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Application of supercritical carbon dioxide for the preservation of strawberry juice: Microbial and physicochemical quality, enzymatic activity and the degradation kinetics of anthocyanins during storage

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ABSTRACT

Supercritical carbon dioxide (SCCD) was applied for strawberry juice preservation. SCCD under selected parameters was effective for microflora and enzyme inactivation. For storage studies samples preserved at 30 and 60 MPa, at 45 °C for 30 min were selected. Yeasts and moulds were not detected after SCCD treatment in these conditions, whereas TMC was ~1.7 log CFU/g. Polyphenol oxidases were inactivated, whereas peroxidases decreased by ~85%. SCCD treatment resulted in the hydrolysis of sucrose and ~30% losses of vitamin C, which was totally decomposed after the 4th week of storage. Anthocyanins were not affected by the SCCD process and the kinetic rate constant of degradation during storage ranged from 2.12×10^{-2} to 3.10×10^{-2} ($days^{-1}$), and the half-time from 22.4 to 32.7 days, depending on the monomer and pressure applied. Overall, SCCD treatment seems to be a promising technique to obtain high quality strawberry juice as a safe alternative to non-pasteurized juices.

Industrial relevance: Strawberries are highly appreciated for their nutritional quality, colour and taste. Strawberry compounds, i.e. anthocyanins and vitamin C, are heat sensitive. These nutrients as well as colour are degraded when the fruits are processed using high temperature treatment. Supercritical carbon dioxide (SCCD) is an interesting method among the innovative non-thermal technologies for the preservation of fruit products. In this study, the data proved that SCCD processing is a promising non-thermal alternative to pasteurization to preserve fresh cloudy strawberry juice.

1. Introduction

Strawberries (*Fragaria x ananasa*) are a popular fruit grown in many countries. For more than ten years, Poland has been one of the leading countries in terms of strawberry production, with the highest harvest per capita in the world (Kalisz, Marszałek, & Mitek, 2009, FAOSTAT). Strawberries are greatly appreciated for their nutritional value, taste and colour. Their high nutritional value is due to phenolic compounds, i.e. ellagic acid and vitamins, mainly vitamin C. A high amount of anthocyanins contributes to the attractive red colour of strawberry products (Marszałek, Mitek, & Skąpska, 2015a, Patras, Brunton, De Pieve, & Butler, 2009). However, pathogenic and spoilage microorganisms can grow in unprocessed strawberry juice despite the product's low acidity. Thermal treatment is the traditional effective method to inactivate microorganisms in foods (Lambert, 2003; Mann, Kiefer, & Leuenberg, 2001; Marszałek, Mitek, & Skąpska, 2015b). Treatment at a high temperature results not only in microbial stability but also in the inactivation of tissue enzymes, which is desirable in terms of product storage stability. Unfortunately, many of the bioactive compounds present in strawberry juice, i.e. anthocyanins and vitamin C, are thermo-sensitive and are destroyed by heat treatment. A high temperature also affects the fresh flavour of fruits. The processing of strawberries is also difficult because of the high activity of the tissue enzymes responsible for enzymatic browning (Aguiló-Aguayo, Sobrino-López, Soliva-Fortuny, & Martin-Belloso, 2008, Lopez-Serrano & Ros-Barcelo, 2001, Marszałek, Mitek & Skapska, 2015a, Marszałek, Woźniak, & Skąpska, 2015c). Hence, it is necessary to use efficient non-thermal preservation techniques in the production of strawberry juices. Attempts have been made to use, e.g. high pressure processing for preserving strawberry products, but the use of industrial pressures did not enable the total destruction of active enzymes (Marszałek, Mitek & Skapska, 2015a, Terefe, Buckow, & Versteeg, 2013, Terefe, Yang, Knoerzer, Buckow, & Versteeg, 2010).

Dense phase carbon dioxide (DPCD) processing, a collective term for liquid carbon dioxide (LCD) and supercritical carbon dioxide (SCCD — CO_2 above the critical point of 31.1 °C and 7.38 MPa) or high pressure carbon dioxide (HPCD), are emerging, non- or mild-thermal preservation method, alternative to high-pressure processing or traditional heating of fruit juices (Chen, Zhang, Song, Jiang, Wu & Hu, 2010). HPCD near-critical CD and SCCD can be used at temperatures and pressures which are relatively safe for heat-labile

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compounds, as well as sufficient for the inactivation of microorganisms and tissue enzymes such as polyphenol oxidases (PPO) and peroxidases (POD) (Bi, Wu, Zhang, Xu, & Liao, 2011, Damar & Balaban, 2006, Fabroni, Amenta, Timpanaro, & Rapisarda, 2010, Ferrentino & Spilimbergo, 2011, Gui, Wu, Chen, Liao, Hu, Zhang & Wang, 2007, Liu, Hu, Zhao, & Song, 2012, Marszałek, Skapska & Woźiak, 2015c). The results reported by Zhong, Black, Davidson, and Golden (2008) demonstrated that the microbial reduction obtained under supercritical carbon dioxide conditions was significantly higher than in a subcritical state under the same conditions of temperature and time. Damar and Balaban (2006) claimed that HPCD retained the fresh-like sensory, nutritional, and physical properties of many liquid foods by avoiding the thermal effects of traditional pasteurization. Other studies with orange juice showed that HPCD treatment could improve some physical and nutritional attributes such as cloud formation and stability, colour, and ascorbic acid retention (Arreola, Balaban, Marshall, Replow, Wei & Cornell, 1991; Kincla, Hill, Balaban, Portier, Sims, Wei, & Marshall, 2006.

HPCD can inactivate microorganisms by lowering the pH of cells, inducing the precipitation of intracellular carbonate calcium and magnesium, disrupting cells, modifying the cell membrane and extracting cellular components (Liu, Hu, Zhao & Song, 2012). Recent reports have dealt with the influence of carbon dioxide on enzymes. Polyphenol oxidase (PPO, EC 1.14.18.1) and peroxidase (POD, EC 1.11.1.7) are important, and the most pressure resistant enzymes present in many fruit and vegetables. Their residual activity negatively affects the quality of processed fruit and vegetable products, resulting in browning, the formation of off-flavour and loss of vitamins and pigments. Therefore, the inactivation of PPO and POD in the processing of fruit and vegetables is the major quality indicator.

The reports available on the use of HPCD 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 (Bi, Wu, Zhang, Xu & Liao, 2011, Chen, Zhang, Song, Jiang, Wu & Hu, 2010, Damar & Balaban, 2006, Gasperi, Aprea, Biasioli, Carlin, Endrizzi, Piretti & Spilimbergo, 2009, Liu, Hu, Zhao & Song, 2012, Spilimbergo, Komes, Vojvodic, Levaj, & Ferrentino, 2013). To our best knowledge, there is no report on the application of SCCD treatment on the quality of strawberry juice, as well as the application SCCD using pressures over 50 MPa.

Therefore, the aim of this study was to investigate the effects of SCCD treatment with different pressure parameters (10–60 MPa), time (10–30 min) and temperature (35–65 °C) on the inactivation of microorganisms and enzymes (i), changes in sugar profiles, vitamin C content and colour during storage (ii), as well as the inactivation kinetics and half-life of anthocyanin pigments in strawberry juice during storage at 6 °C (iii).

2. Materials and methods

2.1. Strawberry juice- control sample (CS)

Strawberries cv. 'Senga Sengana', were harvested in June 2013 and pre-treated (washing, destalking, freezing) at Ulmer Sp.j. company (Stare Zadybie, Poland). After freezing in a fluidization tunnel (UniDex, Poland), the strawberries were sorted (Niagara Sortex, Bühler, Switzerland) according to colour and size (45–55 mm) and stored at −24 °C. After defrosting (at 6 ± 2 °C), the fruits were mashed up in a food processor (CL-30, Robot Coupe, France) and treated with commercial pectinolytic enzymes (0.03% m/m, Klerzyme 150, DMS, France) at 50 °C for 1.5 h. The resultant liquid strawberry pulp was pressed on a frame hydraulic press (TPZ 7, Bücher-Guyer, Switzerland) and deaerated at 0.06 MPa (LVE, Fryma, Switzerland). This cloudy strawberry juice was used as the control sample (CS).

2.2. Supercritical carbon dioxide (SCCD) preservation

SCCD treatment was performed with a Spe-ed SFE 4 (Applied Separations, USA) system. The minimum pressure (10 MPa) was chosen slightly above the critical pressure for carbon dioxide (7.36 MPa) and the maximum pressure (60 MPa) was slightly less than the equipment's operating limit (65 MPa). All the data relating to the temperature of the chamber (not in the sample) and pressure were displayed on a control panel.

For each experiment, 7 mL of strawberry juice was placed in a 12 mL glass tube without the cap and then placed in a rinsed and sanitized (in the autoclave at 120 °C) pressure vessel (25 mL) which had been preheated to the experimental temperature 35, 45 and 65 °C and then exposed to a pressure of 10, 30, 60 MPa for 10, 20, 30 min. Pressure was generated by high pressure pump and applied directly to the vessel. 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 process time. After treatment, the tube with strawberry juice was removed from the pressure vessel in the laminar chamber and immediately cooled. After equilibration to the ambient temperature, the residual activity of PPO and POD, microorganisms, anthocyanins, vitamin C, sugars and colour were determined. Experiments and measurements were performed in triplicate.

Selected samples were stored in glass bottles without light at 6 °C for 12 weeks.

3. Chemical reagents

The following standards and chemicals were used in the study: pelargonidin-3-glucoside (Pg-3-Glc) and cyanidin-3-glucoside (Cy-3-Glc) of HPLC purity (Extrasynthese, France); L-ascorbic acid (AA) of HPLC purity (Supelco, USA); DL-dithiothreitol (DTT) (>99%); polyvinylpyrrolidone (PVP) (~110 μm) (Fluka, USA); catechol (>99%), hydrogen peroxide (30%) and Triton X-100 (Sigma-Aldrich, USA). The remaining reagents were purchased in POCh (Warsaw, Poland). Demineralized water used for the analyses was purified using Direct-Q 3 apparatus (Millipore, USA).

4. Analyses

4.1. Microbiological analyses

Yeasts and moulds (Y&M) were analysed according to the EN ISO 21527-1, 2008 standard. All samples were diluted using sterile normal saline (0.85% sodium chloride), spread plated on sterile nutrient agar with dichloran rose bengal chlortetracycline (DRBC) and incubated at 25 °C for 5 to 7 days.

The total microbial count (TMC) was determined according to the EN ISO 4833, 2003 standard. Samples diluted with sterile normal saline were plated on count agar (PCA); plates were incubated at 30 °C for 72 h. All the experiments were conducted in duplicate and the mean values of Log 10 CFU/g sample have been reported.

4.2. PPO and POD activities

The activity of selected tissue enzymes was determined as described by Terefe, Yang, Knoerzer, Buckow & Versteeg (2010). 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 strawberry juice and the mixture (4.5 : 4.5 g, *w*/*w*) were homogenized for 3 min and centrifuged (MPW-350R, MPW Med. Instruments, Poland) at 14,000 × g for 30 min at 4 °C. The supernatant, after filtration through blotting filter paper, 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