Innovative Food Science and Emerging Technologies 36 (2016) 212–220

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

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

Biochemical and microstructural assessment of minimally processed peaches subjected to high-pressure processing: Implications on the freshness condition

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ARTICLE INFO

Article history:
Received 4 April 2016
Received in revised form 4 June 2016
Accepted 30 June 2016
Available online 4 July 2016

Keywords:
High pressure processing
Peaches
Viability
Enzymes
Freshness
Microstructure

ABSTRACT

Since the condition of freshness is closely linked to the definition of minimally processed fruits, it is generally assumed that they should be constituted by living tissues. High-pressure processing (HPP) has been proven to be successful in maintaining the freshness of foods, although the integrity of some tissues could be altered. The aim of this work was to assess different biochemical and microstructural changes experimented by the HPP-treated tissues. The following determinations were conducted in control and treated diced peaches: viability assay, microstructure analysis, relative expression of RNAm, and enzyme activities related to browning and anaerobic metabolism. Results showed that although HPP-treated tissues were not viable, changes in the expression of the RNAs of the enzymes evidenced that some metabolic processes were still active. In turn, the microstructure remained rather unaltered, while the enzymes tested were significantly inhibited. Then, although some characteristics of living tissues were modified in the HPP-treated fruits, they could be considered as fresh in appearance. *Industrial relevance:* It is well known that conventional thermal treatments applied to preserve fruit products usually cause important changes in flavor, color, texture, and consequently the loss of fresh appearance. However, in the case of novel technologies such as high-pressure processing (HPP), this causality is not straightforward. Interestingly, the claim of "freshness" in foods has become a controversial topic, since some regulations indicate that for a food to be regarded as fresh, it should not have been subjected to any preservation processing. However, a list of exceptions has been considered for processes such as irradiation at low doses, or application of edible coatings. This list of exception is expected to increase, accordingly to the raise in successful experiences with the application of novel technologies such as HPP. This work provides information on the effect of HPP on different biochemical and structural aspects of treated peaches, with the aim to contribute toward the commercial application of HPP to minimally processed fruits.

1. Introduction

According to the definition, minimally processed fruits should have been subjected to processes that do not substantially modify the characteristics of the raw material, with the aim to be transformed into a product suiting the convenience of consumers and/or retailers. Since the products under this category implicitly require a condition of freshness, some authors assume that these products should be entirely constituted by living tissues (Artés & Artés-Hernández, 2003; Wiley, 1994). When fruits are preserved by conventional thermal treatments, the difference between fresh and processed product is clear, since these treatments interrupt all metabolic processes, with the consequent loss of the fresh appearance, causing significant changes in the flavor, color, and texture. However, when novel "nonthermal" technologies such as high-pressure processing (HPP) are applied to plant products, this causality is not straightforward (Sánchez-Moreno et al., 2005). Therefore, it becomes apparent that some traditional definitions should be reviewed and accordingly adapted, by setting apart, for example, the concept of "freshness" from the condition of "living tissue."

Although canned peaches, which constitute one of the most popular canned desserts, can be considered as an alternative for the consumption of fruits (Saura, Laencina, Pérez-López, Lizama, & Carbonell-Barrachina, 2003), from a nutritional point of view, one of the major problems associated with this kind of products is the need of adding sugar and the loss of heat-labile nutrients because of the use of

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http://dx.doi.org/10.1016/j.ifset.2016.06.026
1466-8564/

preservative thermal treatments (Miller, Pang, & Broomhead, 1995; Rickman, Barrett, & Bruhn, 2007). Therefore, consumer preferences have been evolved until leaning nowadays toward the selection of fresh or, at least, minimally processed products without additives.

The application of HPP for the preservation of minimally processed fruits constitutes an innovative area of research. Therefore, the literature about specific topics such as the effect of HPP on structural and biochemical characteristics of pressurized fruit tissues is rather scarce. However, some of the subjects already studied are the effect of HPP on the microstructure, texture, and some bioactive compounds (Vázquez-Gutiérrez, Hernández-Carrión, Quiles, Hernando, & Pérez-Munuera, 2012) and the development of biochemical reactions taking place in certain plant tissues (Van der Plancken et al., 2012).

Considering the well-established success of HPP in preserving the nutritional value and the sensory characteristics of foods (i.e. their freshness), one of the aims of this work was to analyze the biochemical response of the tissues subjected to HPP, through the determination of the viability of cells conforming the tissues, the relative expression of enzymes linked to enzymatic browning (phenylalanine ammonia-lyase—PAL and polyphenol oxidase—PPO) and anaerobic metabolism (alcohol dehydrogenase-ADH and pyruvate decarboxilase-PDC), and the direct measurement of the activity of related enzymes (PPO and ADH). The conditions of the HPP applied in the present study were defined, according to our preview experience (Denoya et al., 2016), as those leading to the minimal alteration of peach texture while causing the maximal inactivation of microorganisms and enzymes.

Another aspect evaluated in the present work was the microstructure of tissues, by assessing the changes brought about by the HPP treatments. These changes can be caused by both enzymatic and non-enzymatic transformations of the cell wall polymers, as well as by the compression of the cell structure after the degassing of the tissue. In this regard, several publications (Denoya, Vaudagna, & Polenta, 2015; Perera, Gamage, Wakeling, Gamlath, & Versteeg, 2010; Vercammen et al., 2012) pointed out the absence of significant changes in the texture of pressurized fruit or vegetable products. Therefore, it is expected that the assessment of the microstructural aspects underlying the application of HPP can provide with a better explanation for the visual changes experimented by fruits, such as the appearance of translucency, and can also help to unveil issues related to the cell viability.

2. Material and methods

2.1. Plant material

Peaches (*Prunus persica* (L.) Batch) cv. Flavorcrest were harvested from an experimental orchard in San Pedro Buenos Aires, Argentina (Latitude 33°41 S, Longitude 59°41 W). Fruits were carefully selected according to their uniform size and maturity by ground color. Soluble solids were 12° Brix on average. Firmness of fruit was between 20 N and 30 N. The pH was between 3.4 and 3.5. The fruit were stored at 0 °C in a cold chamber until processing.

2.2. Sample preparation

Prior to processing, the peaches were washed in running tap water. Cylinders (15 mm length, 15 mm diameter) from the parenchyma tissue were obtained using a cork borer and a stainless steel knife. In order to obtain homogeneity in the samples, the cylinders were taken from the middle zone of the mesocarp, parallel to the major axis of the fruit. Subsequently, the cylinders were dipped into tap water containing 20 ppm of HClO for 2 min. After being drained, the cylinders were dipped into an aqueous solution containing 1% ascorbic acid (ACS, Biopack, Argentina) and 1% citric acid (USP, Anedra, Austria) for 2 min to prevent surface browning and to wash the remaining HClO. The cylinders were drained again and vacuum-packed in Cryovac BB2800 bags (O_2 transmission rate: 6–14 $cm^3/m^2/24$ h at 23 °C, 1 atm) filled with eight units each, using a double-chamber vacuum-packing machine (Rapivac, Model Maximax 800, Argentina). The samples were divided into two different groups: 1) HPP-treated samples (P); 2) control samples (C), with no additional treatments.

HPP-treated samples were packed into an additional outer bag to prevent leakages. HPP was performed in a high hydrostatic pressure system Stansted Fluid Power Ltd. High Pressure IsoLab System model FPG9400:922 (Stansted, UK) with a vessel of 2 L capacity and a maximum working pressure of 900 MPa. A mixture of propylene glycol 30% (v/v) and distilled water was used as the pressure-transmitting medium. The process parameters of the HPP treatment were selected considering the optimal conditions determined in a preliminary study (Denoya et al., 2016) and were 600 MPa for 5 min. Compression rate was 5 MPa s^{-1}. The pressurization was carried out at an initial temperature of 22 °C, which was only modified by adiabatic heating (up to 38 °C).

2.3. Viability assay

Viability assay was determined according to the method described by Martínez, Nieto, Castro, Salvatori, & Alzamora (2007) with some modifications. Cross-sections of peach tissue were immersed for 5 min in a Fluorescein Diacetate (FDA, MP Biomedicals, France) solution prepared from a 5 mg mL^{-1} FDA solution in acetone that was diluted with phosphate buffer 0.05 M pH 6 to a final concentration of 0.1% w/v. Sections were observed with an epifluorescent microscope Axiolab HB050X (Carl Zeiss, Germany) using a blue filter (excitation: 450–500 nm and emission: 520 nm).

2.4. Change in a* color parameter

The color parameter a* of peach cylinders was measured with a Minolta CR-400 chromameter (Konica Minolta Sensing, Inc. Osaka, Japan), using the CIE scale, where a* represents chromaticity on a green (−) to red (+) axis. The parameter was measured on the surface of the cylinders immediately after opened the packages and after 180 min. The change of a* in time was calculated as a ratio (Δa*/min) and was measured as a browning rate parameter. The instrument was set up for illuminant D_{65} and 2° observer angle.

2.5. Relative expression of enzymes at RNAm level

2.5.1. RNA extraction

Total RNA was isolated from 4 g of tissue using the method described by Meisel et al. (2005). RNA pellets obtained were then dissolved in 40 μL of GIBCO® ultra-pure water (Invitrogen, Massachusetts, USA) and incubated at 56 °C for 5 min.

In order to avoid DNA contamination, samples were then treated with Deoxiribonuclase I (Invitrogen, Massachusetts, USA) according to the manufacturer instructions.

2.5.2. Primers design

Primer Express 3.0 (Applied Biosystems, California, USA) was used to design the qPCR primers, based on EF1 (elongation factor 1), TEF2 (translational elongation factor 2), ADH, PAL, and PDC gene sequences from species of *Prunus* genus, preferable *persica*, which were all retrieved in GenBank (Table 1). These sequences were aligned with Clustal W2 software (available at the European Bioinformatics Institute website: http://www.ebi.ac.uk/Tools/clustalw2/). In order to check in silico specificity, BLAST software *(NCBI—www.ncbi.nlm.nih.gov)* was employed. The nucleotide sequences of the primers obtained for each gene are listed in Table 2.

2.5.3. Quantitative real-time RT-PCR

Relative gene expression was determined by quantitative one-step real-time RT–PCR in a PCR Real-Time StepOne Plus System (Applied