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Effectiveness of combined preservation methods to extend the shelf life of *Morcilla de Burgos*

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ABSTRACT

Morcilla de Burgos is the most famous blood sausage in Spain. However, while producers are interested in extending its shelf life, the consumer is increasingly demanding more natural food. This situation has led to the current search for new and mild preservation technologies. Two batches of four different products: control without any treatment, control with organic acid salts (CnOAS; a 3% mixture of potassium/sodium L-lactate), control with high hydrostatic pressure processing (CnHPP; 600 MPa–10 min), and a combination of both treatments (OAS + HPP), were carried out to evaluate any synergistic effect that occurs when combining OAS and HPP, and the influence of different preservative treatments on the spoilage bacterial population and their evolution. HPP (with or without addition of OAS) can be considered the most suitable method for preserving *morcilla de Burgos* as it does not produce negative changes in sensory attributes. No clear selective effect of different treatments on the composition of the spoilage bacteria was seen and similar spoilage patterns were observed independently of the preservation treatment used.

1. Introduction

The introduction of food quality labels, especially in the EU (designations of origin or protected geographical indications), has resulted in a renaissance of traditional meat products that were originally produced by small craft producers and commercialized in local markets. Blood sausages are traditional meat products that are popular in many parts of the world. Nowadays, these products are receiving attention since they have become gourmet products in several countries, thus leading to an increase in their production and potential market. *Morcilla de Burgos* is the most popular blood sausage in Spain. Despite its popularity, however, its short shelf life limits its distribution, therefore extending its shelf life is a priority for producers in order to expand their markets (Diez, Santos, Jaime, & Rovira, 2008a; Diez et al., 2008b; Santos, Diez, González-Fernández, Jaime, & Rovira, 2005a; Santos, González-Fernández, Jaime, & Rovira, 2003; Santos et al., 2005b). Recent food safety crises (BSE, dioxins, food poisoning outbreaks) have alarmed consumers, who continue to demand high quality and convenient meat products with a natural flavour and taste and who appreciate the fresh appearance of minimally processed food. Different mild preservation methods have been proposed for *morcilla*, such as the use of vacuum or modified atmosphere packaging (Santos et al., 2003) and the application of organic acid salts (OAS) and high hydrostatic pressure processing (HPP) to vacuum-packaged products (Diez et al., 2008a, 2008b). Vacuum-packed *morcilla* normally begins to spoil after 14–21 days of chill storage, mainly due to vacuum loss and the formation of slime and a sour odour and taste caused by the growth of lactic acid bacteria (LAB) (Diez et al., 2008b; Koort et al., 2006; Santos et al., 2005b). This pattern of spoilage has also been reported for other cooked (Chenoll, Macián, Elizaquivel, & Aznar, 2007; Korkeala & Björkroth, 1997), and dry meat products (Rantsiou & Cocolin, 2006).

Sodium lactate has been used in the meat industry because of its ability to increase flavour, prolong shelf life, and improve the microbiological safety of products. The antimicrobial effects of lactates are due to their ability to lower water activity and the inhibitory effects of the lactate ion (Houtsma, de Wit, & Rombouts, 1993; Koos, 1992). Several researchers have successfully extended the shelf life of cooked (Bloukas, Paneras, & Fournitzis, 1997; Papadopoulos, Miller, Acuff, Vanderzant, & Cross, 1991; Rondinini, Maifreni, & Marino, 1996) and various other meat products (Brewer, McKeith, Martin, Dallmier, & Meyer, 1991; Cegielska-Radziejewska & Pikul, 2004; Kuo & Chen, 2004; Sallam & Samejima, 2004; Serdengecti, Yildirim, & Gokoglu, 2006) by adding sodium lactate.

HPP is a mild, non-thermal technology that is receiving increasing attention as pressures of between 300 and 600 MPa inactivate product-spoiling microorganisms and enzymes at low temperatures without changing the majority of the sensory and nutritional characteristics of the product (De Heij et al., 2003; Denys & Hendrickx, 1999; Hugas, Garriga, & Monfort, 2002; Smelt, 1998; Ting, Balasubramaniam, & Raghubeer, 2002). The efficacy of this

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treatment is influenced by such parameters, as the pressure level, temperature, and exposure time, as well as by intrinsic factors of the food itself, such as pH, microflora types, or composition (Garriga, Grèbol, Aymerich, Monfort, & Hugas, 2004; Smelt, 1998). The ability of bacteria to survive HPP increases when they are treated in nutritionally rich media such as meat or meat products, which contain carbohydrates, proteins, and fat, therefore experimentation with real food matrices is strongly recommended (Garriga et al., 2004). HPP has been found to contribute to the maintenance of sensory properties and extension of shelf life (Garriga et al., 2004; Huang, Moreira, & Murano, 1999; Rubio, Martínez, García-Cachán, Rovira, & Jaime, 2007; Yuste, Mor-Mur, Capellas, Guamis, & Pla, 1998).

To further increase the shelf life and safety of pressurized products, HPP is being investigated in combination with other technologies such as bacteriocins or other natural antimicrobials (Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007). According to Raso and Barbosa-Cánovas (2003), combining antimicrobials with HPP could enhance the effectiveness of pressurization, with concomitant advantages in product quality and safety.

Previously (Diez et al., 2008a), the application of a 3% mixture of potassium/sodium L-lactate and HPP treatment at 600 MPa, separately, to vacuum packaged *morcilla* was found to be better in terms of delaying LAB growth than other preservative treatments such as modified atmospheres (Santos et al., 2005a). However, the inhibitory effect was lower than initially expected. The aim of this work was: firstly, to evaluate whether there is any synergistic effect of combining OAS and HPP under the optimal conditions established by Diez et al. (2008a), and secondly, to determine whether the application of different preservative treatments affects the bacterial population.

2. Materials and methods

2.1. Treatments

Two batches of morcilla were manufactured following the procedure described by Diez et al. (2008a), and treated with a combination of OAS and HPP. In order to evaluate the potential synergic effect of both treatments, three types of controls were also manufactured: control without any treatment, control with organic acid salts (CnOAS), and control with high pressure treatment (CnHPP).

In CnOAS treatment, a 3% mixture of potassium/sodium L-lactate (Purasal™ LITE S/6, Purac, Amsterdam, The Netherlands) was added to the product. When high pressure treatment was applied, vacuum-packaged sausages were treated in a discontinuous hydrostatic pressurization unit (Wave 6000/135 NC, Hyperbaric, Burgos, Spain) with a cylinder bore measuring 0.30 m in diameter and 2.2 m in length and with a working volume of 0.135 m^3. A pressure level of 600 MPa was tested for 10 min. The pressure transmission fluid was water. The initial water temperature of 15 °C increased, by around 3 °C per 100 MPa during high pressure processing due to adiabatic heating. The time required to reach a pressure of 600 MPa was 260 s and the holding time was 10 min. The depressurization time was less than 5 s. When OAS and HPP were combined, the *morcillas* with 3% potassium/sodium L-lactate (Purasal™ LITE S/6, Purac) were treated at 600 MPa for 10 min.

After treatment, the *morcillas* were brought to the laboratory in ice boxes and stored in the dark at 4 °C for 50 days. Each batch was tested microbiologically and its sensory characteristics analyzed in triplicate at 0, 1, 8, 15, 22, 29, 36, and 50 days. Analysis at day 0 corresponded to the product before HPP treatment. A control batch containing no organic acid salts and with no HPP treatment was included in both repetitions.

2.2. pH and microbiological analysis

Blood sausage pH and the following microbial parameters: Total viable count (TVC) lactic acid bacteria (LAB), enterobacteria, pseudomonads, *Staphylococcus* sp. and *Clostridium perfringens* were determined according to Diez et al. (2008a).

2.3. Sensory analysis

Two types of sensory analysis were performed: a difference test and a descriptive profile.

2.3.1. Difference test

Paired comparisons between different treatments were performed at day 2 by 44 or 45 untrained panellists. *Morcilla* was served to the panellists in 1-cm thick slices and cooked in a microwave to a core temperature of 70–75 °C. This test was done for each treatment and a comparison made with the control samples and between the different treatments. The panellists were also asked about their preferences.

2.3.2. Profile analysis

A quantitative descriptive analysis was carried out according to Santos et al. (2005a) by a trained panel who scored on a five-point scale where one corresponds to complete absence and five to the maximum intensity of each parameter. The panel sessions took place at days 8, 15, 22, 29, and 36 (day 0 was omitted because the products presented optimal sensory conditions in all treatments and day 50 as the products were considered unfit for consumption because of the high microbiological counts). The sensory profile consisted of seven descriptors grouped in two blocks. The first block was related to visual attributes like vacuum loss, presence of spots, and presence of slime. The second block included an evaluation of the texture and olfactory attributes like sour odour and off-flavour and sour taste. The samples were evaluated at room temperature for the first two blocks while the *morcillas* were cut into 1-cm thick slices and cooked in a domestic microwave oven to a core temperature of 70–75 °C for texture and flavour evaluation. A score of over three for any parameter meant that the product was considered unfit for consumption.

2.4. Bacterial identification

Origin of strains: to evaluate the spoilage bacterial population pattern in each product, 192 strains of LAB (48 strains from each treatment (C, CnOAS, CnHPP, and OAS + HPP)) were isolated from the MRS plates corresponding to analysis at days 1, 8, and 36. These days were selected because they showed the most marked changes in LAB; day 1: there were no significant differences between the treatments; day 8: significant differences between the treatments were detected; day 36: LAB counts exceeded a population of 7 log cfu/g in all samples, therefore the *morcillas* were considered to be spoiled according to the criteria established in previous studies (Diez et al., 2008a, 2008b). Similar criteria have been used to assess cooked meat products (Korkeala, Lindroth, Ahvenainen, & Alanko, 1987; Vermeiren, Devlieghere, De Graef, & Debevere, 2005). Colonies were randomly selected from MRS plates containing less than 300 colonies and purified on MRS agar. All isolates were initially examined for Gram reaction and catalase and oxidase activity. Only gram-positive, catalase-negative, and oxidase-negative isolates were isolated and stored at −80 °C in MRS broth (Oxoid) with 20% glycerol (Panreac, Badalona, Spain) for further studies. For identification, the isolates were cultured at 30 °C in MRS broth for 24 h or on MRS agar for 2–3 days at 30 °C.

Ribotyping and ribotyping data management: was applied according to the procedures describe in Santos et al. (2005b).