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Kinetic modelling of tissue enzymes inactivation and degradation of pigments and polyphenols in cloudy carrot and celery juices under supercritical carbon dioxide

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ABSTRACT

Supercritical carbon dioxide (SCCD) was applied to inactivate tissue enzymes in carrot and celery juices. The polyphenol oxidases (PPO), peroxidases (POD), pectin esterases (PE) and polygalacturonases (PG) inactivation kinetics were significantly dependent on the SCCD process parameters. Traditional pasteurization (TP) resulted in a higher level of inactivation of all the enzymes tested in celery juice as well as POD and PG in carrot juice, compared to SCCD. The lowest and highest decimal reduction time was noted for PE (200 min) and PG (1645 min) in celery juice, respectively. The carotenoids in carrot and polyphenols in celery juices were resistant to SCC treatment, whereas the chlorophylls in celery and polyphenols in carrot juices were degraded up to approx. 20 and 45%, respectively. SCCD treatment is a promising technique for obtaining high quality vegetable juices but further studies should be concentrated on additional factors which facilitate the inactivation of tissue enzymes.

1. Introduction

Fresh vegetable juices are becoming increasingly popular as a low-sugar alternative to fruit juices [1]. The consumption of vegetable juices that contain many essential nutrients, such as vitamins and minerals, which are beneficial for health and the overall well-being of the human body, is noted especially in health-awareness societies. Cloudy vegetable juices represent almost all the nutritional values of vegetables and, therefore, can be used as an alternative to ready to eat fresh vegetables.

Carrot (*Daucus carota* L.), one of the important root crops cultivated throughout the world, is a good source of natural antioxidants such as carotenoids and phenolic compounds [2]. On the other hand, it poses some problems when processed into juice due to the high level of microbial contamination of the carrot root and high activity of tissue enzymes, affecting its colour and texture [3].

Leaf celery (*Apium graveolens* L. var. secalinum) is a hypo-caloric vegetable. Every edible 100 g of celery gives only 20 kcal, yet nutritionally it has good vitamin A levels, and is rich in potassium. Similarly to carrot, the active enzymatic system of fresh celery gives rise to problems during storage and processing. The first signs of deterioration in the quality of celery are the loss of its green colour. Moreover, during handling, cutting, and mechanical damage, the green pigments are quickly degraded by oxidative enzymes [4].

Almost all thermal preservation methods have significant drawbacks when applied in fruit and vegetable processing, because of their destructive effect on thermolabile nutrients, pigments and flavour compounds. Therefore, there is a need for non-thermal technologies, which would ensure product safety and stability while, at the same time, having a minimal effect on the nutritional and sensory quality. High-pressure processing (HPP) is one such non-thermal technique, used increasingly in the food industry, and which has the potential to improve the balance between the safety and quality characteristics of, among other things, fruit and vegetable products. Supercritical carbon dioxide (SCCD) preservation, although not currently used in the processing industry, appears to be promising in fruit and vegetable technology, because of its better efficiency at inactivating tissue enzymes [5–7]. SCCD can be used at temperatures and pressures which are relatively safe for heat-labile compounds, and at the same time sufficient to inactivate microorganisms and tissue enzymes [1,5,8–13].

There are some report with the study on the influence of carbon dioxide on fruit and vegetable tissue enzymes including their inactivation kinetics [2,3,8,11,16] but the limit number of this report were focused on the inactivation kinetic parameters. Polyphenol oxidase (PPO, EC 1.14.18.1) and peroxidase (POD, EC 1.11.1.7) are

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the principal enzymes involved in the enzymatic browning of vegetable tissues. PPO is a copper-containing enzyme responsible for the hydroxylation of monophenols to *o*-diphenols and, as a consequence, the reaction of this compound with endogenous amino acids and proteins to form complex brown pigments [14]. POD, as another oxidoreduction enzyme, participates in several metabolic plant processes such as the catabolism of auxins, lignification of the cell wall, as well as in browning reactions, resulting in products which catalyzes the oxidation of various electron donors with H_2O_2 and carries a 'b'-type haem as a prosthetic group [15].

The degradation of the cell wall pectin is catalyzed by the action of two groups of endogenous enzymes, namely pectin esterase (PE, EC 3.1.1.11) and polygalacturonase (PG, EC 3.2.1.15). PE deestrifies the pectin yielding methanol and pectic acid with a lower degree of esterification whereas PG catalyzes the hydrolytic cleavage of the glycosidic α-D–*(1–4)* bonds in the pectic acid [16]. The residual activity of oxydoreductases and hydrolases negatively affects the quality of processed fruit and vegetable products, resulting in browning, the formation of an off-flavour, loss of vitamins and pigments and reduces the viscosity, negatively affecting the organoleptic properties. Therefore, the inactivation of PPO, POD, PE and PG in the processing of fruit and vegetables is the major indicator of quality.

The reports available on the use of SCCD to preserve fruit and vegetable products were focused on the inactivation of microorganisms and enzymes in apple, orange, carrot and watermelon juices, employing a continuous, semi-continuous or batch system, using pressures below 50 MPa [1,3,8,12,17,18] or strawberry juice up to 65 MPa [5,6]. To our best knowledge, there is no report on the dynamic of vegetable enzyme inactivation under SCCD treatment at higher pressure. Therefore, the aim of this study was to determine the *k*- and *D-value* of PPO, POD, PE and PG inactivation in carrot and leaf celery cloudy juices under SCCD up to 60 MPa and to investigate the degradation of the main pigments during this processing.

2. Materials and methods

2.1. Reagents

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 in POCh (Warsaw, Poland). Ultrapure water was obtained using a Direct-Q 3 UV system from Merck Milipore (Darmstadt, Denmark).

2.2. Sample preparation

Fresh carrots and celery leaves were purchased from a local market, peeled, washed with water, cut into smaller pieces and squeezed to obtain juices (J 80 Ultra, Robot Coupe, France). Both juices were uniform in all experiments. Untreated juices were used as the control samples (CS_{carrot}, CS_{celery}).

2.3. Supercritical carbon dioxide (SCCD) preservation

SCCD treatment was performed with a Spe-ed SFE 4 (Applied Separations, USA) system. A minimum pressure of 10 MPa was chosen, slightly above the critical pressure for carbon dioxide (7.36 MPa), and the maximum pressure was 65 MPa. All the data relating to the pressure and temperature of the chamber were displayed on a control panel. Moreover, during processing, the unique miniaturized stainless steel capsule with an iButton® temperature logger (−40 °C–+85 °C, DS1922L, Maxim Integrated, USA), was used to record the time-temperature history inside the sample.

For each experiment, approx. 60 mL of juice was poured into a 160 mL glass jar, without the cap, and then placed in a rinsed and sanitized (in an autoclave at 120 °C) pressure vessel (500 mL) preheated to the experimental temperature 31, 39, 47 or 55 °C and then exposed to a pressure of 10, 30, 60 MPa for 10, 20, 30 min. The pressure ramp was 60 MPa/min, and at the end of the SCCD treatment, the vessel was slowly depressurized over a period of 5 min. Pressurization as well as depressurization time were not added to the processing time. After treatment, the jars with juices were removed from the pressure vessel in the laminar chamber and immediately cooled. After equilibration to the ambient temperature, the enzyme activity and pigment content were determined. 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 an MS REF 090 L refractometer (My Soft, Warsaw, Poland).

3.2. HPLC analysis of sugars

Glucose, fructose, and sucrose were determined according to the EN 12630:1999 standard [19]. For the analysis, a Sugar-Pak I, 10 μm, 6.5 mm × 300 mm analytical column with a Sugar-Pak and Guard-Pak insert, 10 μm (both Waters, USA), was used. Separation of the 10 μL samples was performed within 18 min at a flow rate of 0.5 mL/min and a column temperature of 90 °C. Samples were eluted isocratically using 0.1 mM calcium disodium EDTA. The compounds were quantified using a 2414 refractive index detector (Waters, USA).

3.3. PPO and POD activities

The activity of oxidoreductases was determined as described by Terefe, et al. [20]. The extraction mixture comprised 0.2 M phosphate buffer (pH = 6.5) containing 4% (w/v) polyvinylpyrrolidone (PVPP), 1% (v/v) Triton X–100 and 1 M NaCl. The juice and the mixture (4.5: 4.5 g) were homogenized (T10 rotor-stator, X120, CAT Scientific, USA) for 3 min and centrifuged (380R, Hettich Instruments, Germany) at 14,000 x *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 M phosphate buffer (pH 6.5) containing 0.07 M 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 M phosphate buffer (pH = 6.5) was added to the mixture containing 200 μL of the supernatant, 200 μL of 0.05 M phosphate buffer containing 1% p–phenylenediamine (w/v) and 200 μL of 1.5% (v/v) 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.