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Enzymatic, physicochemical, nutritional and phytochemical profile changes of apple (*Golden Delicious* L.) juice under supercritical carbon dioxide and long-term cold storage

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ABSTRACT

The impact of supercritical carbon dioxide (SCCD) (10–60 MPa/45 °C/30 min) and subsequent 10 weeks storage at 4 °C on polyphenol oxidase (PPO), peroxidase (POD) activities, phenolic profile, vitamin C, sugars, physicochemical properties of cloudy apple juices was investigated.

No significant changes in sugars and total polyphenols were observed, whereas significant degradation (≈28%) of vitamin C and individual polyphenols (≈18%) was noted after SCCD treatment. After 4 weeks storage only 34% of vitamin C was retained and no vitamin C was detected after this time. Ten weeks of storage caused hydrolysis of sucrose in 15%, whereas degradation of individual polyphenols ranged from 43 to 50% depending on the pressure applied. The highest pressure was applied the highest retention of polyphenols was observed. The lightness of juice significantly increased by 15% after SCCD and decreased during storage. Moreover, the synergistic effect of both enzymes with chlorogenic acid and catechol was found.

1. Introduction

Apples and their derived products (eg. apple juices) contain a significant amount of nutrients, dietary fibers and bioactive secondary plant metabolites such as polyphenols, which are associated with several benefits in the prevention of noncommunicable diseases (Poulsen et al., 2011). Moreover, phenolic compounds have an important influence on organoleptic and sensorial characteristics of apples (Putnik, Bursać Kovačević, Herceg, Pavkov, et al., 2017). The phenolic compound concentration in apples differs according to variety as well as environmental and agricultural conditions. In case of apple juices, another factor that strongly influences polyphenol content is the technology used for juice production (Will, Roth, Olk, Ludwig, & Dietrich, 2008).

Apple juice is commonly consumed as a clear juice, but there is a growing market for not from concentrate (NFC) juices with much higher concentration of polyphenols (Markowski, Mieszczakowska, & Płocharski, 2009). Phenolic compounds can belong to different subgroups such as: benzoic acids, hydrocinnamic acids, flavonols, flavanols, flavones, flavanones, chalcones, dihydrochalcones, dihydroflavonols, and anthocyanins (Chen et al., 2010) but not all of these groups can be found in all apple varieties and apple products. All of these compounds exhibit antioxidant capacity and numerously health-promoting properties but unfortunately during clear juice processing ≈50-90% of these compounds are lost (Will et al., 2008).

Nowadays consumers prefer light, whitish yellow NFC apple juices with significant cloudiness and minimal symptoms of sedimentation, harmonized in terms of taste (Niu et al., 2010). Unfortunately, during juice production and storage time, browning reactions are initiated by oxidoreductive enzymes due to degradation of polyphenols to colored quinones, which are substrates to further reactions, leading to pigment formation (Putnik, Bursać Kovačević, Herceg, & Levaj, 2017). Enzymatic browning is a significant problem for NFC juices due to the lowering of sensorial properties and nutrients. Fresh apple juice is exposed to enzymatic browning, because enzymes from oxidoreductase group such as polyphenoloxidase (PPO, EC 1.14.18.1) and peroxidase

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(POD, EC 1.11.1.7) are highly active (Manzocco, Plazzotta, Spilimbergo, & Nicoli, 2017; Marszałek, Kruszewski, Woźniak, & Skąpska, 2017, Marszalek et al., 2018). From this point of view control of the browning reactions has gained growing attention in the fruit and vegetable industry (Lee, Seo, Rhee, & Kim, 2016; Putnik, Bursać Kovačević, Herceg, Roohinejad, et al., 2017).

Thermal treatments have been traditionally used for microorganism inactivation as well as to effectively control the enzymes that negatively affect the quality of foods (Marszałek, Woźniak, Skąpska, & Mitek, 2017; Misra et al., 2017; Putnik, Roohinejad, et al., 2017). However, the use of conventional thermal treatments can promote some nutritional and quality losses. Therefore, there is an increased interest in innovative approaches alternative to thermal treatment (Misra et al., 2017). For instance, supercritical carbon dioxide processing (SCCD) can be used as alternative technology to thermal treatment and can be comparable to other innovative technologies such as high pressure processing (HPP) or high pressure homogenization (HPH), as it similarly preserves heat-labile compounds and inactivate microorganism, whereas allows decreasing enzyme activity in a more efficient way (≈10-time lower pressures compared to HPP or HPH) (Ferrentino & Spilimbergo, 2011; Illera et al., 2018; Liu, Hu, Zhao, & Song, 2012; Marszałek, Woźniak, Kruszewski, & Skąpska, 2017; Spilimbergo, Komes, Vojvodic, Levaj, & Ferrentino, 2013).

It was already demonstrated that SCCD assures satisfactory effect on enzyme and microbial inactivation in apple, strawberry and vegetable juices (Marszałek, Kruszewski, et al., 2017; Marszałek, Krzyżanowska, Woźniak, & Skąpska, 2016, 2017; Marszałek, Skąpska, Woźniak, & Sokołowska, 2015). On the other hand, the effect of SCCD treatment on the chemical quality of fruit and vegetable products are still under study. In the recent literature, there are only limited reports evaluating the influence of SCCD on enzyme activity, nutritional and bioactive compounds in cloudy apple juice, while no reports are available on the impact of long time storage changes of phenolic compounds profile under SCCD treatment at pressures up to 60 MPa. However, these issues are fundamental to be investigated in order to boost the commercialization of this technology.

In these regards, the aim of this study was to study the shelf-life of cloudy apple (*Golden delicious*) juice after SCCD treatment at different process pressure (10–60 MPa) in terms of enzyme activity and nutritional, bioactive and quality characteristics. The analysis were focused on the color changes, sugar and polyphenol profile, vitamin C concentration, PPO and POD enzymes activity immediately after SCCD treatment and during subsequent cold storage up to 10 weeks. Additionally, the substrate specificity and synergistic effect in relation to PPO and POD enzymes was investigated.

2. Materials and methods

2.1. Reagents

Ultrapure water was obtained from a Direct-Q 3 UV system (Merck Milipore, Darmstadt, Denmark). Polyvinylpyrrolidone (PVP) (~110 μm) (Fluka, USA); catechol (> 99%), hydrogen peroxide (30%), p-Penylenediamine, Triton X–100 as well as the polyphenols standards (procyanidin B_1 (PB_1), procyanidin B_2 (PB_2), phloridzin (PHL) , chlorogenic acid (CHL), (+)-catechin (CAT), *p*-coumaric acid (p-COU), (−)-epicatechin (EPI), gallic acid (GAL) and L-tyrosine) and the enzymes used in the study (horseradish peroxidase from *Armoracia rusticana* roots and mushroom polyphenol oxidase from *Agaricus bisporus*) were purchased from Sigma-Aldrich (Poznan, Poland). Other reagents were bought from POCh (Warsaw, Poland).

2.2. Sample preparation

Fresh apples (*Malus domestica* Borkh. cv. *Golden Delicious*) were purchased from a local supermarket (Warsaw, Poland). Before processing the fruits were washed, disintegrated and immediately squeezed to obtain juice (J 80 Ultra, Robot Coupe, France). Fresh squeezed apple juice was divided into two portions. The first portion was untreated – fresh juice, whereas the second was immediately (up to 30 min) processed by SCCD at different process conditions.

2.3. Supercritical carbon dioxide (SCCD)

SCCD experiment was performed using a Spe-ed SFE 4 (Applied Separations, USA) equipment with vessel of 500 mL. The chamber temperature was measured at the center of the reactor within a miniaturized stainless steel capsule with iButton® temperature logger (−40 °C to +85 °C, DS1922L, Maxim Integrated, USA), while the pressure was measured at inlet valve; data were displayed on the control panel.

For each experiment, ca. 60 mL of juice were placed in a sterile 160 mL glass jars and then placed in the high pressure vessel which was preheated to the experimental temperature. Before each experiment the vessel was sanitized (in the autoclave at 120 °C for 30 min).

The pressure were chosen: 10, 30, 60 MPa for 30 min. The lowest pressure was selected above the critical point for carbon dioxide (7.36 MPa), 30 MPa as a mild pressure while the maximum pressure for safety of the equipment. Treatment temperatures (45 °C) and times (30 min) were selected based on previous studies (Marszałek, Kruszewski, et al., 2017). The pressurization ramp was 60 MPa/min, while depressurization at the end of the SCCD treatment took place in 5 min. Pressurization and depressurization time were excluded from the process time. After the treatment, the samples were removed from the high pressure vessel under laminar cabinet, closed and cooled up to 4 ± 2 °C. The SCCD-treated apple juices were stored in a chamber at 4 ± 2 °C. The juice was analyzed immediately after SCCD treatments and then after 4, 14, 28, 46, 56 and 70 days (10 weeks). Experiments and measurements were performed in duplicate. Microbial stability of fruit juices up to 10 weeks of storage has been proved in our previous works (Marszałek et al., 2015) under the conditions evaluated in the present study.

2.4. Physicochemical analysis

The pH was measured using pH meter HI 2210 (Hanna Instruments, Woonsocket, USA). The total soluble solid (TSS) content was estimated using a MS REF 090L refractometer (My Soft, Warsaw, Poland). The color changes were measured using a ColorQuest XE colorimeter (Hunter Lab, Germany), in glass cuvettes with an optical path of 5 mm. The measurement was made using the CIE*Lab* system, using illuminant D_{65}. The color parameters were expressed as *L* (whiteness or brightness/darkness), *a* (redness/greenness), and *b* (yellowness/blueness). The total color differences (ΔE) (Eq. (1), were calculated from the Hunter *L, a* and *b*-values and used to describe the color changes.

$$\Delta E = \sqrt{(L_0-L)^2 + (a_0-a)^2 + (b_0-b)^2} \quad (1)$$

2.5. HPLC analysis of sugars

Sugars profile were determined according to the EN 12630:1999 technical standard. The HPLC analyses were carried out using a Waters 2695 (USA) system with Sugar-Pak I column (10 μm, 6.5 mm × 300 mm) and Guard-Pak column, 10 μm (both Waters, USA). The separation was performed at a flow rate of 0.5 mL/min and a column temperature of 90 °C during 18 min. Samples were eluted isocratically using 0.1 mM calcium disodium EDTA as a eluent. The sugars were quantified using a refractive index detector 2414 (Waters, USA). The contents of sugars were expressed in mg of each sugar per L of juice.