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Synergistic effect of carbon dioxide atmospheres and high hydrostatic pressure to reduce spoilage bacteria on poultry sausages

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ABSTRACT

Synergistic effect of combining high hydrostatic pressure (HHP) and CO_2 atmospheres has been studied against *Leuconostoc carnosum*, *Brochothrix thermosphacta*, *Salmonella enteritidis*, *Campylobacter jejuni* and *Listeria innocua* separately inoculated in poultry sausages. The microbial counts of the HHP treated samples (350 MPa 10 min at room temperature) and CO_2 atmosphere packaged were compared with non pressure treated and air packaged ones, analyzed at 20 h and 7 days after the treatment. The results showed a synergistic effect of the combination of these two preserving technologies against all the microorganisms studied, except in *S. enteritidis* which showed a greater resistance under CO_2 atmospheres, and *C. jejuni*, that is especially sensitive at high pressures. It seems that cell damage produced by high pressure facilitates the penetration of carbon dioxide into the microorganisms' cells, affecting their metabolism and consequently their growth. Using CO_2 atmospheres in combination with HHP treatments, pressure could be lowered without compromising the reduction of microbial counts.

1. Introduction

During the last decade, the consumption of chicken meat has increased rapidly all over the world and poultry production has become the fastest growing meat sector. Poultry and poultry products are a highly perishable food and their shelf-life varies between 4 and 10 days under refrigeration (Marenzi, 1986). Deterioration depends mainly on the microbiological quality of the poultry carcasses, as poultry meat offers the perfect environment, pH, nutrients and humidity conditions for microorganism development.

Some of the common microorganisms present in poultry meat products are responsible for the most usual food-borne intoxications. Is the case of *Campylobacter jejuni*, *Listeria monocytogenes* and *Salmonella enteritidis*. *Campylobacter* is the most common human food-borne bacterial pathogen worldwide. It is responsible for campylobacteriosis in humans: a disease ranging from a self-limiting gastroenteritis to a more serious systemic infection (Young, Davis, & Dirita, 2007). Furthermore, *Campylobacter* is usually considered as fragile as other bacterial pathogens and is known to be fastidious in its growth requirements. However, the human foodborne infections related to *Campylobacter* suggest its great survival rate throughout the food chain, from live animals to the meat retail level (Bièche, De Lamballerie, Chevret, Federighi, & Tresse, 2012). More than 2 million people in the United States and 0.2 million in the European Union are annually infected by *Campylobacter*, being the most frequently reported food-borne illness; and the actual number of cases is believed to be around nine million each year (European Food Safety Authority, 2014).

Listeria monocytogenes is a Gram-positive rod-shaped bacterium and is an important pathogen in acidified food and other food products, such as dairy products and ready-to-eat meats and fishes. As a food borne microorganism, *L. monocytogenes* requires food processors to be particularly careful during storage because of its moderate heat resistance and the ability to grow anaerobically under refrigeration. It is a psychrotrophic organism and therefore can grow and multiply in chilled food products before consumption (Yuste, Mor-Mur, Capellas, & Pla, 1999). Cross-contamination from raw meat to other foods and survival of the microorganism in processed poultry are possible causes of its high incidence in outbreaks (Pini & Gilbert, 1988).

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Salmonella is a Gram-negative facultative anaerobe mesophilic bacterium, widely distributed in nature and in the gastrointestinal tract of animals and humans. Is one of the most important pathogens associated with gastrointestinal diseases, and the majority of human cases are linked to contaminated foods. The illness caused by *Salmonella* in humans is called salmonellosis. In the European Union, over 100,000 human cases are reported each year (European Food Safety Authority, 2014). Moreover, *S. enteritidis* is one of the *Salmonella* serotypes most commonly associated with morbidity and mortality in humans (Ahmed et al., 2000). At the present moment, the control of this microorganism is a priority for the food industry and especially in the case of poultry products.

About non-pathogenic microorganisms commonly present in poultry meat, *Brochothrix thermosphacta* is one of the most current spoilage organism associated with meat products stored aerobically or under modified atmospheres. The main alteration is due to the production of malodorous end-products such as acetoin and acetic, butyric, isobutyric and isovaleric acids causing off-flavors that render the affected meat unpalatable (McClure, Baranyi, Boogard, Kelly, & Roberts, 1993). As a facultative anaerobe, *B. thermosphacta* has the capability to grow on meat during both aerobic and vacuum storage, which makes it a significant meat colonizer, at times having the potential to be the dominant organism (Doulgeraki, Ercolini, Villani, & Nychas, 2012). The successful spoilage of chilled products is due mainly to its psychrotrophic nature, growing between 0 °C and 30 °C. Its pH growth range (pH 5–9) is suitable for most meat products.

Other spoilage microorganisms usually present in poultry meat are strains belonging to *Leuconostoc* genus, which are Gram-positive aerotolerant lactic acid bacteria (LAB) with an economic importance in the food industry. They are related to numerous positive processes such as fermentation of foodstuff (sauerkraut, pickles, meat products, etc.), production of gas (CO_2) in cheese presenting openness (in particular Blue-veined cheeses), production of flavor compounds in multiple dairy products and also adding potential roles in functional foods (Hemme & Foucaud-Scheunemann, 2004). On the other hand, species of *Leuconostoc* are one of the main genera associated with spoiled meat products packaged with air, vacuum or modified atmosphere (Doulgeraki et al., 2012). Good manufacturing practices and hygienic conditions during the slaughtering and processing of the meat are critical to produce a safety and durable product.

With the aim to obtain safe and durable products and motivated for the consumers demand and the progress of science and technology, food industry has introduced new methodologies and technologies to preserve food. Even though pasteurization and sterilization still are the most extensively used methods for its preservation, new processes tend to use non-thermal treatments such as magnetic or electrical fields, ionization, light pulses, high pressures, ultrasounds, and the application of chemical or biological products like carbon dioxide, polycationic polymers, bacteriocins and lytic enzymes (Mertens & Knorr, 1992). Some of these systems already have regulatory approval and are commonly used in the industry, while others continue to be developed and evaluated for potential commercial application (Trujillo et al., 2000). High hydrostatic pressure (HHP), as an alternative to heat pasteurization, is one of the most promising non-thermal processing techniques for the inactivation of microorganisms in liquid and solid food systems while preserving nutritional and sensory characteristics (Wang, Pan, Xie, Yang, & Lin, 2010). At refrigeration, ambient or moderate heating temperature, HHP allows inactivation of pathogenic and spoilage microorganisms in foods with fewer changes in texture, color and flavor as compared to conventional technologies (Cheftel, 1995; Knorr, 1993; Velazquez, Gandhi, & Torres, 2002). Unlike thermal processing and other preservation technologies, the effects of HPP are uniform and nearly instantaneous throughout the food and thus independent of food geometry and equipment size (Torres & Velazquez, 2005). It was first reported by Hite (1899) who treated milk at 670 MPa for 10 min and detected a 5–6 log-cycle reduction in total counts. Since then, its efficacy has been demonstrated several times in a wide variety of food products, not only against spoilage bacteria (Gervilla, Capellas, Ferragut, & Guamis, 1997; Rodríguez-Calleja, Cruz-Romero, O'Sullivan, García-López, & Kerry, 2012; Wilson, Dabrowski, Stringer, Moezelaar, & Brocklehurst, 2008) but on the improvement of food properties and production processes (Boluda-Aguilar, Taboada-Rodríguez, López-Gómez, Marín-Iniesta, & Barbosa-Cánovas, 2013; Juan, Trujillo, Guamis, Buffa, & Ferragut, 2007; Saldo, McSweeney, Sendra, Kelly, & Guamis, 2002; Trujillo et al., 2000). Because of the complexity of the reactions that can take place in a biological system, it is difficult to predict the effect of high pressure on any particular bacterial population. The effectiveness of the treatment depends primarily on the pressure applied and on the holding time. The resistance of microorganisms is highly variable, depending mainly on the type of organism and the food matrix (Rendueles et al., 2011). Nevertheless, it is well established that high pressure mainly leads to the denaturation of proteins, specifically enzymes by affecting the catalytic function and cell membrane damages (De Angelis & Gobbetti, 2004).

However, the economic costs of the equipment needed to reach pressures up to 600 MPa or more, which are required for efficient bacterial inactivation, have limited the commercial breakthrough of this technology. Owing to this, the use of other technologies in combination with HHP at lower pressures has been thoroughly studied over the last two decades. Recently, to reduce the needed inactivation pressure, various effective synergistic treatments have attracted much more attention, and several combined treatments have been investigated to optimize the processes and elucidate the mechanism of HHP treatment. These combined factors include antimicrobials, pH, moderate temperature and modified atmospheres (Amanatidou et al., 2000; Garcia-Graells, Valckx, & Michiels, 2000; Somolinos, García, Pagán, & Mackey, 2008; Wang et al., 2010).

Modified atmosphere packaging (MAP) is a technology consisting in package food product and replace the air inside the package for another atmosphere usually composed by oxygen (O_2), nitrogen (N_2) or carbon dioxide (CO_2) alone or mixed among them in binary or ternary gas mixtures. CO_2 is the most significant gas of MAP due to its antimicrobial activity. When CO_2 is introduced into the package, it is partly dissolved in the water and lipid-phase of the food. This results after reaching equilibrium in a certain concentration of dissolved CO_2 in the water-phase of the product (Devlieghere, Debevere, & Van Impe, 1998). Tan and Gill (1982) suggested different possible mechanisms for CO_2 inhibition, which include slowing down the intracellular activity of enzymes and microorganisms due to a decrease in intracellular pH as a consequence of the carbonic acids formed, which can alter the properties of the cell membranes. Nonetheless, it is evident that the inhibition of the growth of microorganisms in MAP foods is significantly related to the concentration of the CO_2 dissolved in the product (Devlieghere & Debevere, 2000; Devlieghere et al., 1998).

The synergistic effect between HHP and CO_2 against certain enzymes and microorganisms has been previously reported. Corwin and Shellhammer (2002) tested how combining these technologies would affect the activity of pectin methylesterase and polyphenol oxidase and also the destruction of *Lactobacillus plantarum* and *Escherichia coli*, using pressures greater than 500 MPa for enzymes and 365 MPa and 455 MPa for microorganisms, both inoculated in orange juice. Also Park, Park, and Park (2003) studied this synergistic effect by packaging broth inoculated with *Bacillus*

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