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Analysis of the bacterial biodiversity of peaches under refrigerated storage after treatment by high hydrostatic pressure

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ABSTRACT

This study aimed at determining the effect of high hydrostatic pressure (HHP) treatments on the bacterial biodiversity of processed peaches. Peach cubes in peach juice inoculated with epiphytic microbiota recovered from peach surfaces were pressurized at 600 MPa for 8 min at temperatures of 22 °C and of 45 °C. Non-pressurized samples were used as controls. Samples were chill stored for 15 days. Application of HHP treatments reduced viable counts of total aerobic mesophiles and yeasts and molds, and delayed recovery of survivors. Pyrosequencing analysis revealed that 35.7% of operational taxonomic units (OTUs) from peaches samples belonged to *Actinobacteria* (mainly *Couchioplanes*, *Bifidobacterium* and *Propionibacterium*), 31.1% to *Firmicutes* (mainly *Streptococcus*, *Lactobacillus* and *Enterococcus*) and 28.8% to *Proteobacteria* (mainly *Reyranella*, *Methylobacterium*, *Acetobacter*, *Dyella*, *Sphingomonas*, *Tatumella*, *Enterobacter*, *Prevotella* and *Gluconobacter*). During storage of control samples, the relative abundances of *Gluconobacter* and *Lactobacillus* increased. Application of HHP treatments reduced the relative abundance of *Actinobacteria* and *Firmicutes* while *Proteobacteria* increased. The opposite was observed during storage of the treated samples. Increasing the treatment temperature to 45 °C decreased the relative abundance of *Actinobacteria*. Results from the present study indicate that peach microbiota is strongly influenced by refrigeration storage and by application of HHP treatments.

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1. Introduction

The development of high-throughput sequencing technologies (HTS) has fueled the interest for studying the microbial diversity of foods and the dynamics of microbial populations during food storage (Cocolin and Ercolini, 2015; Ercolini, 2013). A pioneer study by Leff and Fierer (2013) compared the surface microbiota of several fruits and vegetables (including apples, grapes, lettuce, mushrooms, peaches, peppers, spinach, sprouts, strawberries and tomatoes) by 454 pyrosequencing analysis targeting the 16S rRNA region. Pyrosequencing has also been applied to study the changes in the microbiota of vegetable foods (cherimoya pulp and green asparagus) treated by high hydrostatic pressure (HHP) during refrigerated storage (Pérez Pulido et al., 2015; Toledo del Árbol et al., 2016). However, there are no previous studies on the microbiota of peaches or how it can be affected by HHP treatments.

HHP processing has shown to be an effective method to improve the microbiological safety and shelf-life of various types of fruit juices and purées (Guerrero-Beltrán et al., 2005; Rastogi et al., 2007). HHP inactivates the vegetative cells of foodborne pathogens and spoilage bacteria that spoil the foodstuffs, with minimal changes in the organoleptic and nutritional properties (Barba et al., 2012, 2015; Rendueles et al., 2011). Previous studies have addressed the application of HHP treatments for preservation of peaches (Guerrero-Beltrán et al., 2004; Denoya et al., 2015, 2016), but none addressed the study of microbial diversity.

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Among the treatment conditions tested, 600 MPa for 5 min achieved highest enzyme inactivation while preserving texture and color of minimally processed peaches (Denoya et al., 2016). HHP treatments efficiently inactivated the foodborne pathogens *Listeria monocytogenes* and *Salmonella typhimurium* and reduced the total microbial load in peach juice (Dogan and Erkmen, 2004; Erkmen, 2011). However, aerobic bacteria required longer treatment time (25 min) for complete inactivation (Erkmen, 2011). The growth of spoilage microorganisms during refrigerated storage of peach pieces in sucrose solution after HHP treatment at 600 MPa for 5 min was studied by Argyri et al. (2014), who reported that total viable counts, lactic acid bacteria and yeasts remained below the limit of detection for 104 days in the HHP-treated samples while microbial growth was observed in the untreated controls. Studies carried out in vitro and in other food systems have shown that increasing the process temperature in the range 45–50 °C increase the rate of inactivation of food pathogens and spoilage microbes (Alpas et al., 2000; Raso and Barbosa-Canovas, 2003). In spite of the extended use of HHP processing, little is known about its effects on the diversity of microbial populations in foods or the dynamics of the surviving fractions during storage of the treated foods.

The aim of the present study was to determine the changes in the microbiota of a mixture of peach cubes in peach juice (with or without HHP treatments) during refrigeration storage by means of HTS technology. Since the efficacy of HHP treatments can be improve by increasing the treatment temperature, this study also aimed at determining the effect of treatment temperatures (22 °C and 45 °C) on the microbial load and on the dynamics of the surviving bacterial microbiota during refrigerated storage.

2. Materials and methods

2.1. Sample preparation

Peaches (*Prunus persica* (L.) var. BabyGold) were bought in the same day from 4 different stores at the province of Jaen (Southern Spain) in the month of June 2014. Peaches (3.12 kg) were placed in sterile stomacher bags and washed by hand rubbing with 300 mL sterile saline solution in order to recover the surface microbiota. The resulting cell suspension was centrifuged (3500 × *g*, 30 min) and the sediment was resuspended in 10 mL sterile saline solution. The obtained suspension was stored under refrigeration until use (not more than 3 h). In parallel, peaches were peeled under aseptic conditions and sliced with a sterile knife. Peach juice was prepared from peach slices by using a Moulinex EASY FRUIT (Moulinex, Berkshire, UK) fruit juice extractor, under aseptic conditions. At the same time, peach slices obtained as above were cut into cubes (approx. 1 × 1 cm). Then, 10 mL of the obtained peach juice and 10 ± 1.0 g peach cubes were packed inside a zip lock bag and placed on ice. The different bags were inoculated (200 μL each) with the suspension of microbial cells recovered from peach surfaces. Then, each zip lock bag was placed inside a polyethylene–polyamide bag and vacuum-sealed. Eight bags were used as controls and sixteen were used for HHP treatments as will be described below.

2.2. High hydrostatic pressure treatments

High hydrostatic pressure (HHP) treatments were carried out by using a Stansted Fluid Power LTD HHP equipment (SFP, Essex, UK) suited with a 2.5 L vessel and a temperature control module. Peach samples were pressurized at 600 MPa for 8 min, based on experience from previous trials using lower pressure and shorter time conditions. Come-up speed was 75 MPa/min. Decompression was almost immediate. Pressurization fluid was water with added 10% propylenglycol. HHP treatments were carried out at room temperature (22 °C), and also at 45 °C. The temperature inside the vessel ranged between 23.0 and 27.0 °C during treatments carried out at room temperature, and between 44.5 and 48.0 °C for treatments carried out at 45 °C. Samples were placed on ice immediately after application of HHP treatments. Controls and HHP-treated samples were stored at 4 °C for up to 15 days.

After treatments and also at desired times during storage (1, 7, and 15 days), two bags from controls and two from each HHP treatment were mixed individually with sterile saline solution (20 mL per bag) and pummeled in stomacher bags for 2 min. The obtained pulp suspension was serially diluted in sterile saline solution and plated in triplicate on Trypticase Soy Agar (TSA, Scharlab, Madrid) for total aerobic mesophilic counts and on Yeast Mannitol Agar (Scharlab) with added Chloramphenicol (Sigma Aldrich, Madrid) at 100 mg/L (YMA–CM) for yeasts and molds. Plates were incubated at 30 °C for 24 h (TSA) or 48 h (YMA–CM). The pH of pulp suspensions was measured with a pH meter (Crison Instruments, S.A., Barcelona, Spain).

2.3. DNA extraction, amplicon library preparation and sequencing

Aliquots (1.5 mL) of homogenized peach samples obtained as described above were centrifuged at 600 × *g* for 5 min in an Eppendorf centrifuge in order to sediment pulp solids. The supernatant was transferred to a new Eppendorf test tube and centrifuged at 13.500 × *g* for 5 min to recover microbial cells. The pellets obtained from each sample were resuspended in 0.5 mL sterile saline solution each. Then, Propidium Monoazide (PMA^{TM}, Biotium, UK) was added to block subsequent PCR amplification of the genetic material from dead cells (Nocker et al., 2006, 2007) as described by Elizaquivel et al. (2012). DNA from PMA-treated cells was extracted by using a GenElute^{TM} Bacterial Genomic DNA Kit (Sigma-Aldrich, Madrid), following instructions provided by the manufacturer. DNA recovered from duplicate samples was then pooled into a single sample. DNA concentration and quality were measured with a NanoDrop spectrophotometer (Thermo Scientific, United Kingdom).

For pyrosequencing, V3–V5 region of the 16S rRNA gene was amplified using key-tagged bacterial primers prepared by LifeSequencing S.L. (Valencia, Spain) based on Sim et al. (2012) and Pérez Pulido et al. (2015). Library preparation, pyrosequencing, and bioinformatic analysis were carried out by LifeSequencing S.L. services as described by Pérez Pulido et al. (2015).

2.4. Statistical analysis

The average data ± standard deviations from viable cell counts were determined with Excel programme (Microsoft Corp., USA). A *t*-test was performed at the 95% confidence interval with Statgraphics Plus version 5.1 (Statistical Graphics Corp, USA), in order to determine the statistical significance of data corresponding to viable cell counts. Data on the microbial composition of samples for the different treatments applied and at different storage times were analyzed by principal component analysis (PCA) using XLSTAT 2014 evaluation version (2015.1, Addinsoft, France). The Pearson correlation coefficient (r) was applied, and correlations were defined as very weak (0.00–0.19), weak (0.20–0.39), moderate (0.4–0.59), strong (0.60–0.79) or very strong (0.80–0.99), with a *P* significance of <0.05. Only data for genera with relative abundances ≥ 1.0 were used for the PCA analysis.

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