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High-pressure processing effects on foodborne bacteria by mid-infrared spectroscopy analysis

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ABSTRACT

High pressure processing is an emergent technology for food preservation that causes minimal changes in the characteristics of the foods, preventing food spoilage and foodborne diseases.

In this work, 12 different foodborne bacteria were submitted to high pressure (300 MPa, 15 min, room temperature) in order to assess the bacterial reduction and the alterations induced with this processing in cellular components. It was observed that all the Gram-negative bacteria were inactivated to undetectable levels while Gram-positive showed resistance to pressure, being *Staphylococcus aureus* and *Bacillus cereus* the most resistant, decreasing only 2 logs. *Listeria monocytogenes* decreased about 5 logs. IR spectroscopy was used to investigate the differences in the spectra of the cells after the pressurization. Regarding cellular modifications, it was possible to notice that changes in hydrogen bounds appear to be on the basis of the modifications observed in the spectra after high pressure processing.

1. Introduction

Fish and meat have a high nutritional value and are considered easily perishable foods, as they can be contaminated from the environment and/or during its processing. In the case of fish, the high post mortem pH attained in the flesh, the high water activity and the presence of free amino acids and nucleotides make this food a readily available bacterial growth substrate (Gram & Huss, 1996; Luten, Börresen, & Oehlenschläger, 1997). The same happens in meat, in which the high water activity, moderate pH and readily available sources of energy, carbon and nutrients, make this product ideal for microbial growth (Varnam & Sutherland, 1995).

Food preservation methods have been successfully used to preserve fish and meat products, such as cooking, canning, curing or freezing. However, many characteristics of fresh fish and meat are lost with these processing methods. High-pressure processing (HPP) is an emerging food preservation method that offers numerous advantages over other food processing procedures. This procedure inactivates or reduces spoilage and pathogenic bacteria, but nutrients, flavour and colour remain largely unaffected, allowing the production of foods with almost complete retention of their nutritional and sensory qualities. Moreover, as pressure is uniformly transmitted independent of the shape or size of the food, it is ensured that the whole food is adequately processed (Grandison, 2011; Patterson, Linton, & Doona, 2007). In order to select the optimal conditions of the HPP treatment, the characterization of the cell targets of pressure is very important. It is known that high pressure does not alter the low-energy covalent bonds, which have low compressibility and does not break these bonds within the ranges that are usually applied in high pressure processing of food. As a consequence, the primary structure of molecules such as proteins and fatty acids remains intact, however, modifications may occur in secondary, tertiary and quaternary structures, for instance in the form of protein unfolding. The resistance of microorganisms to pressure varies considerably depending on the pressure range applied, temperature and duration of the treatment, as reviewed by some authors (Huang, Lung, Yang, & Wang, 2014; Mota, Lopes, Delgadillo, & Saraiva, 2013). The inactivation of bacteria by HPP is the result of a combination of factors and cell membranes are the primary sites to be damaged by pressure, altering cell permeability, transport systems, loss of osmotic state, organelle disruption and inability to preserve pH (Campus, 2010). There are other components and cellular functions sensitive to high pressures that are modified or inhibited, such as the ribosome, protein synthesis, and enzyme activity (Considine,

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Kelly, Fitzgerald, Hill, & Sleator, 2008; Rendueles et al., 2011). However, nucleic acids are relatively resistant to high pressures and as the structure of the DNA helix is largely the result of hydrogen bond formation, it is also stable under pressure (Patterson, 2005). It is known that Gram-positive bacteria are more resistant to high pressures than Gram-negative cells (Farkas & Hoover, 2000; Smelt, 1998; Wuytack, Diels, & Michiels, 2002). Gram-positive cells have a thick cell wall constituted by peptidoglycan that is less affected than the thinner peptidoglycan cell wall overlaid by the outer membrane of Gram-negative cells. The cell membrane is considered to be a primary site of pressure damage in microorganisms, which affects the cell integrity (Casadei, Mañas, Niven, Needs, & Mackey, 2002; Patterson, 2005). Apparently, the double layered phospholipids of the external membrane are packed tightly in the compression stage, promoting the transition to a gel state. During decompression, the membrane structure is lost and pores are formed. In order to maintain its functions and properties, the membrane should preserve its fluid state, which is determined by the composition of unsaturated fatty acids. High pressure reduces fluidity on cell membranes due to the increasing packing of the fatty acyl chains of phospholipids (Bartlett, 1999; Casadei et al., 2002). Factors such as cells growth phase or age tend to influence the resistance to high pressure as well. However, the physiological state of bacteria does not appear to be a significant factor if the HPP treatment is applied in order to inactivate all microorganisms of safety concern.

Rapid methods such as mid-Infrared spectroscopy (MIR) have been successfully used to analyse food microorganisms in a simple, fast and inexpensive way (Davis & Mauer, 2010; Whittaker et al., 2003). Infrared spectra of microorganisms are very complex fingerprint-like patterns typical of each different bacteria (Davis & Mauer, 2010; Helm, Labischinski, Schallehn, & Naumann, 1991; Naumann, Fijala, & Labischinski, 1988). This methodology studies the interaction of the infrared radiation with samples representing an “image” of their chemical composition. There are studies in which cell components can be identified by IR spectroscopy. Differences in cell lipids and proteins after ultraviolet irradiation of bacteria were investigated by this technique and spectral modifications were detected (Helm & Naumann, 1995; Santos et al., 2013). IR spectroscopy was also used to assess the modifications of a *Staphylococcus aureus* strain caused by pulsed UV light and infrared heating food processing methods (Krishnamurthy, Tewari, Irudayaraj, & Demirci, 2008) and to study the changes induced in a piezotolerant and a wild-type strains of *Listeria monocytogenes* with HPP (Karatzas & Bennik, 2002). Moreover, the biochemical changes of bacterial spores after a pressure-assisted thermal processing were investigated using IR spectroscopy (Subramanian, Ahn, Balasubramaniam, & Rodriguez-Saona, 2007).

The objective of this work was to use MIR in order to identify modifications in the cellular components of bacteria isolated from food samples after HPP processing. This approach intends to develop a fast and effective technique to screen food pathogens before and after HPP processing, in order to understand at which extent pressure damages the cell structure in the studied bacteria.

2. Materials and methods

2.1. Isolation of bacteria from fish, meat and cooked ham

Three pieces of hake (*Merluccius merluccius*), 3 pieces of dried salted cod (*Gadus morhua*), 3 pieces of pork meat and cooked ham (*Sus scrofa domesticus*), 3 pieces of chicken meat (*Gallus gallus domesticus*) and 3 pieces of cattle meat (*Bos taurus*) were obtained in 3 different commercial surfaces.

Cod was desalted in sterile distilled water (fish:water ratio, 1:10) during 24 h at 4 °C before microbial analysis and the water was changed 3 times (every 8 h) to simulate the soaking method adopted by the consumers. Cod was analysed immediately after desalting procedure. Hake, pork, chicken and cattle meat and ham were analysed immediately after the sample acquisition.

A total of 18 randomly selected sub-samples were aseptically cut: 3 samples of hake, 3 samples of cod, 3 samples of pork meat, 3 samples of pork ham, 3 samples of chicken meat and 3 samples of cattle meat.

2.2. Bacterial quantification in fish and meat

Each food sub-sample was aseptically homogenized with an Ultra-Turrax (T25, Janke & Kunkel - IKA Labortechnik) in Ringer’s Solution (Merck) (1:10, weight: volume). One hundred microliters of serially diluted samples were pour plated, in duplicate, in Tryptic Soy Agar (TSA) medium (Merck), Violet Red Bile Dextrose Agar (VRBD) medium (Merck) and in Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS) medium (Merck). Cod samples were additionally pour plated in TSA medium supplemented with 3% NaCl. After 3 days of incubation at 37 °C, some colonies presenting different morphologies (colour, shape, size and density) were selected. The colonies were purified by three repeated streaking steps on TSA plates.

2.3. Bacterial identification

For the identification of the selected colonies, bacterial DNA was extracted using the Instagene Matrix (Biorad, USA). 16S rDNA was amplified using the universal 27f forward primer (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1512r reverse primer (5′-CGGCTACCTTGTTACGACT-3′). The reaction occurred in a Multigene Gradient Thermal Cycler (MIDSCI). The reaction mixture contained 1 μL of bacterial DNA, 3.75 μL of MgCl2, 2.5 μL of KCl buffer, 2.5 μL of dNTP, 0.25 μL of each primer, 0.5 μL of BSA, 1 μL of Taq polymerase (MBI Fermentas, Lithuania) and Milli-Q water (Millipore). The PCR running conditions included a 5 min initial denaturation of template DNA at 94 °C, 25 denaturation cycles at 94 °C for 1 min, annealing at 55 °C for 2 min, extension at 72 °C for 2 min and a final extension at 72 °C for 10 min. The selected PCR amplicons were purified using Ron’s PCR-Pure purification kit (BIORON, Germany). Automated DNA sequencing was performed by GATC biotech (Konstanz, Germany) and the sequences were analysed using BLAST database.

Acinetobacter, Aeromonas hydrophila, Bacillus cereus, Enterobacter, Klebsiella, Staphylococcus aureus, and *Pseudomonas aeruginosa* were selected. *Escherichia coli* NCTC 10418, *Listeria monocytogenes* NCTC 11994, *Photobacterium damselae damselae* DSM-7482, *Salmonella enterica* sv. Nottingham NCTC 7832 and *Vibrio anguillarum* DSM-21597 from culture collections were also used as they are important pathogenic bacteria that can infect fish, meat and cooked ham. Bacteria were streaked on TSA medium (Merck) and used after 18 h of incubation at 37 °C.

2.4. High-pressure processing (HPP)

For determination of microbial concentration before and after HPP, bacterial mass was weighted and diluted with the appropriate amount of Ringer solution (Merck) in order to posteriorly determine the colony forming units (CFU) per mL. This mixture was very well mixed and 500 μL were inserted in polyethylene tubes without air. The tubes were afterwards inserted in a polyamide-polyethylene bag (PA/PE-90, Albipack-Packaging solutions, Portugal), manually heat-sealed (vacuum packager Packman,