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Effect of high pressure processing on the survival of *Salmonella* Enteritidis and shelf-life of chicken fillets

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ABSTRACT

High pressure processing (HPP) is a preservation technology alternative to heat treatment that is mild for food, but effectively inactivates the spoilage microbiota and foodborne pathogens of several foods. The purpose of the current study was to evaluate the effect of HPP on *Salmonella* ser. Enteritidis, indigenous microbiota and shelf-life of chicken fillets. Chicken fillets were inoculated with *S.* Enteritidis at three different initial inocula (3, 5, 7 log CFU/g), packed under vacuum, treated or not with HPP (500 MPa/10 min) and stored at 4 and 12 °C. Total viable counts, *S.* Enteritidis, pseudomonads, *Brochothrix thermosphacta*, lactic acid bacteria, *Enterobacteriaceae* and yeasts/molds populations were determined in parallel with sensory analysis of non-inoculated samples. The HPP resulted in the reduction of the pathogen population below the detection limit of the enumeration method (0.48 log CFU/g), irrespective of the inoculum. During the shelf life of the HPP samples, the pathogens population remained below or near the detection limit of the enumeration method at both temperatures, except from the high inoculum case that an increase was observed at 12 °C. At the low inoculum level, the pathogen could not be detected with the enrichment method after the first storage days (2nd day for 4 °C and 0 day for 12 °C). The survival of *Salmonella* strains was assessed by pulsed field gel electrophoresis and it was shown that the survival of the different strains depended on the inoculum and storage temperature. Regarding the indigenous microbiota, *Br. thermosphacta* was reported for the first time to be the main spoilage microorganism that survived and dominated after the HPP. From the results it was evident that, HPP may enhance the safety and increase the shelf life (6 at 4 °C and 2 days at 12 °C) of chicken meat.

1. Introduction

Global meat production rose by almost 20% over the last decade, mostly by the growth in poultry and pigmeat. It is estimated that in 2024 the production will be 17% higher than the base period (2012–14). Global annual meat consumption per capita is expected to reach 35.5 kg retail weight equivalent (r.w.e.) by 2024 (1.6 kg r.w.e. higher from the base period). This additional consumption will consist mainly of poultry, which is expected to surpass pigmeat as the preferred source of protein consumed worldwide (OECD/FAO, 2015).

As fresh poultry meat is a highly perishable food, due to its biological composition, the high consumption of poultry products leads to concerns pertaining to product safety, shelf life quality and associated desirable sensory characteristics. Spoilage of poultry muscles encompasses changes of the available low molecular weight compounds (e.g. lactate, glucose, amino acids), during the proliferation of the bacterial population that comprise the microbial association of the stored meat. The dominance of a particular microbial association for poultry muscle depends on factors that persist during processing, transportation and storage in the market (Argyri et al., 2012). Thus, the chicken's meat shelf-life is affected by the growth of different spoilage bacteria and may also be contaminated by pathogenic foodborne bacteria (Del Olmo et al., 2012).

Although pathogenic genera do not constitute a part of the spoilage association per se, their occurrence is possible due to their presence in the raw meat or transfer during unhygienic processing of a product (Argyri et al., 2012). *Salmonella* is a foodborne pathogen present in raw poultry meat products and in the processing environment that is known as a major cause of foodborne disease,

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frequently associated with consumption of poultry meat (Carrasco et al., 2012; Foley et al., 2008; Lerasle et al., 2014). According to the Commission Regulation (EC) No 1441/2007 (2007) on microbiological criteria for foodstuff, meat products made from poultry meat intended to be eaten cooked, absence of *Salmonella* in 25 g is required for products placed on the market during their shelf-life.

On the other hand, consumers in the 21st century are demanding high quality, innovative food products that are free from additives, fresh tasting, microbiologically safe and with an extended shelf-life. One food technology that has the potential to meet these demands is high pressure processing (Patterson, 2005; Simonin et al., 2012). High pressure processing (HPP) is a preservation technology that has the potential to increase the safety and shelf life of foods (Bravo et al., 2014; Realini et al., 2011). At refrigeration, ambient or moderate heating temperature, HPP allows inactivation of pathogenic and spoilage microorganisms in foods with fewer changes in texture, color and flavor, as compared to conventional thermal technologies (Cheftel, 1995; Torres and Velazquez, 2005). The effectiveness of HPP on suppressing the growth and survivability of spoilage and pathogenic microorganisms, as well as inactivating food enzymes, depends on different factors, such as process parameters utilised, Gram type, strain and growth stage of microorganisms concerned and food matrix to be processed (Garriga et al., 2004; Rivas-Cañedo et al., 2009; Smelt, 1998).

Among products processed using high pressure, the number and variety of meat and meat products has risen dramatically worldwide (Garriga and Aymerich, 2009). Pressure levels higher than 400 MPa are generally necessary to achieve efficient microbial inactivation, depending on the product microbiota and on the meat product itself (Simonin et al., 2012). Additionally, Patterson et al. (2010) that have investigated the microbiological quality of vacuum-packaged cooked minced chicken meat treated using a range of pressures (400–600 MPa) and holding times (1, 2 and 10 min), followed by storage at 4, 8 or 12 °C, reported that as the pressure level and hold time increased, the number of surviving microorganisms decreased significantly. However, increasing the processing time and pressure level may have an adverse effect on the organoleptic characteristics of the product.

It is known that complex food matrices, such as meat or milk, which contain carbohydrates, proteins and fat, are nutritionally rich substrates that increase the ability of bacteria to survive (Del Olmo et al., 2012; Escriu and Mor-Mur, 2009; Garcia-Graells et al., 1999). Rivas – Cañedo et al. (2009) demonstrated the effect of high-pressure treatment (400 MPa, 10 min at 12 °C) on vacuum packed minced beef and chicken breast. They found that Gram-negative bacteria in beef and chicken breast were more affected by HPP than Gram-positive bacteria. Additionally, it is well documented that the species of foodborne pathogens contain strains that are relatively resistant to pressure in comparison to other strains of the species (Alpas et al., 1999; Benito et al., 1999). This could be the reason for the variation in results obtained by researchers using different strains of the same species (Alpas et al., 2000).

Nonetheless, HP-processed foods are usually nonsterile and therefore must be refrigerated to maintain their sensory characteristics and microbiological stability (Rendueles et al., 2011). In these circumstances, evolution of survivors or recontaminants over the shelf-life period should be studied. Cells with sublethal damage, under appropriate conditions (nutrient-rich substrates, appropriate temperature and storage time), can be resuscitated (Bozoglu et al., 2004).

Based on the above, the aim of this study was to evaluate the effect of HPP on *Salmonella* ser. Enteritidis, indigenous chicken microbiota and shelf-life of chicken fillets. To our knowledge, this is the first time that a composite experiment that includes measurements of the indigenous microbiota as well as of the presence/absence and measurements of *Salmonella* Enteritidis in a cocktail mixture of several strains and in several inoculum levels, in parallel with sensory analysis (non-inoculated samples) is conducted. Additionally, it is of great importance that the strain distribution of the inoculated *Salmonella* was evaluated by using molecular tools.

2. Materials and methods

2.1. Experimental design

To investigate the effectiveness of different HPP treatments, preliminary inactivation tests were designed concerning pressure levels of 400 MPa for 10 or 20 min, 500 MPa for 10 or 20 min and 600 MPa for 10 min and samplings were performed at days 0, 5, 10 of storage (Del Olmo et al., 2010; Del Olmo et al., 2012; Kruk et al., 2011; Ma et al., 2007; Patterson et al., 2010; Rodríguez-Calleja et al., 2012). Based on the results of the preliminary experiments (sensory analysis and indigenous microbiota and *Salmonella* inactivation), the main experiment was then designed, where chicken breast fillets were inoculated with *S.* Enteritidis at three different initial inoculum levels, packed under vacuum with or without the application of the high pressure of 500 MPa for 10 min and then stored at 4 and 12 °C. Microbiological analysis was assessed in parallel with molecular analysis and sensory evaluation of non-inoculated samples.

2.2. Preparation of chicken fillets samples

Chicken breast fillets from two different batches were obtained from a meat processing plant located in Athens (Greece) and transported under refrigeration to the laboratory within 30 min, where it was held at 1 °C for 1–2 h. The meat was then aseptically cut and weighted into portions of 30 g and subsequently inoculated with *Salmonella enterica* ser. Enteritidis (cocktail culture of 3 strains) as follows. A portion of samples were not inoculated and served as samples for sensory analysis.

2.3. Inoculation of the chicken fillet samples

In the current study, a 3 strains cocktail of *Salmonella enterica* subsp. *enterica* serovar Enteritidis (FMCC B-56 PT4; FMCC B-57 PT7; ATCC 13076) was prepared. All the strains were kindly provided from the Food Microbiology Culture Collection (FMCC) of the Agricultural University of Athens. The strains were revived from a stock culture stored at −80 °C, subcultured into 10 ml Brain Heart Infusion Broth (BHI, LabM, LAB049) and incubated overnight at 37 °C. A subculture was prepared in fresh 10 ml BHI and incubated for 18 h 37 °C. Cells were then harvested by centrifugation (5000 × g, 10 min, 4 °C), washed twice with sterile 10 ml Ringer solution (BR0052G, Oxoid), resuspended in Ringer solution and the cultures of each strain were mixed in equal volumes. This cocktail was used with the appropriate dilutions to reach 3 final inoculum levels of *ca.* 10^3, 10^5, 10^7 CFU/g on the chicken fillets samples. The samples were then packed under vacuum, into plastic pouches (100 mm wide - 100 mm long, 90 mm thickness), of O_2 permeability at 23 °C and 75% relative humidity of *ca.* 75 cc/m2/24 h/1 atm (Flexo-Pack S.A., Athens, Greece), using a HenkoVac 1700Machine (Howden Food Equipment B.V., The Netherlands). Half of the prepared samples were treated with HPP.

2.4. High pressure processing (HPP)

HPP experiments were conducted by applying 500 MPa