of the tested enzymes than SCCD. The lowest decimal reduction time was noted for PG and the highest for POD. The PG, PE, PPO and POD inactivation kinetic constants were calculated as 0.90, 0.85, 0.82 and 0.6 $\times$ 10^{-2} min^{-1} for *k-values* and 256, 271, 281 and 384 min for *D-values*, respectively, based on the first order kinetic model. The highest degradation of bethacyanins, bethaxanthins and polyphenols occurred at 60 MPa at 55 °C, for 30 min: 58, 32 and 30%, respectively. Overall, SCCD treatment is a promising technique to obtain high quality non-acidified beetroot juice but further studies should concentrate on additional factors affecting the inactivation of tissue enzymes.

1. Introduction

Red beet (*Beta vulgaris* L.) is a rich source of polyphenols and a group of water soluble nitrogen pigments called betalains. Betalains consist of two sub-classes: betacyanins (red-violet pigments) and betaxanthins (yellow-orange pigments) (Ravivhandran et al., 2013). The betalains family is considered to be a cancer preventive agent and shows a high antioxidant effect and radical scavenging capacity. Similarly to other natural pigments, betalains are very sensitive to degradation by heat, light, enzymes and oxygen (Paciulli, Medina-Meza, Chiavaro, & Barbosa-Canovas, 2016). Therefore, non-thermal processing techniques are recommended to preserve the nutritious value and colour of red beet products.

An interesting non-thermal method of food preservation is the use of high pressure carbon dioxide (HPCD), including supercritical state (SCCD). An advantage of this method, compared to well-known and already commercially used high hydrostatic pressure (HHP) processing, is the use of much lower pressures and greater ability to destroy active tissue enzymes (Marszałek, Skąpska, Woźniak, & Sokołowska, 2015, Marszałek, Krzyżanowska, Woźniak, & Skąpska, 2016a; Marszałek, Woźniak, & Skąpska, 2016b).

The most important enzymes that may have a negative impact on the quality of vegetable products during storage, are polyphenoloxidase (PPO), peroxidase (POD), pectin esterase (PE) and polygalacturonase (PG). PPO (Enzyme Commission number - EC: 1.14.18.1) is responsible for the browning and degradation of natural pigments and other polyphenols, leading to discoloration and the loss of antioxidant activity (Chakraborty, Baier, Knorr, & Mishra, 2015). POD (EC: 1.11.1.7) participates in several metabolic plant processes, such as the catabolism of auxins, lignification of the cell wall as well as browning reactions which catalyzes the oxidation processes (Elstner & Heupel, 1976). PE (EC: 3.1.1.11) and PG (EC: 3.2.1.15) are involved in the breakdown of pectin and other cell wall materials, resulting in a product with reduced viscosity and undesirable organoleptic properties (Chakraborty et al., 2015). Therefore, the inactivation of these groups of enzymes is extremely important in the processing of fruit and vegetables.

The reports available on the use of SCCD to preserve fruit and vegetable products focused on the inactivation of microorganisms and enzymes in apple, orange, carrot and Hami melon juices, employing a continuous, semi-continuous or batch system, using

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pressures below 50 MPa (Bi, Wu, Zhang, Xu, & Liao, 2011; Chen et al., 2010; Fabroni, Amenta, Timpanaro, & Rapisarda, 2010; Gasperi et al., 2009; Gui et al., 2007; Spilimbergo, Komes, Vojvodic, Levaj, & Ferrentino, 2013) or strawberry juice up to 65 MPa (Marszałek et al., 2015). To the best of our knowledge, there is no report on the kinetics of enzyme inactivation under SCCD treatment in beetroot juice. Therefore, the aim of this study was the kinetic modelling of POD, PPO, PG and PE inactivation and the degradation of betalains and polyphenols of non-acidified beetroot juices (pH > 6.0) under SCCD.

2. Materials and methods

2.1. Reagents

Ultrapure water was obtained using a Direct-Q 3 UV system from Merck Milipore (Darmstadt, Denmark). The following standards and chemicals were used in the study: polyvinylpyrrolidone (PVP) (~110 μm) (Fluka, USA); catechol (>99%), hydrogen peroxide (30%), *p*-Penylenediamine and Triton X−100 (Sigma-Aldrich, USA). Other reagents (analytical grade) were purchased at POCh (Warsaw, Poland).

2.2. Sample preparation

Fresh beetroots were purchased from a local market, peeled, washed, cut into pieces and squeezed to obtain cloudy juice using J 80 Ultra (Robot Coupe, Monceau-les-Mines, France). The juice samples were subjected to SCCD or thermal pasteurization (TP). Freshly squeezed and unpreserved juice was called as the control sample (CS).

2.3. Supercritical carbon dioxide (SCCD) treatment and thermal pasteurization (TP)

SCCD treatment was performed using a Spe-ed™ SFE apparatus (Applied Separations, Alletown, USA) system described in our previous study (Marszałek et al., 2015) and illustrated in Fig. 1. For each experiment, approx. 60 mL of beetroot juice was placed in a pressure vessel (500 mL) which had been preheated to the experimental temperature 31, 39 or 55 °C, exposed to a pressure of 10, 30, 60 MPa for 10, 20, 30 min, and immediately cooled in an ice bath. The actual temperature of the sample was measured and registered using an iButton® temperature logger (DS 1922L, Maxim Integrated, San Jose, USA). Pressurization and depressurization time were not added to the process time.

TP treatment was performed in 100-mL glass jars in a stainless steel, electric-heated and thermostated bath pasteurizer (Labo Play, Bytom, Poland) for 10 min at 55, 75 and 95 °C. The come-up and down time in all the TP processes was 2.5 min.

Experiments and measurements were performed in duplicate.

3. Analyses

3.1. Physicochemical analysis

The pH was measured using an HI 2210 pH meter (Hanna Instruments, Woonsocket, USA). The total soluble solid content (TSS) was measured using a MS REF 090 L refractometer (My Soft, Warsaw, Poland). The glucose, fructose, and sucrose were determined using HPLC in accordance with the EN 12630 (1999) standard.

3.2. PPO and POD activities

The activity of oxidoreductases was determined as described by Terefe, Yang, Knoerzer, Buckow, and Versteeg (2010). The extraction mixture comprised 0.2 mol/L phosphate buffer (pH = 6.5) containing 40 g/L polyvinylpyrrolidone (PVPP), 10 mL/L Triton X−100 and 1 mol/L NaCl. The juice and the mixture (4.5: 4.5 g) were homogenized (T10 rotor-stator, ×120, CAT Scientific, Fairfield, USA) for 3 min and centrifuged (380R, Hettich Instruments, Boston, United States) at 14,000×*g* for 30 min at 4 °C. After filtration through blotting filter paper, the supernatant was used to determine PPO and POD activity.

For the PPO activity assay, 100 μL of the supernatant was introduced into 3 mL of 0.05 mol/L phosphate buffer (pH 6.5) containing 0.07 mol/L catechol, and the absorbance was measured spectrophotometrically (6705 UV-VIS Spectrophotometer, Jenway, UK) at λ = 420 nm and 25 °C for 10 min. A blank sample was prepared in the same way by substituting the supernatant with a phosphate buffer. The PPO activity was expressed as a change in the absorbance/min/g of fresh weight (FW) of the sample analyzed.

For the POD activity assay, 1.5 mL of 0.05 mol/L phosphate buffer (pH = 6.5) was added to the mixture containing 200 μL of the supernatant, 200 μL of 0.05 mol/L phosphate buffer containing 10 g/L *p*-phenylenediamine and 200 μL of 15 mL/L hydrogen peroxide. Mixture absorbance was measured at λ = 485 nm, 25 °C for 10 min. The POD activity was expressed as a change in the absorbance/min/g of FW of the sample analyzed.

3.3. PE activity

The PE activity was determined according to Kimball (1991, pp. 117–125) with modifications of the sample volume and

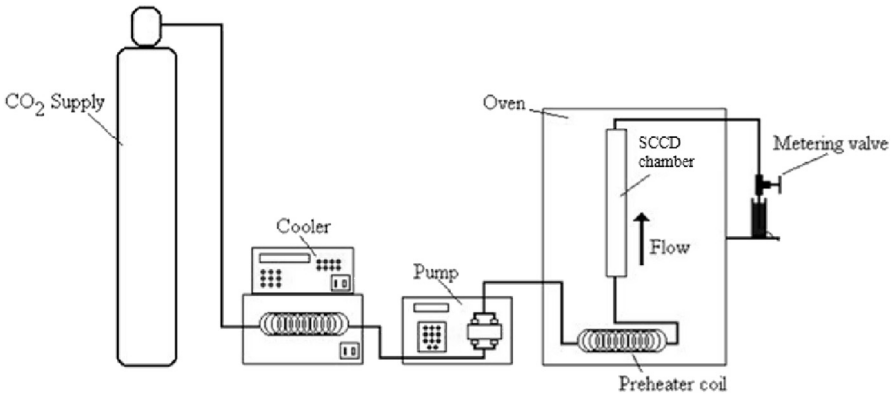

Fig. 1. Scheme of SCCD apparatus. SCCD: supercritical carbon dioxide.