Innovative Food Science and Emerging Technologies 39 (2017) 230–240

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

The effect of high pressure processing on clingstone and freestone peach cell integrity and enzymatic browning reactions

Chukwan Techakanon [a,b], Gary M. Smith [a], Judy Jernstedt [c], Diane M. Barrett [a,*]

[a] Department of Food Science and Technology, University of California, Davis, One Shields Avenue, Davis, CA 95616, United States
[b] Faculty of Science and Industrial Technology, Prince of Songkla University, Surat Thani Campus, 31 Makham Tia, Muang Surat Thani, Suratthani 84000, Thailand
[c] Department of Plant Sciences, University of California, Davis, CA 95616, United States

ARTICLE INFO

Article history:
Received 18 March 2016
Received in revised form 11 December 2016
Accepted 19 December 2016
Available online 21 December 2016

Keywords:
High pressure processing
Membrane integrity
^{1}H NMR
Polyphenol oxidase
Peaches
Enzymatic browning

ABSTRACT

HPP-treated fruits and vegetables may undergo undesirable enzymatic browning reactions due to loss of membrane permeability and sub-cellular compartmentalization. Clingstone and freestone peaches were treated from 100 to 500 MPa for 10 min and evaluated for polyphenol oxidase (PPO) activity, color, total phenols, and for cell integrity using light microscopy and ^{1}H NMR. Significant changes in membrane integrity following HPP above 200 MPa were determined by T_2 shifts in the vacuolar compartment from initial levels of 0.79 (clingstone) or 0.88 (freestone) to approximately 0.60–0.68. Clingstone peaches treated at 300, 400 and 500 MPa showed significant decreases (5, 12 and 7%) in % water of the vacuolar compartment and simultaneous increases in the cytoplasmic compartment (4, 8 and 5%). Additionally, there was a reduction in the number of viable cells from an initial 57–58% to 0 and 14% in clingstone and freestone peaches, respectively. These results correlated with the development of increased browning.

Industrial relevance: Clingstone peaches are firm-textured and therefore are preserved primarily through canning, which desirably softens the texture. In this study we evaluated the use of high pressure processing – at a range of MPa levels – for preservation, and found that enzymatic browning took place after 2 weeks in refrigerated storage if processing occurred above 200 MPa. Analytical tools were developed to follow the onset of the browning, and in future work preventative measures will be studied to minimize this reaction.

1. Introduction

High pressure processing (HPP) is a novel food preservation method that is currently receiving the most attention from the food sector, because it is successful in creating products of high nutritional value and sensorial quality. The advantage of this preservation method is that it is not only a microbiologically safe means of providing longer shelf life but also retains the fresh characteristics of products.

In general, pressures in the range of 100–600 MPa are used for food preservation (Palou et al., 2000), but these pressures may also result in loss of cell and therefore tissue integrity. In plant-based materials, cell integrity plays an important role in final product characteristics, in particular color and texture. Loss of cell integrity involves increased membrane permeability, resulting in opening of subcellular organelles and movement of water and metabolites within the cell. High pressure treatment was previously reported to affect the peach enzymes involved in changes in color, polyphenol oxidase activity (Rao et al., 2014) and activity of the enzyme involved in textural changes, pectin methylesterase (Boulekou, Katsaros, & Taoukis, 2010). Undesirable color changes in HPP-processed fruit are a result of enzymatic browning reactions induced by the loss in cell integrity. This loss allows the interaction between the enzyme, initially located in the plastid of the intact fruit, and its substrates, which are initially located in the vacuole. Color is a primary quality attribute in fruits, therefore enzymatic browning reactions have been a crucial problem in HPP treated fruits e.g. mango puree (Guerrero-Beltrán, Barbosa-Cánovas, & Swanson, 2005), banana puree (Palou, López-Malo, Barbosa-Cánovas, Welti-Chanes, & Swanson, 1999), tomato puree (Sánchez-Moreno, Plaza, De Ancos, & Cano, 2006) and navel orange juice (Polydera, Stoforos, & Taoukis, 2005). The complexity of the browning reaction and its substantial impact on food quality has motivated food scientists to explore it at the cellular level. Numerous authors have considered that PPO (1,2-benzenediol; oxygen oxidoreductase, EC 1.10.3.1) and phenolic compounds are the major factors involved in the enzymatic browning reaction (Lee, Kagan, Jaworski, & Brown, 1990; Cheng & Crisosto, 1995; Coseteng & Lee, 1987). However, Cantos, Tudela, Gil, and Espín (2002) observed different results in potatoes, where there was no significant correlation between the degree of browning and any biochemical attribute they tested, e.g. PPO, peroxidase or initial phenolic content. These observations led us to the idea that cell integrity and ability of the enzyme and its substrate to interact may be an important factor controlling browning reactions.

* Corresponding author.
E-mail address: dmbarrett@ucdavis.edu (D.M. Barrett).

http://dx.doi.org/10.1016/j.ifset.2016.12.011
1466-8564/

A combination of NMR-relaxometry and light microscopy were used in this study to characterize the integrity of membranes and cells. These techniques were successfully employed for quantification of onion cell integrity following thermal and HPP processing (Gonzalez, Barrett et al., 2010) and for determining the effect of HPP on strawberry parenchyma tissue (Marigheto, Vial, Wright, & Hills, 2004). ^{1}H NMR relaxometry is widely applied in plant research for probing sub-cellular changes. The proton spin–spin (T_2) relaxation time is related to water content, the properties of water in different cellular locations, and the interaction of water with macromolecules (Snaar & Van As, 1992). Therefore the change in permeability of a membrane, in particular the tonoplast, which encloses the large percentage of cellular water located in the vacuole, should be evident by a change in the T_2 spectrum. The observation of cell integrity using light microscopy is accomplished using a cell viability staining method. In this study, neutral red (NR) is the viability stain used for discriminating intact vacuoles (Admon, Jacoby, & Goldschmidt, 1980). The main property of this initially yellow dye is to diffuse across the tonoplast membrane into the acidic environment of the vacuole, where it undergoes a color change to an intense red. Once damage occurs in plant cells, the tonoplast loses its integrity, releasing the neutral red dye throughout the cell and resulting in a quantifiably less-intense red color.

In general, peaches are classified into two major types, clingstone and freestone. The clingstone type has a stone (endocarp) that tightly clings to the flesh (mesocarp) and usually has a firm texture, which is desired for commercial canning. Freestone peaches are those in which the flesh is easily freed from the stone, and are consumed fresh due to their soft texture. Because of genetically controlled differences between these two types, e.g. integrity of the mesocarp as well as level of PPO activity and concentration of phenolic compounds, browning scenarios in clingstone and freestone peaches following HPP treatment hypothesized to differ. The objectives of this study are to determine the effect of pressure levels in the range of 100–500 MPa on cell integrity in clingstone and freestone peaches, using NMR and microscopic studies, and to correlate that to the development of brown color, PPO activity and total phenols content.

2. Materials and methods

2.1. Raw materials

The clingstone type peach cultivar, Carson, and the freestone type, Summerset, were harvested by hand from Foundation Plant Services, at the University of California, Davis, CA. Peaches were sorted and only fruit at the mid-ripe stage, which corresponded to a firmness of 35–40 N using a TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK), were stored at 4 °C for approximately two days until processing. Three fruit at the same range of firmness were hand peeled, sliced into approximately 3 cm thick slices and placed into polyethylene bags (4 mil vacuum pouches, Ultrasource, Missouri, USA). Each bag contained 3 peach slices of the same peach type, 1 from each of the 3 different fruits, and separate bags were created for each analysis method. Approximately 2 mL of peach extracts were vacuum packed in polyethylene bags. All of the samples were kept at ambient temperature (22 ± 2 °C) for 30 min after packaging prior to HPP treatment. The same fruit was analyzed for all parameters, e.g. difference in lightness, the paramagnetic study using NMR, PPO activity, total phenols and determination of viable cells using light microscopy. On each replicate day of processing, six packages per each of the peach types were processed at each of the five pressures (100–500 MPa). The control treatment was an unprocessed, sliced peach sample in a vacuum package (approximately 0 MPa).

2.2. High pressure processing (HPP)

The packaged samples were processed at 100, 200, 300, 400, and 500 MPa for 10 min in a high pressure processing unit (Avure Technologies Inc., Kent, WA). The initial high pressure unit temperature (Ti) was around 23 °C. The maximum temperature in the high pressure chamber was dependent on the set pressure, which for 100–500 MPa was 25, 28, 30, 33, and 35 °C, respectively. The high pressure unit had a 2.0 L vessel and 600 MPa maximum pressure level. The pressurizing medium was water. In each operation, there will be a come-up stage during which pressure is built up to the target pressure, a constant pressure stage for 10 min and a decompression stage. At the end of the holding period, pressure is released to atmospheric pressure within a few seconds. Three replicates were performed on three separate days.

2.3. Nuclear magnetic resonance (NMR) relaxometry

Following high pressure processing, one cylindrical piece was obtained from each of three slices using a cork borer with a 15 mm diameter and 15 mm height. The samples were blotted dry before being placed into a covered NMR tube, which was placed in a plastic sample holder. NMR relaxometry measurements were performed after samples reached room temperature using an NMR spectrometer (Aspect AI, Industrial Area Havel Modi'in, Shoham, Israel) with a magnetic field of 1.02 T and frequency of 43.5 MHz. T_2 was measured using the Carr-Purcell-Meiboom-Gill sequence with an echo time of 0.5 ms and 15,000 echoes. T_2 spectrum inversion using Laplace transformation was performed on the raw data to determine the change in each plant cell compartment. Raw data were then processed by a non-negative least square algorithm using Prospa (Magritek, Wellington, New Zealand).

2.4. Light microscopy

2.4.1. Section preparation

Following the HPP process, the sliced samples were further cut into small rectangular cuboids approximately 1.0 × 0.5 × 0.3 cm and placed in a sample holder. Sections approximately 200 μm in thickness were obtained using a Vibratome1000 Plus (The Vibratome Co., St. Louis, Missouri, U.S.A.).

2.4.2. Neutral red staining

Stain was prepared using 0.5% neutral red in acetone stock solution, which was filtered twice with Whatman paper # 1, and diluted to 0.04% in 0.55 M mannitol–0.01 M HEPES (*N*-[2-hydroxyethyl] piperazine-*N*′-[2-ethane-sulfonic acid]) buffer, pH 7.8. Peach sections were soaked in the staining solution for a period of 2 h, after which they were rinsed for 0.5 h in the 0.55 M mannitol–0.01 M HEPES buffer solution.

2.4.3. Toluidine blue O staining

This technique allows for observation of cell walls following HP treatment and determination of cell size (O'Brien, Feder, & McCully, 1964). Peach sections of 200 μm thickness were immersed in a solution of 0.025% TBO in water for 10 min before being transferred to a microscopic slide.

A drop of de-ionized water was added to the section and covered with a cover slip. Sections were observed with a light microscope (Olympus System Microscope, Model BHS, Shinjuku-Ku, Tokyo, Japan) at 40× and 100× objective magnification. A digital color camera (Olympus MicroFire, Olympus, Tokyo, Japan) was attached to the microscope to capture images (Olympus software, Olympus America, Melville, N.Y., U.S.A.) providing color photomicrographs (800 × 600 pixel resolution).

2.5. Image processing and analysis

Image processing software, Image J (NIH, U.S.A.), was used for micrograph processing and analyzing. Fifteen micrographs were randomly