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High pressure processing of cocoyam, Peruvian carrot and sweet potato: Effect on oxidative enzymes and impact in the tuber color

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ABSTRACT

High pressure processing (HPP) is a non-thermal technology used to activate or inactivate enzymes. This study investigated the effects of HPP (600 MPa for 5 or 30 min at 25 °C) on cocoyam, Peruvian carrot and sweet potato color, and the polyphenoloxidase (PPO) and peroxidase (POD) activities in tuber cubes, puree, and enzyme extract subjected to HPP. The results showed enzyme inactivation by HPP in cocoyam (up to 55% PPO inactivation in puree and 81% POD inactivation in extract) and Peruvian carrot (up to 100% PPO and 57% POD inactivation the extract). In contrast, enzyme activation was observed in sweet potato (up to 368% PPO and 27% POD activation in puree). The color results were compatible to enzyme activity: the color parameters remained unchanged in cocoyam and Peruvian carrot, which showed high PPO and POD inactivation after HPP. Furthermore, the impact of HPP on the enzymes was influenced by the matrix in which HPP was carried out, evidencing that the enzyme structure can be protected in the presence of other food constituents.

Industrial relevance: The enzymes PPO and POD are an important concern for vegetable processing, due its ability to induce browning after vegetables are cut. The HPP at 600 MPa for 5 or 30 min can be used to inactivate these enzymes in cocoyam and Peruvian carrot, guaranteeing the color and freshness of the tubers similar to the fresh cut vegetable.

1. Introduction

Cocoyam (*Dioscorea rotundata*), Peruvian carrot (*Arracacia xanthorrhiza*) and sweet potato (*Ipomoea batatas*) are tubers used as a source of carbohydrate in human nutrition. It is commonly marketed in fresh form, and the lack of industrialization limits its consumption, due to the growing consumer demand for easy-to-prepare and ready-to-eat products.

Cutting tubers can result in color and flavor changes during storage, due to the action of the enzymes peroxidase (POD) and polyphenoloxidase (PPO) (Hendrickx, Ludikhuyze, Van Den Broeck, & Weemaes, 1998; Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007). The browning mechanism is intensified after cutting, once cell disruption leads to the interaction between polyphenolic substrates and enzymes in the presence of oxygen. The PPO catalyzes two reactions: (1) hydroxylation of monophenols to diphenols, and (2) oxidation of diphenols to quinones. Although POD activity may be partially responsible for browning reactions that occur in tubers (according to several authors), it occurs only in the presence of hydrogen peroxide (H_2O_2), which is generally found at very low concentrations in plant cells (Degl'Innocenti, Guidi, Pardossi, & Tognoni, 2005; Richard-Forget & Gauillard, 1997; Underhill & Critchley, 1995; Veljovic-Jovanovic, Noctor, & Foyer, 2002).

PPO and POD inactivation is necessary to maintain the quality of these tubers due to enzymatic browning reactions in foods. Although thermal blanching is widely used for this propose, it impacts in sensory and nutritional damage of the vegetable (Tribst, Sant'ana, & de Massaguer, 2009).

High pressure processing (HPP) – also called as high hydrostatic pressure (HHP) or high isostatic pressure – is a non-thermal emerging technology developed to be an alternative to traditional food thermal processing (Mújica-Paz, Valdez-Fragoso, Samson, Welti-Chanes, & Torres, 2011). The gradual cost reduction of the process (Buzrul & Alpas, 2012) makes the technology increasingly accessible for processing of various foods.

The effects of HPP on microorganism inactivation are well established, once processes at 600 MPa for a few minutes at room temperature are considered similar to thermal pasteurization (Mújica-Paz et al., 2011; Rastogi et al., 2007). Furthermore, the FDA recommends the use of elevated pressure (580 MPa) for pasteurization of low acid food,

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aiming to inactivate microorganisms as *Escherichia coli* O157:H7, *Listeria* spp., *Salmonella* spp. and *Staphylococcus* sp. However, the process time has not been established by FDA (FDA, 2010); thus, pressure around 600 MPa can be considered adequate for commercial proposes.

Therefore, the application of HPP technology to process vegetables has emerged, aiming to keep their nutritional and healthy characteristics. Therefore, the application of HPP on vegetables, mainly for fruit products, represents the main application of the technology (Mújica-Paz et al., 2011).

The effect of HPP on enzymes was less intensively studied, but in general, HPP is described as a process that changes enzyme functionality, inactivating enzymes at high pressures (>400 MPa) and, in several cases, activating enzymes at lower pressures (up to 200 MPa) (Eisenmenger & Reyes-De-Corcuera, 2009; Mozhaev, Lange, Kudryashova, & Balny, 1996; Mújica-Paz et al., 2011; Sila et al., 2007). Unfortunately, POD and PPO are normally related as baroresistent, remaining active at pressures up to 600 MPa in various vegetable products (Cano, Hernandez & De Ancos, 1997; Bayindirli, Alpas, Bozoglu, & Hizal, 2006; Huang et al., 2013). On the other hand, some authors have found significant inactivation of these enzymes in other vegetables sources (Cao et al., 2011; Hernández & Cano, 1998; Soysal, Söylemez, & Bozoğlu, 2004; Yamaguchi, Kato, Noma, Igura, & Shimoda, 2010), possibly highlighting that the baroresistance is dependent on the food matrix and pre-process applied to it before HPP.

Enzyme activation is normally due to interactions between food constituents and release of enzymes and substrates from vegetable membranes under pressure (Huang et al., 2013; Rastogi et al., 2007). Also, the process can activate latent isoenzymes (Guerrero-Beltrán, Barbosa-Cánovas, & Swanson, 2005) due to the changes in the enzyme conformation that possibly exposes active sites, leading to an increase in enzyme activity (Huang et al., 2013).

Therefore, the different sensitivity of PPO and POD to HPP reported in previous studies may be due to the source of enzyme, substrate, pressure, time, and temperature of the process (Mújica-Paz et al., 2011). Additionally, according to Akyol, Alpas, and Bayindirli (2006), the environment to which the enzyme is subjected can affect its resistance, once enzyme extracts are more resistant than the enzyme in fruit or vegetable tissue. However, the lack of studies about the HPP effects on the enzymes from vegetables subjected to process at different forms impairs the evaluation of how cellular integrity and presence of substrate can affect the enzyme activity.

Studies on the effect of HPP on oxidative enzymes in tubers are scarce. Previous studies have shown that processes at 600 MPa for 5 or 30 min are interesting conditions to obtain cocoyam, Peruvian carrot, and sweet potato which are semi ready-to-eat due to changes on their physical structure (softening) and starch gelatinization (Oliveira, Tribst, Leite Júnior, Oliveira, & Cristianini, 2015). Complementary to these results the impact of HPP on PPO and POD in these tubers should be investigated, aiming to evaluate whether the HPP process can limit undesirable browning and preserve the visual characteristics of the vegetables.

2. Material and methods

2.1. Vegetables and sample preparation

The cocoyam (*D. rotundata*), Peruvian carrot (*A. xanthorrhiza*), and sweet potato (*I. batatas*) were purchased from a local market (Campinas, Brazil), cleaned up and peeled. The tubers were sliced in cubes of 0.5 cm and immersed in water at 15 °C to minimize the initial browning. After, 20 cubes were packaged in flexible bags (LDPE-Nylon-LDPE, 16 μm thickness — TecMaq, Brazil) under vacuum to be processed as cube samples. A puree was prepared by milling of 20 pieces of tubers in an analytical mill (Model A11 B S32, IKA®, Germany). The puree was immediately packaged under vacuum to minimize initial browning. Additionally, an enzyme extract was prepared according to Section 2.4.1. Non-processed tuber, cubes, puree, and enzyme extract were used as the control.

2.2. Experimental set-up

The experiments were performed to determine the impact of HPP on different tubers. Color was determined in the cubes and puree samples, simulating two possible commercial products. For the analysis of enzyme activity, HPP was carried out in different matrices (cube, puree, and enzyme extract), aiming to elucidate how the food matrix can affect enzyme activation/inactivation, considering that:

i) Tuber cubes have enzymes and substrates initially separated by the preserved physical structure of the plant (cell), which is partially disrupted during pressurization (Oliveira et al., 2015);
ii) Tuber purees have enzymes and substrates susceptible to chemical reactions due to the disruption of cellular tissue during milling, with consequent physical proximity of both;
iii) Enzyme extracts contain only the enzymes diluted in 0.2 M phosphate buffer (pH 6.5) during pressurization.

Considering the general equation of the enzyme reaction ($E + S \leftrightarrow ES \rightarrow P$), the enzyme may be in different configurations at the time of the HPP (the enzyme was processed isolated in the extract, and complexed (ES) and isolated (different degrees) in the cube or puree). Furthermore, the enzyme can interact (cube and puree) or not (extract) with other constituents of the tubers. Thus, the evaluation of each sample allows explaining the reactions that occurred in each matrix, and consequently the effect of HPP under different conditions.

2.3. High pressure processing

High pressure processing was carried out using an Avure (QFP 2L-700) system produced by Avure SPA Vasteras (Avure Technologies, OH, USA). The HPP system has a 2 l-capacity chamber, and reaches pressures of 690 MPa and works at temperatures between 10 and 90 °C. The temperature of the chamber was measured by a type K thermocouple inserted into the chamber, one located in the top and other in the middle. The pressure was captured by a pressure transducer. The compression time to reach 600 MPa was around 122.7 ± 6.9 s and the decompression was practically instantaneous (2.2 ± 0.3 s). The temperature of the chamber block was set at 25 °C. The initial temperature of the water in the chamber was set at 8–10 °C reaching 27.1 ± 1.3 °C (due to adiabatic heating) at the beginning, and 25.2 ± 1.7 °C at the end of the process. The process time was 5 and 30 min at 600 MPa, considering a regular HPP for inactivation of pathogens (5 min), and a process with an extended time (30 min) to confer more changes in enzyme activity. Each tuber was processed at each process condition in triplicate.

2.4. Analysis

2.4.1. Enzyme extraction and activity

The enzyme activity was measured in cubes, puree, and extracts subjected to HPP. To determine the enzyme activity, the enzyme was extracted as follows: 10 g tubers and 0.5% of polyvinylpyrrolidone (PVPP) were homogenized in a mill (Model A11 B S32, IKA®, Germany). Then, 50 mL of phosphate buffer 0.1 M (pH 6.5) was added and homogenized in a high shear mixer (ULTRA-TURRAX®, Model T-25D, IKA®, Germany) at 16,000 rpm for 2 min. The homogenized sample was centrifuged at 15,300 g/4 °C/15 min (Beckman Coulter®, Brea, CA, USA) and the supernatant containing the enzymes polyphenoloxidase (PPO) and peroxidase (POD) (crude extract) were used to determine the enzyme activity (Cano, Hernandez & De Ancos, 1997). For the puree samples subjected to HPP, the extraction procedure was carried out as described above using 10 g of processed puree