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Survival of *Salmonella* spp. in minced meat packaged under vacuum and modified atmosphere

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ABSTRACT

The effect of different modified atmosphere packaging regimes on the behavior of *Salmonella* spp. on minced meat was studied. Minced meat was experimentally contaminated with a *Salmonella* spp. cocktail (*S.* Enteritidis, *S.* Typhimurium, *S.* Infantis and *S.* Arizonae), packaged under vacuum or modified atmosphere with initial headspaces containing 20%O_2/50%CO_2/30%N_2 and 20%O_2/30%CO_2/50%N_2) and stored at 3 ± 1 °C for 12 days. Samples were analyzed for *Salmonella* spp., viable and lactic acid bacteria count every third day. *Salmonella* spp. counts decreased during storage in all packaging types, with reductions of about 1.5 log CFU/g. A significant difference ($p < 0.01$) was noted between *Salmonella* spp. counts in meat packaged in vacuum and modified atmospheres, although there was no significant difference in *Salmonella* spp. count between meat packaged in 50%CO_2, and meat packaged in 30%CO_2. At the end of the study, there were significant differences ($p < 0.01$; $p < 0.05$) in total viable and lactic acid bacterial counts between meat packaged in vacuum and modified atmosphere, and the lowest counts were noted in meat packaged in modified atmosphere with 50%CO_2.

Introduction

Pork and beef minced meat are widely consumed in Europe.[1] In Serbia as well as in other Balkan and some Mediterranean countries, minced meat is an inseparable part of traditional dishes (e.g. moussaka, sarma), and meat products (e.g. sausages, ćevapčići, hamburger). The mincing process disrupts the meat cellular structure, releasing tissue fluids and making the minced meat a highly nutritious medium supporting bacterial growth; mincing also allows migration of surface bacteria throughout the product.[2] Therefore, it presents a highly perishable product that need to be wrapped or packaged and chilled immediately to an internal temperature of not more than 2 °C or frozen to -18 °C during storage and transport (Regulation (EC) 853/2004).[3]

Despite measures to control foodborne pathogens from farm to fork the burden of diseases caused by foodborne pathogens remains important health and economic issue.[4–8] Some of these pathogens, such as *Salmonella* spp., continue

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to cause major human public health and economic problems in both developed and developing countries.[9] *Salmonella* spp. are the second most often reported bacteria causing food-borne disease in humans, following *Campylobacter* spp.[10,11] Meat can be contaminated with *Salmonella* during the slaughter, dressing and deboning processes, or during processing, transport, storage and household use, as a result of cross-contamination.[12,13] *S.* Enteritidis and *S.* Typhimurium are the most frequently reported serotypes causing human salmonellosis in both the EU and the United States, while the incidence of *S.* Infantis is increasing.[14–18] This highlights the need for improved prevention and control of *Salmonella* spp. in food.

The food industry has developed numerous preservation techniques in order to prevent and control *Salmonella* and other food-borne pathogens and spoilage microorganisms in fresh meat products, making the meat safer for consumption and extending its shelf life. Vacuum packaging (VP) and modified atmosphere packaging (MAP) are the most commonly used packaging methods for meat and meat products. MAP is considered to be an effective technique for raw meat preservation.[19–23] These methods are based on removal of the surrounding atmosphere (VP) or flushing it out and replacing it with a gas mixture (MAP) before sealing in gas barrier materials.[20–23] Gases most often used in MAP are carbon dioxide, which inhibits bacterial growth, oxygen, which prevents anaerobic growth and retains meat color, and nitrogen, which avoids oxidation of fats and pack collapse. Depending on the type of food or effect desired, these gases can be used separately or in combination in various concentrations.[19,21]

Considering the prevalence of *Salmonella* in minced meat and the frequency of its consumption via many traditional products and, taking into account that packaging of meat is the most common method of food preservation, there is a need to explore the effect of packaging methods on *Salmonella* spp. survival, especially in mixed minced meat (pork and beef). Therefore, the aim of this study was to compare the effects of vacuum and two initial headspace-modified atmosphere conditions ($20\%O_2/50\%CO_2/30\%N_2$ and $20\%O_2/30\%CO_2/50\%N_2$) on the survival of *Salmonella* spp., total viable bacteria and lactic acid bacteria in minced meat stored at $3 \pm 1\,^{\circ}C$.

Materials and methods

Pork and beef muscles from leg of different carcasses used in the study were provided 48 h post-slaughter by a local slaughterhouse (Pećinci-Subotište, Serbia). Connective tissues and visible fat were trimmed after which the pieces of meat were minced separately in a sterile grinder (4 mm perforation diameter in the meat grinder plate), mixed in a 50:50 ratio of pork:beef and transported under refrigeration to the laboratory within an hour.

Four serovars of *S. enterica* (*S.* Enteritidis ATCC 13076, *S.* Typhimurium ATCC 14028, *S.* Arizonae ATCC 13314 and *S.* Infantis ATCC 51741) (www.atcc.org) were used in this study. The serovars were stored in Brain Heart Infusion (BHI; Merck, Germany) with 20% glycerol at −80 °C until needed. One ml of each frozen *Salmonella* serovar was added to 10 ml of BHI (Merck, Germany), incubated at 37 °C for 24 h, then were streaked on Xylose Lysine Tergitol-4 Agar (XLT_4) (Merck, Germany) to verify their characteristics. In order to get a second subculture isolated, black colonies were picked from the XLT_4 plates and inoculated into BHI tubes (1 colony per tube) and further incubated for another 24 h at 37 °C. After incubation, the cultures were centrifuged at $5000 \times g$ (Eppendorf, Hamburg, Germany) for 10 min and suitable dilutions were prepared in BHI. A *Salmonella* cocktail was prepared by combining equal portions of standardized cell suspensions to yield approximately 8 log CFU/ml of each serovar in the mixture. *Salmonella* counts were determined by serial dilution and subsequent enumeration on XLT_4. This *Salmonella* cocktail (40 ml of the cocktail) was used to inoculate 9 kg of minced meat in the sterile mixer in the experimental laboratory of the Faculty of Veterinary Medicine, University of Belgrade. According to legal requirement for the absence of *Salmonella* in 25 g of raw meat, meat used in the present study was not naturally contaminated with *Salmonella*. Minced meat was divided in portions of 100 g, and packaged in three different conditions: VP, modified atmosphere package 1 (MAP1, containing $20\%O_2/50\%CO_2/30\%N_2$) and modified atmosphere package 2 (MAP2, containing $20\%O_2/30\%CO_2/50\%N_2$). MAP treatments were conducted considering ratio of 1:3 (v/w) between the volume of gas and weight of the minced meat (G/P ratio). A Variovac packaging machine (Variovac Primus, Zarrentin, Germany) was used for VP and MAP. Minced meat was packaged in a OPA/EVOH/PE foil (oriented polyamide/ethylene vinyl alcohol/polyethylene Dynopack, POLIMOON, Kristiansand, Norway), with low gas permeability (O_2 – 3.2 cm^3/m^2/day at 23 °C, N_2 – 1 cm^3/m^2/day at 23 °C, CO_2 – 14 cm^3/m^2/day at 23 °C, water vapor – 15 g/m^2/day at 38 °C). All minced meat samples weighed 100 ± 5 g and were refrigerated at $3 \pm 1\,^{\circ}C$.

Minced meat was analyzed for *Salmonella* spp., total viable count (TVC-mesophiles, 30 °C), and lactic acid bacteria (LAB) count immediately and on days 3, 6, 9 and 12 of storage. For bacterial enumeration, approximately 10 g of meat were weighed aseptically after package opening, transferred into sterile Stomacher bags and 90 ml of Buffered Peptone Water (BPW) (Merck, Germany) was added to each sample. Meat samples were homogenized in a Stomacher blender (Stomacher 400 Circulator, Seward, UK) for 2 min. Serial decimal dilutions were prepared in buffered peptone water (Merck, Germany) and 1 ml or 0.1 ml of appropriately diluted homogenized meat was inoculated directly on the surface of XLT_4 (Merck, Germany) for *Salmonella* spp. enumeration[24] and incubated for 24 h at 37 °C, Plate Count Agar (PCA; Merck, Germany) for TVC-mesophiles enumeration according to ISO 4833:2003,[25] and incubated at 30 °C for 72 h and MRS Agar (Merck, Germany) for LAB enumeration according to ISO 15214:1998,[26] and incubated at 30 °C for 72 h. After incubation, plates were examined visually for typical colonies and morphological characteristics associated with each growth medium, the number of colonies was counted and results were recorded as colony forming units per g (CFU/g). Suspect colonies of *Salmonella* spp. were tested using API 20e (BioMerieux Italia-Bagno a Ripoli, Florence), while suspect colonies of lactic acid bacteria were stained by Gram and catalase test was done.

The meat pH was measured after 10 min at room temperature using a hand-held pH meter, Testo 205 (Testo AG, Lenzkirch, Germany), equipped with a penetrating glass electrode.

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