LWT - Food Science and Technology 78 (2017) 289–295

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Changes in bacterial diversity of refrigerated mango pulp before and after treatment by high hydrostatic pressure

Rubén Pérez Pulido, Mª José Grande Burgos, Antonio Gálvez*, Rosario Lucas

Área de Microbiología, Departamento de Ciencias de la Salud, Facultad de Ciencias Experimentales, Universidad de Jaén, 23071 Jaén, Spain

ARTICLE INFO

Article history:
Received 7 August 2016
Received in revised form
20 December 2016
Accepted 26 December 2016
Available online 28 December 2016

Keywords:
Mango
High hydrostatic pressure
Bacterial diversity

ABSTRACT

The purpose of the present study was to determine the effect of high hydrostatic pressure treatment (HHP) on the microbiota of mango pulp. Mango pulp was artificially contaminated with its own epiphytic microbiota to simulate a worst-case scenario of contamination during pulp preparation. Controls and samples treated by HHP (600 MPa, 8 min) were chill stored for 30 days. HHP treatment significantly ($P < 0.05$) reduced viable cell counts. Pyrosequencing analysis of the bacterial community revealed that the relative abundances found in the starting control samples for *Actinobacteria* (45.63%), *Firmicutes* (42.55%), *Proteobacteria* (10.68%) and *Bacteroidetes* (1.0%) changed during storage, with a strong increase of *Proteobacteria*. HHP treatment also induced a strong increase in *Proteobacteria*, followed by a late recovery of *Firmicutes* and to a less extent *Actinobacteria*. *Lactobacillus* was the main operational taxonomic unit (OTU) detected both in controls during early storage and in HHP-treated samples during late storage. Results from the present study indicate how bacterial populations of both controls and HHP-treated mango pulp samples undergo complex changes during refrigeration storage.

1. Introduction

The mango (*Mangifera indica* L. Anacardiaceae) is one of the most commercialised fruits in tropical countries (Muchiri, Mahungu, & Gituanja, 2012). Mango puree, slices in syrup, nectar, leather, pickles, canned slices and chutney are the main industrial products obtained from mango fruits (Jahurul et al., 2015). Mango fruit is an excellent source of antioxidants and vitamins (Abbasi et al., 2015; USDA, 2010). Mango polyphenolics including gallic acid and gallotannins have been shown to elicit cytotoxic and anti-inflammatory properties (Banerjee, Kim, Krenek, Talcott, & Mertens-Talcott, 2015; Kim et al., 2016).

The marketability of fresh mango is limited by its short shelf-life. Mango is usually processed and preserved by thermal pasteurization, which often leads to deterioration of organoleptic attributes and loss of nutritional quality (Ahmed, Shivhare, & Kaur, 2002; Miller & Silva, 2012; Ndiaye, Xu, & Wang, 2009). High hydrostatic pressure treatments (HHP) do not affect covalent bonds, and have been reported to only cause minor loss of low molecular weight compounds in vegetables, such as pigments, vitamins, and flavor substances (Butz et al., 2002). HHP processing of mango has been reported to result in moderate to non-significant losses in ascorbic acid content (Kaushik, Kaur, Rao, & Mishra, 2014; Liu, Li, Wang, Bi, & Liao, 2014), while its measurable carotenoid content was not affected or even increased (Liu, Wang, Li, Bi, & Liao, 2013; Liu et al., 2014). HHP treatment inactivates *Escherichia coli* and *Aspergillus niger* in mango nectar (Bermúdez-Aguirre, Guerrero-Beltrán, Barbosa-Cánovas, & Welti-Chanes, 2011; Tribst, Franchi, Cristianini, & de Massaguer, 2009) and prolongs the product shelf life (Jacobo-Velázquez, Ramos-Parra, & Hernández-Brenes, 2010). HHP treatment at 600 MPa has been reported to achieve maximum reduction in microflora and moderate changes in quality attributes in fresh mango pulp (Kaushik et al., 2014).

Most studies on the effects of HHP processing on the food microbiota have been based on culture-dependent methods. Recently, high-throughput sequencing (HTS) technology has been applied to identify changes in microbial populations during the shelf-life of HHP-processed foods stored under different conditions (Pérez Pulido, Toledo, Grande, Gálvez, & Lucas, 2015; Toledo del Árbol et al., 2016). Moreover, HTS can also provide insights into the microbiota of raw materials and sources of contamination (Cocolin & Ercolini, 2015; Ercolini, 2013), and to study the bacterial communities associated with the surfaces of fruits and vegetables

* Corresponding author. Present address: Área de Microbiología, Departamento de Ciencias de la Salud, Facultad de Ciencias Experimentales, Edif. B3, Universidad de Jaén, Campus Las Lagunillas s/n, 23071 Jaén, Spain.
E-mail address: agalvez@ujaen.es (A. Gálvez).

http://dx.doi.org/10.1016/j.lwt.2016.12.050
0023-6438/

(Leff & Fierer, 2013; Lopez-Velasco, Carder, Welbaum, & Ponder, 2013). However, there are no previous studies on the bacterial diversity of mango pulp processed by HHP.

The purpose of the present study was to determine the effects of HHP treatments on the bacterial diversity of mango pulp stored under refrigeration. Mango pulp was inoculated with its own epiphytic microbiota in order to simulate a worst-case surface contamination scenario during processing. In order to determine the effects of HHP treatments on the microbiota of mango pulp, high-throughput sequencing of the 16S rRNA gene was carried out after treatment and during sample storage.

2. Materials and methods

2.1. Sample preparation

Mangoes (*Mangifera indica* L. var Kent) were purchased from four different local food stores and kept under refrigeration until processing (for no more than 24 h). A microbial suspension from mango surfaces was obtained by washing 10 representative mango units (4230 g total weight) with sterile saline solution. The obtained suspension was washed with sterile saline solution, resuspended in 5 ml saline and stored under refrigeration for no longer than 18 h before it was inoculated into freshly-made mango pulp. For preparation of mango pulp, mangoes were peeled and sliced under aseptic conditions with a sterile knife. Slices were processed with a Braun Vario 350W blender (Braun GmbH, Germany) to obtain the final mango pulp.

Mango pulp (250 g) was inoculated (2%, vol/vol) with the suspension of epiphytic microbiota obtained as described above to yield a final concentration of 4.4 $\log_{10}$ CFU/g total aerobic mesophilic counts and distributed in 10-ml aliquots in sterile zip-lock bags. Then, samples were placed individually inside polyethylene-polyamide bags and sealed under vacuum. Ten bags were used as controls and ten treated by HHP 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 capable of operating in a pressure range of 0–700 MPa. Mango pulp samples were pressurized at 600 MPa for 8 min. Come-up speed was 75 MPa/min. Decompression was almost immediate. Pressurization fluid was water with added 10% propylenglycol. The temperature inside the vessel during treatments ranged between 23 and 27 °C. All samples (treated or not by HHP) were stored at 4 °C for up to 30 days.

After treatments and also at desired times during storage (1, 7, 15 and 30 days), two bags from controls and two from HHP-treated samples were removed. Each bag was mixed with sterile saline solution (10 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 tryptic soya 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 mango samples obtained as described above were centrifuged at 600×*g* for 5 min in an Eppendorf centrifuge in order to remove pulp solids. The supernatants were transferred to new Eppendorf test tubes 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™, Biotium, UK) was added to block subsequent PCR amplification of the genetic material from dead cells (Nocker, Cheung, & Camper, 2006; Nocker, Sossa-Fernandez, Burr, & Camper, 2007) as described by Elizaquivel, Sánchez, and Aznar (2012) and Toledo del Árbol et al. (2016). DNA from PMA-treated cells was extracted by using a GenElute™ 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 Life-sequencing S.L. (Valencia, Spain) based on Sim et al. (2012). PCR reactions were performed with 20 ng of community DNA, 200 μM of each of the four deoxynucleoside triphosphates, 400 nM of each primer, 2.5 U of FastStart HiFi Polymerase, and the appropriate buffer with $MgCl_2$ supplied by the manufacturer (Roche, Mannheim, Germany), 4% of 20 mg/ml BSA (Sigma, Dorset, United Kingdom), and 0.5 M Betaine (Sigma). Thermal cycling consisted of initial denaturation at 94 °C for 2 min followed by 30 cycles of denaturation at 94 °C for 20 s, annealing at 50 °C for 30 s, and extension at 72 °C for 5 min. To obtain sufficient material, PCR reactions were repeated in triplicate and pooled prior to purification by running the PCR amplicons on 1% (w/v) agarose gels. Amplicons were quantified using the PicoGreen assay (Quant-iT, PicoGreen DNA assay, Invitrogen) and combined in a single tube in equimolar concentrations. The pooled amplicon mixture was purified twice (AMPure XP kit, Agencourt, Takeley, United Kingdom) and the cleaned pool requantified with PicoGreen assay. Amplicons were submitted to the pyrosequencing services offered by Life Sequencing S.L. (Valencia, Spain) where EmPCR was performed and subsequently, unidirectional pyrosequencing was carried out on a 454 Life Sciences GS FLX+ instrument (Roche) following the Roche Amplicon Lib-L protocol.

2.4. Bioinformatic analysis

Bioinformatic analysis was carried out by Life Sequencing S.L. services. Raw reads were first filtered according to the 454 processing pipeline. Pyrosequencing reads were filtered with Q20 FASTX_tool_kit version 0.0.14, and reads were excluded from the analysis if they had an average quality score < Q20 and if there were ambiguous base calls (Ns). Reads were trimmed for adaptors and PCR primers, and only reads greater than 300 nts were retained for analysis. Chimera were eliminated using the Uchime algorithm under default mode (UCHIME version 4.2.40). The average final lengths of reads ranged from 559 to 588 nt. Sequences were compared with NCBI 16 S rRNA database using BLASTN. Operational taxonomic units (OTUs) were defined by a 97% similarity. The read clusters were further assigned to taxonomies using the RDP classifier.

2.5. Statistical analysis

All experiments were carried out in duplicate. 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 viable cell count data. 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 (2014.1.03, Addinsoft, France). The Pearson correlation coefficient (r) was applied. 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.

3. Results

3.1. Effect of treatments on viable counts

Mango pulp was artificially contaminated with its own epiphytic microbiota to simulate a worst-case scenario of contamination during pulp preparation. In control samples without any treatment, total aerobic mesophilic (TAM) counts remained stable (4.37–4.19 $\log_{10}$ CFU/g) for the first day, but then increased significantly ($P < 0.05$) to 6.7 $\log_{10}$ CFU/g at day 7 and reached a maximum of 7.58 $\log_{10}$ CFU/g at day 15 (Table 1). Application of HHP treatment reduced TAM counts significantly ($P < 0.05$) to 1.74 $\log_{10}$ CFU/g. The surviving fraction did not increase or decrease significantly ($P > 0.05$) during the whole storage period (Table 1). Counts for yeasts and molds (YM) also remained stable for the first day in control samples but then increased by day 7, reaching similar levels as TAM during the remaining storage period. However, in the HHP-treated samples, YM counts remained below detectable levels for most of the storage period, except at day 15 (Table 1). The pH of samples increased slightly during storage from 3.37 to 3.93 in control samples and to 3.56 in the HHP-treated samples at the end of storage.

3.2. Impact of treatments and storage time on bacterial diversity

The main operational taxonomic units (OTUs) detected in mango pulp belonged to *Actinobacteria* (45.63%) and *Firmicutes* (42.55%), followed by *Proteobacteria* (10.68%) and *Bacteroidetes* (1.0%) (Fig. 1A). The most representative genera detected were *Lactobacillus* (*L. salivarius*), *Propionibacterium* (*P. acnes*), *Bifidobacterium* (*B. longum*), *Kocuria* (*K. palustris*) and *Flavonifractor* (*F. plautii*) (Fig. 1B and C). Main changes in microbial populations during storage included an increase in relative abundances of *Microbacterium* (*M. hydrocarbonoxydans*) and *Dyella* (*D. jiangningensis*) at day 7, and a remarkable increase of *Erwinia* (*E. billingiae*) becoming the predominant OTU (97.21%) at day 15. By day 30, the predominant OTUs belonged to *Pantoea* (*P. vagans*, 57.19%) and *Curtobacterium* (*C. plantarum*, 40.44%).

Most OTUs (91.33%) detected after application of HHP treatment belonged to *Proteobacteria*, while the relative abundance of *Firmicutes* and *Actinobacteria* decreased remarkably. The main genera detected were *Sphingomonas* (*S. paucimobilis*, 42.07%), *Delftia* (*D. tsuruhatensis*, 27.87%), *Lysobacter* (*L. thermophilus*, 6.47%) and *Sphingobium* (*S. yanoikuyae*, 4.77%).

During storage of the HHP-treated samples, the relative abundance of OTUs belonging to *Proteobacteria* decreased, while *Firmicutes* increased progressively from day 1 (Fig. 1). Among *Proteobacteria*, only *Dyella* (*D. jiangningensis*) showed a relatively stable abundance during storage (from days 1–30). Among *Firmicutes*, the relative abundance of *Lactobacillus (L. salivarius*) increased progressively during storage. *Enterococcus* (*E. faecalis*) was also detected at high relative abundance, but only at day 15 (24.04%). The relative abundance of *Actinobacteria* also increased during storage, specially at day 7, with *Propionibacterium*, *Bifidobacterium*, *Corynebacterium* and *Microbacterium* as main representatives.

Principal component analysis (PCA) of relative abundances at genus level revealed that untreated control samples stored from days 0–7 clustered closely (Fig. 2A) and had moderate or strong ($r = 0.5567–0.7140$) and significant ($P < 0.05$) positive correlations between them (Table 2). These samples were associated with *Lactobacillus*, *Propionibacterium* and *Bifidobacterium* (Fig. 2B) In contrast, samples from storage times 15 and 30 showed weak, non-significant ($P > 0.05$) negative correlations ($r = -0.0782$ to -0.8147), indicating strong changes in microbial composition that were associated with high relative abundances of *Erwinia*, *Pantoea* and *Curtobacterium*. HHP treatment samples from time 0 were associated with *Sphingomonas* and *Delftia,* while treated samples for storage times 7–30 clustered separately and were associated with *Lactobacillus*, *Propionibacterium* and *Dyella* (Fig. 2). HHP-treated samples from time 0 showed weak negative correlations with most other samples ($P > 0.05$), while treated samples from storage times 7–30 had significant ($P < 0.05$) moderate positive correlations ($r = 0.4707–0.5857$) between them and also significant ($P < 0.05$) positive correlations with control samples from day 1 (moderate, $r = 0.5098$; strong, $r = 0.6978$, or very strong, $r = 0.9368$) (Table 2).

4. Discussion

Surface microbiota is often the main source of microbial contamination of processed fruits and vegetables. In the present study, mango pulp was inoculated with surface-recovered microbiota to simulate a worst-case scenario of contamination during pulp preparation and also to facilitate the study of changes in the surviving fraction during storage of the treated samples. Preliminary tests indicated that microbial load in pulps under normal contamination conditions could be lower, of approximately between 2 and 3 $\log_{10}$ CFU/g (unpublished results). After application of HHP treatment at 600 MPa for 8 min, there was a residual surviving fraction of ca 1.7 $\log_{10}$ CFU/g total aerobic mesophilic counts that tended to persist or decrease during storage (Table 1). This has been also reported in previous studies, and could be attributed to survival of bacteria showing greater tolerance to HHP treatments but also to the generation of sublethally injured cells. The yeast and mold fraction seemed to have a greater sensitivity to HHP

Table 1
Effect of HHP treatment and refrigeration storage on total aerobic mesophilic counts and yeast and molds in mango pulp inoculated with its own epiphytic microbiota.

Total aerobic mesophiles	Viable counts (Log_{10} CFU/g) at different storage times (days)				
	0	1	7	15	30
Control	4.37 ± 0.04	4.19 ± 0.12	6.69 ± 0.15	7.58 ± 0.16	7.13 ± 0.17
HHP	1.74 ± 0.19	1.40 ± 0.19	1.48 ± 0.22	1.65 ± 0.42	1.00 ± 0.47
Yeasts and molds					
Control	3.35 ± 0.12	3.14 ± 0.19	6.39 ± 0.19	7.13 ± 0.18	7.37 ± 0.09
HHP	<1.0	<1.0	<1.0	1.17 ± 0.24	<1.0

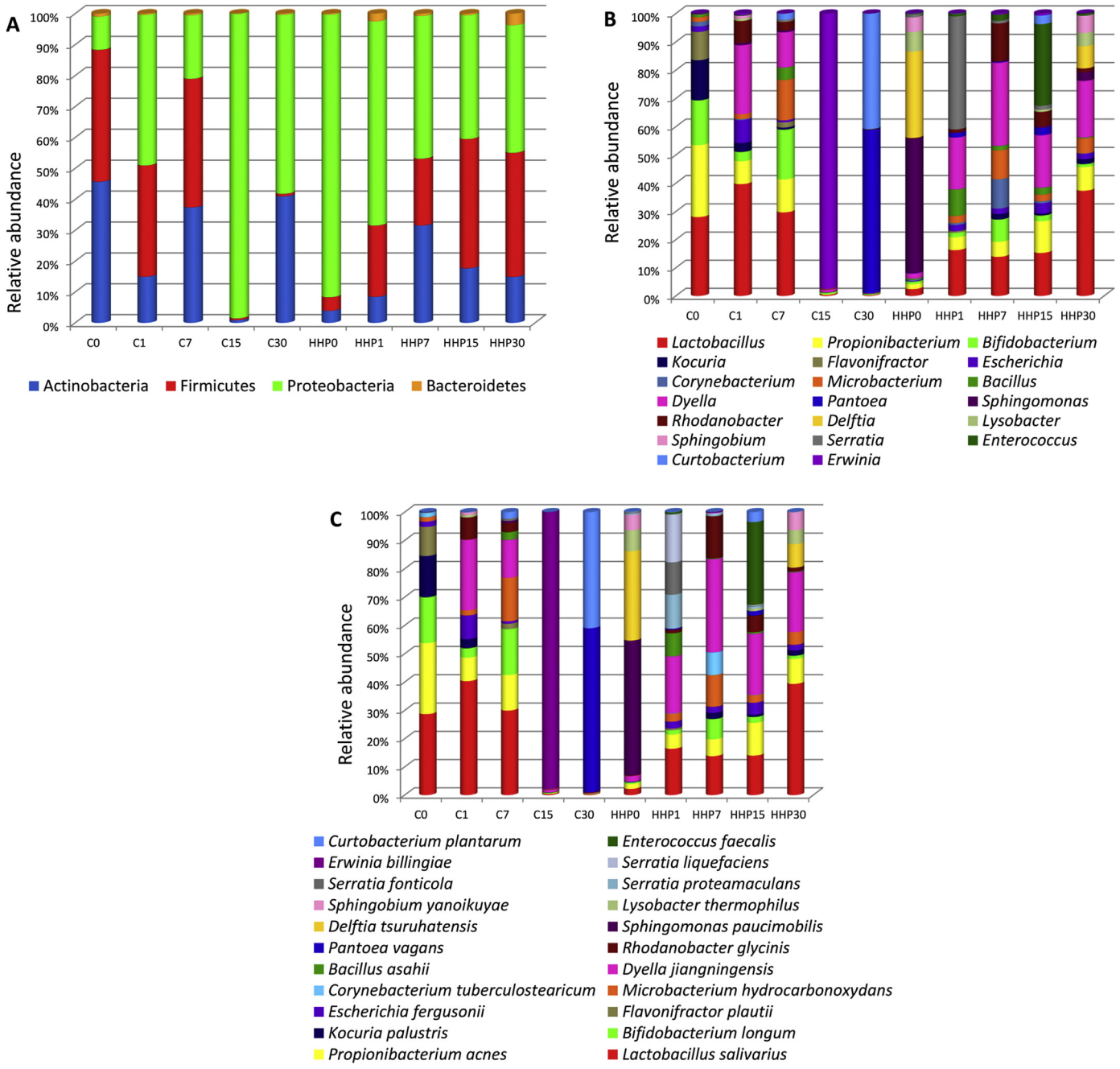

Fig. 1. Relative abundance of OTUs based on 16S rRNA gene pyrosequencing analysis of DNA from mango pulp treated or not by HHP. Mango pulp inoculated with epiphytic microbiota without any treatment ("C"), or treated by high hydrostatic pressure at 600 MPa for 8 min ("HHP"). Samples were analyzed at T0, 1, 7, 15 and 30 days of storage at 4 °C. OTUs were sorted by *Phylum* (**A**), *Genus* (**B**) and *Species* (**C**) taxonomic levels. The numbers indicate storage time (days).

treatments, since no survivors were detected after the HHP treatment (except at one storage point). The dynamics of the residual bacterial fraction during storage of the treated food product may be important to evaluate its microbiological stability. Yet, only limited studies have been carried out on the microbial dynamics of food products during storage.

In the present study, *Firmicutes* (mainly *Lactobacillus*) and *Actinobacteria* (mainly *Propionibacterium* and *Bifidobacterium*) were the predominant OTUs detected at initial stages, while Enterobacteriaceae only became relevant during late storage (Fig. 1A). This situation is also different from previous reports on surface microbiota from cherimoya pulp and from green asparagus, in which *Proteobacteria* (mainly *Pantoea* in the case of cherimoya pulp or *Pantoea* and *Pseudomonas* in the case of green asparagus) were the predominant OTUs found at early stages (Pérez Pulido et al., 2015; Toledo del Árbol et al., 2016). There are scarce reports on the microbiota from mango surfaces. A recent study analyzed the surface microbiota of Ataulfo mangoes by PCR amplification of the V3 region from the 16S rDNA gene followed by denaturing gradient gel electrophoresis and band sequencing (Fernández-Suárez et al., 2013). The predominant genera identified on the surface of mangoes were *Enterobacter*, *Pantoea*, and *Klebsiella*. Other genera (*Escherichia*, *Erwinia*, *Salmonella*, *Lactococcus*, *Weissella*, *Lactobacillus*, *Citrobacter*, *Pseudomonas*, and *Bacillus*) were also detected, in

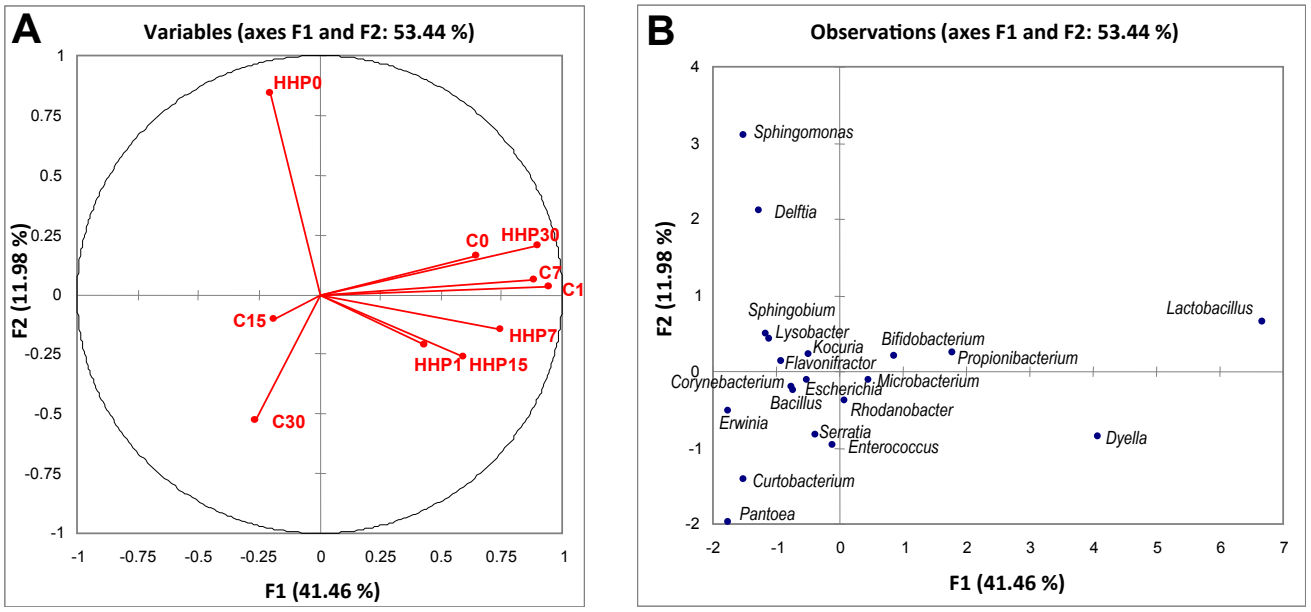

Fig. 2. Principal component analysis of sample variables (storage time and HHP treatment) (**A**) and observations (genus relative abundance) (**B**) of untreated controls ("C") and samples treated with high hydrostatic pressure ("HHP"). The numbers indicate storage time (days).

Table 2
Correlations between controls and HHP-treated samples at different storage times.

Variables	C0	C1	C7	C15	C30	HHP0	HHP1	HHP7	HHP15	HHP30
C0	1									
C1	0.5567	1								
C7	0.7140	0.7833	1							
C15	−0.1290	−0.0973	−0.1399	**1**						
C30	−0.1847	−0.1656	−0.1674	−0.0782	**1**					
HHP0	−0.1787	−0.1257	−0.1782	−0.0983	−0.1377	**1**				
HHP1	0.0753	0.3628	0.2722	−0.1172	−0.1372	−0.1626	**1**			
HHP7	0.1752	**0.6978**	**0.5943**	−0.1370	−0.2174	−0.2128	0.2976	**1**		
HHP15	0.2116	**0.5098**	0.3619	−0.1288	−0.0979	−0.2019	0.1932	**0.4707**	**1**	
HHP30	**0.5380**	**0.9368**	**0.7734**	−0.1250	−0.1848	0.0462	0.3229	**0.5857**	**0.4553**	**1**

Significant correlations at $p < 0.05$ are highlighted in bold.

a smaller proportion.

The OTU with highest relative abundance detected in the mango pulp from this study at time 0 belonged to genus *Lactobacillus* (*L. salivarius*) (Fig. 1B and C). The natural habitat of *Lactobacillus* species varies widely, from food, plants and sewage, to the oral, genital and gastrointestinal (GI) tracts of humans and animals (Claesson, van Sinderen, & O'Toole, 2007; Hammes & Hertel, 2006; Hammes & Vogel, 1995). *Propionibacterium* was the second most abundant OTU detected in mango pulp at time 0. Propionibacteria have been also described from sources other than the classical dairy foods or human skin, such as fermenting olives, olive mill wastewater and from the soil of rice paddies (Stackebrandt, Cummins, & Johnson, 2006), or from spoiled fruit juice (Kusano, Yamada, Niwa, & Yamasoto, 1997). Furthermore, *Propionibacterium acnes* was found in larvae of *Apis mellifera* and *Osmia bicornis* (Mohr & Tebbe, 2006), and was also very common in the bumblebee digestive tract (Killer et al., 2009). The third OTU in relative abundance belonged to *Bifidobacterium*. Bifidobacteria have been detected in the digestive tracts of insects such as honeybees (Scardovi & Trovatelli, 1969), bumblebees (Killer et al., 2009, 2011) and other insects, including wasps, hornets and cockroaches (Mrázek, Štrosová, Fliegerová, Kott, & Kopečný, 2008). Different types of insects, such as fruit piercing moths (*Eudocima* spp.) and fruit flies (*Bactrocera* spp. and others) can cause considerable damage in the mango flesh during suckling, but also by punctures or stings made by the female as she lays her eggs. In addition, secondary-moth feeders often visit fermenting fruit, taking advantage of the access holes (Peña, Mohyuddin, & Wysoki, 1998; Waite, 2002). It is tempting to suggest that the detected OTUs belonging to bifidobacteria and propionibacteria in mangoes could be related to infestation by insect pests. There are scarce reports about association of bifidobacteria with vegetable foods. Recently, *Bifidobacterium* was considered to be important in the acidogenic fermentation of fruit and vegetable wastes by using a simulated waste composed of watermelon, apple and potato (Wu, Ma, Zheng, & Wang, 2015). *Bifidobacterium longum,* being a primary colonizer of the infant gut, has attracted great attention because of its probiotic potential (Underwood, German, Lebrilla, & Mills, 2015). There is a growing interest in developing fruit juices enriched with probiotic bacteria including bifidobacteria (Perricone, Bevilacqua, Altieri, Sinigaglia, & Corbo, 2015; Phoem, Chanthachum, & Voravuthikunchai, 2015). Tentatively, mango pulp or mango juice could be also a candidate for probiotic bifidobacteria adapted to low pH.

Pyrosequencing data clearly indicated that the microbial populations in the untreated mango pulp did change during refrigeration storage, leading to displacement of *Firmicutes* by saprophytic *Proteobacteria* (*Erwinia*, *Pantoa*) and *Actinobacteria*

(*Curtobacterium*) (Fig. 1A and B). The most drastic change was detected at day 15 of storage, where 97.2% of OTUs belonged to *Erwinia*. These plant-associated *Enterobacteria* would most likely be adapted to grow in mango pulp and ferment available sugars, displacing other bacterial groups and allowing late proliferation of other epiphytes such as *Pantoea* and *Curtobacterium*.

High-throughput sequencing data indicated that the HHP treatment remarkably reduced the relative abundance of the main OTUs found in mango pulp, *Firmicutes* and *Actinobacteria* (Fig. 1A). The HHP treatment also had a dramatic influence on the dynamics of the surviving fraction during storage. Compared with the untreated controls, OTUs from *Erwinia, Pantoa* and *Curtobacterium* were not detected or were in a very low relative abundance in the HHP-treated samples, suggesting that these bacteria were inactivated by the HHP treatment. One exception was the gammaproteobacterium *Dyella*, whose OTU remained at relatively stable levels during storage. *D. jiangningensis* has been isolated from the surface of weathered rock (Zhao et al., 2013). Other species of genus *Dyella* have been reported from soils (Son et al., 2013), from living *Sphagnum* (Kostka et al., 2016) and the rhizosphere of tropical trees (Oh et al., 2012), but not from mangoes.

During late storage of the HHP-treated mango pulp, the observed increases in the relative abundances of OTUs belonging to *Lactobacillus*, *Bifidobacterium* and *Propionibacterium* could be explained by loss of viability of other bacterial populations (Fig. 1B). Multiplication of these bacteria in the pulp would also be a possibility, but demonstration of this hypothesis would require further studies using suitable culture media and incubation conditions, especially for the bifidobacteria and propionibacteria. Tentatively, during a late lactic acid fermentation of the pulp, lactic acid production by lactobacilli could provide an additional substrate for growth of propionibacteria while at the same time displace other heterotrophic spoilage bacteria.

In conclusion, results from the present study provide insights on the microbiota from mango surface and the dynamics of microbial populations in the mango pulp during refrigeration storage that lead to its spoilage. HHP treatments not only reduce the microbial load in the pulp but it also influences the dynamics of the surviving fraction during storage, allowing preservation of the refrigerated, HHP-treated pulp.

Acknowledgements

This work was supported by research grant AGL2012-38708/ALI (MICINN) and University of Jaen Plan de Apoyo a la Investigación. (AGR230)Rubén Pérez Pulido and Maria Jose Grande were supported by research grants from Campus de Excelencia Internacional Agroalimentario (CeiA3).

References

Abbasi, A. M., Guo, X., Fu, X., Zhou, L., Chen, Y., Zhu, Y., et al. (2015). Comparative assessment of phenolic content and in vitro antioxidant capacity in the pulp and peel of mango cultivars. *International Journal of Molecular Sciences, 16*(6), 13507–13527.

Ahmed, J., Shivhare, U. S., & Kaur, M. (2002). Thermal colour degradation kinetics of mango puree. *International Journal of Food Properties, 5*, 359–366.

Banerjee, N., Kim, H., Krenek, K., Talcott, S. T., & Mertens-Talcott, S. U. (2015). Mango polyphenolics suppressed tumor growth in breast cancer xenografts in mice: Role of the PI3K/AKT pathway and associated microRNAs. *Nutrition Research, 35*(8), 744–751.

Bermúdez-Aguirre, D., Guerrero-Beltrán, J.Á., Barbosa-Cánovas, G. V., & Welti-Chanes, J. (2011). Study of the inactivation of *Escherichia coli* and pectin methylesterase in mango nectar under selected high hydrostatic pressure treatments. *Food Science and Technology International, 17*(6), 541–547.

Butz, P., Edenharder, R., García, A. F., Fister, H., Merkel, C., & Tauscher, B. (2002). Changes in functional properties of vegetables induced by high pressure treatment. *Food Research International, 35*, 295–300.

Claesson, M. J., van Sinderen, D., & O'Toole, P. W. (2007). The genus *Lactobacillus*—a genomic basis for understanding its diversity. *FEMS Microbiology Letters, 269*(1), 22–28.

Cocolin, L., & Ercolini, D. (2015). Zooming into food-associated microbial consortia: A "cultural" evolution. *Current Opinion in Food Science, 2*, 43–50.

Elizaquivel, P., Sánchez, G., & Aznar, R. (2012). Quantitative detection of viable foodborne *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* in fresh-cut vegetables combining propidium monoazide and real-time PCR. *Food Control, 25*, 704–708.

Ercolini, D. (2013). High-throughput sequencing and metagenomics: Moving forward in the culture-independent analysis of food microbial ecology. *Applied and Environmental Microbiology, 79*, 3148–3155.

Fernández-Suárez, R., Ramírez-Villatoro, G., Díaz-Ruiz, G., Eslava, C., Calderón, M., Navarro-Ocaña, A., et al. (2013). Effect of postharvest UV-C treatment on the bacterial diversity of Ataulfo mangoes by PCR-DGGE, survival of *E. coli* and antimicrobial activity. *Frontiers in Microbiology, 4*, 134.

Hammes, W. P., & Hertel, C. (2006). The genera *Lactobacillus* and *Carnobacterium*. In M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, & E. Stackebrandt (Eds.), *The prokaryotes* (Vol. 4, pp. 320–403). Springer.

Hammes, W. P., & Vogel, R. F. (1995). The genus *Lactobacillus*. In B. J. B. Wood, & W. H. Holzapfel (Eds.), *The genera of lactic acid bacteria* (Vol. 2, pp. 19–54). Glasgow, UK: Blackie Academic & Professional.

Jacobo-Velázquez, D. A., Ramos-Parra, P. A., & Hernández-Brenes, C. (2010). Survival analysis applied to the sensory shelf-life dating of high hydrostatic pressure processed avocado and mango pulps. *Journal of Food Science, 75*(6), S286–S291.

Jahurul, M. H., Zaidul, I. S., Ghafoor, K., Al-Juhaimi, F. Y., Nyam, K. L., Norulaini, N. A., et al. (2015). Mango (*Mangifera indica* L.) by-products and their valuable components: A review. *Food Chemistry, 183*, 173–180.

Kaushik, N., Kaur, B. P., Rao, P. S., & Mishra, H. N. (2014). Effect of high pressure processing on color, biochemical and microbiological characteristics of mango pulp (*Mangifera indica* cv. Amrapali). *Innovative Food Science and Emerging Technologies, 22*, 40–50.

Killer, J., Kopečný, J., Mrázek, J., Koppová, I., Havlík, J., Benada, O., et al. (2011). *Bifidobacterium actinocoloniiforme* sp. nov. and *Bifidobacterium bohemicum* sp. nov., from the bumblebee digestive tract. *International Journal of Systematic and Evolutionary Microbiology, 61*, 1315–1321.

Killer, J., Kopečný, J., Mrázek, J., Rada, V., Benada, O., Koppová, I., et al. (2009). *Bifidobacterium bombi* sp. nov., from the bumblebee digestive tract. *International Journal of Systematic and Evolutionary Microbiology, 59*, 2020–2024.

Kim, H., Banerjee, N., Ivanov, I., Pfent, C. M., Prudhomme, K. R., Bisson, W. H., et al. (2016). Comparison of anti-inflammatory mechanisms of mango (*Mangifera Indica* L.) and pomegranate (*Punica Granatum* L.) in a preclinical model of colitis. *Molecular Nutrition & Food Research, 0*, 1–12.

Kostka, J. E., Weston, D. J., Glass, J. B., Lilleskov, E. A., Shaw, A. J., & Turetsky, M. R. (2016). The *Sphagnum* microbiome: New insights from an ancient plant lineage. *New Phytologist, 211*, 57–64.

Kusano, K., Yamada, H., Niwa, M., & Yamasoto, K. (1997). *Propionibacterium cyclohexanicum* sp. nov., a new acidtolerant ω-cyclohexyl fatty acid-containing *Propionibacterium* isolated from spoiled orange juice. *International Journal of Systematic Bacteriology, 47*, 825–831.

Leff, J. W., & Fierer, N. (2013). Bacterial communities associated with the surfaces of fresh fruits and vegetables. *PLoS One, 8*, e59310.

Liu, F., Li, R., Wang, Y., Bi, X., & Liao, X. (2014). Effects of high hydrostatic pressure and high-temperature short time on mango nectars: Changes in microorganisms, acid invertase, 5-hydroxymethylfurfural, sugars, viscosity, and cloud. *Innovative Food Science and Emerging Technologies, 22*, 22–30.

Liu, F. X., Wang, Y. T., Li, R. J., Bi, X. F., & Liao, X. J. (2013). Effects of high hydrostatic pressure and high temperature short time on antioxidant activity, antioxidant compounds and color of mango nectars. *Innovative Food Science and Emerging Technologies, 21*, 35–43.

Lopez-Velasco, G., Carder, P. A., Welbaum, G. E., & Ponder, M. A. (2013). Diversity of the spinach (*Spinacia oleracea*) spermosphere and phyllosphere bacterial communities. *FEMS Microbiology Letters, 346*, 146–154.

Miller, F. A., & Silva, C. L. M. (2012). Thermal treatment effects in fruit juices. In S. Rodrigues, F. André, & N. Fernandes (Eds.), *Advances in fruit processing technologies* (pp. 363–386). Boca Raton, USA: CRC Press.

Mohr, K. I., & Tebbe, C. C. (2006). Diversity and phylotype consistency of bacteria in the guts of three bee species (*Apoidea*) at an oilseed rape field. *Environmental Microbiology, 8*, 258–272.

Mrázek, J., Štrosová, L., Fliegerová, K., Kott, T., & Kopečný, J. (2008). Diversity of insect intestinal microflora. *Folia Microbiologica (Praha), 53*, 229–233.

Muchiri, D. R., Mahungu, S. M., & Gituanja, S. N. (2012). Studies on Mango (*Mangifera indica*, L.) kernel fat of some Kenyan varieties in Meru. *Journal of the American Oil Chemist's Society, 89*, 1567–1575.

Ndiaye, C., Xu, S. Y., & Wang, Z. (2009). Steam blanching effect on polyphenol oxidase, peroxidase and colour of mango (*Mangifera indica* L.) slices. *Food Chemistry, 113*(1), 92–95.

Nocker, A., Cheung, C. Y., & Camper, A. K. (2006). Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs. dead bacteria by selective removal of DNA from dead cells. *Journal of Microbiological Methods, 67*, 310–320.

Nocker, A., Sossa-Fernandez, P., Burr, M., & Camper, A. K. (2007). Use propidium monoazide for live/dead distinction in microbial ecology. *Applied and Environmental Microbiology, 73*, 5111–5117.

Oh, Y. M., Kim, M., Lee-Cruz, L., Lai-Hoe, A., Go, R., Ainuddin, N., et al. (2012). Distinctive bacterial communities in the rhizoplane of four tropical tree species.

Microbial Ecology, 64(4), 1018–1027.
Peña, J. E., Mohyuddin, A. I., & Wysoki, M. (1998). A review of the pest management situation in mango agroecosystems. *Phytoparasitica, 26*, 1–20.
Pérez Pulido, R., Toledo, J., Grande, M. J., Gálvez, A., & Lucas, R. (2015). Analysis of the effect of high hydrostatic pressure treatment and enterocin AS-48 addition on the bacterial communities of cherimoya pulp. *International Journal of Food Microbiology, 196*, 62–69.
Perricone, M., Bevilacqua, A., Altieri, C., Sinigaglia, M., & Corbo, M. R. (2015). Challenges for the production of probiotic fruit juices. *Beverages, 1*, 95–103.
Phoem, A. N., Chanthachum, S., & Voravuthikunchai, S. P. (2015). Applications of microencapsulated *Bifidobacterium longum* with *Eleutherine americana* in fresh milk tofu and pineapple juice. *Nutrients, 7*(4), 2469–2484.
Scardovi, V., & Trovatelli, L. D. (1969). New species of bifidobacteria from *Apis mellifica* L. and *Apis indica* F. A contribution to the taxonomy and biochemistry of the genus Bifidobacterium. *Zentralblatt Bakteriologie Parasitenkunde Infektionskrankheiten und Hygiene Abteilung II, 123*, 64–88.
Sim, K., Cox, M. J., Wopereis, H., Martin, R., Knol, J., Li, M. S., et al. (2012). Improved detection of bifidobacteria with optimised 16S rRNA gene based pyrosequencing. *PLoS One, 7*(3), e32543.
Son, H. M., Yang, J. E., Yi, E. J., Park, Y., Won, K. H., Kim, J. H., et al. (2013). *Dyella kyungheensis* sp. nov., isolated from soil of a cornus fruit field. *International Journal of Systematic and Evolutionary Microbiology, 63*(Pt 10), 3807–3811.
Stackebrandt, E., Cummins, C. S., & Johnson, J. L. (2006). Family Propionibacteriaceae: The genus *Propionibacterium*. In M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, & E. Stackebrandt (Eds.), *The prokaryotes* (Vol. 3, pp. 400–418). Springer.
Toledo del Árbol, J., Pérez Pulido, R., La Storia, A., Grande Burgos, M. J., Lucas, R., Ercolini, D., et al. (2016). Changes in microbial diversity of brined green asparagus upon treatment with high hydrostatic pressure. *International Journal of Food Microbiology, 216*, 1–8.
Tribst, A. A., Franchi, M. A., Cristianini, M., & de Massaguer, P. R. (2009). Inactivation of *Aspergillus niger* in mango nectar by high-pressure homogenization combined with heat shock. *Journal of Food Science, 74*(9), M509–M514.
Underwood, M. A., German, J. B., Lebrilla, C. B., & Mills, D. A. (2015). *Bifidobacterium longum* subspecies *infantis*: Champion colonizer of the infant gut. *Pediatric Research, 77*(0), 229–235.
USDA. (2010). National nutrient database for standard reference, SR-, fruits reports. In *Raw mango* (p. 449).
Waite, G. K. (2002). Pests and pollinators of mango. In J. E. Peña, J. L. Sharp, & M. Wysoki (Eds.), *Tropical fruit pests and pollinators: Biology, economic importance, natural enemies and control* (pp. 103–129). Wallingford, UK: CABI Publishing.
Wu, Y., Ma, H., Zheng, M., & Wang, K. (2015). Lactic acid reduction from acidogenic fermentation of fruit and vegetable wastes. *Bioresource Technology, 191*, 53–58.
Zhao, F., Guo, X. Q., Wang, P., He, L. Y., Huang, Z., & Sheng, X. F. (2013). *Dyella jiangningensis* sp. nov., a γ-proteobacterium isolated from the surface of potassium-bearing rock. *International Journal of Systematic and Evolutionary Microbiology, 63*(Pt 9), 3154–3157.