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Vacuum impregnation as a tool to introduce biopreservatives in gilthead sea bream fillets (*Sparus aurata*)

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ABSTRACT

The aim of this study was to determine the possible technological uses of biopreservation and vacuum impregnation techniques to extend shelf life of gilthead sea bream fillets. Two impregnation media were studied: a solution containing lactic acid bacteria (LAB) and a nisin solution. Vacuum impregnation was carried out at 4 °C. Fillets were immersed in a vessel containing the impregnation solution and vacuum was applied during 5 min. After this time atmospheric pressure was restored leaving samples under the liquid for 5 min more. Weight gain, physico-chemical properties (moisture, pH, water activity and TVBN), color and microbiological counts were studied during 15 days of storage at 4 °C. The quantities of biopreservative added to the product after impregnation were about 2.16×10^7 CFU/100 g_{fish} for LAB solution and 5294 IU nisin/100 g_{fish} or 0.13 mg_{nisin}/100 g_{fish} for nisin solution. Changes on physico-chemical properties were not significant between fillets impregnated and fillets without impregnation. Impregnation of fillets caused small changes in color attributes, specially an increase the luminosity (L^*). Vacuum impregnation with biopreservative solutions can extend the shelf life of gilthead sea bream fillets, reducing the initial count and/or delaying the growth of microorganisms.

1. Introduction

Consumer demand for fresh refrigerated foods with extended shelf life has been increased in the last years. Considerable research has been directed toward using various preservative technologies to preserve or prolong the shelf life, while ensuring the safety, of fresh foods, including fishery products. These techniques are based on the use of natural or controlled competitive microflora and/or their antimicrobial metabolic products to extend storage life and enhance safety (Devlieghere, Vermeiren, & Debevere, 2004; Rodgers, 2003). Lactic acid bacteria (LAB) are considered as the major group of protective cultures (PC) or antagonistic cultures (Devlieghere et al., 2004; Rodgers, 2001). LABs inhibit other microorganisms through competition for nutrients and/or production of primary or secondary metabolites (Devlieghere et al., 2004). Some of these metabolic compounds have low molecular weight (lactic acid, H_2O_2, CO_2, alcohols, phenyllactic acid, cyclic dipeptides and short or medium chain fatty acids) (Rodgers, 2003; Schnürer & Magnusson, 2005) and some have high molecular weight (polysaccharides and bacteriocins) (Lucke, 2000; Rodgers, 2001).

Therefore, the possible benefits of the application of PC are: the improvement of the safety of the product without changing the parameters of the process, the use of mild conditions of processing (time/temperature combinations in heat treatments), the extension of the temperatures of storage, the increase of shelf life and nutritional quality (since many of them are lactic acid bacteria with probiotic properties). It is necessary to consider that PC have an appreciation of "natural", which is a point to consider due to its use in foods and the acceptance of these on the part of the consumer (Marugg, 1991). The use of PC is also related with the concept of "health through the feeding", since the role that microflora plays in our immune system, acting microorganisms like probiotic agents (Perdigón, VintinI, Alvarez, Medina, & Medici, 1999). Diverse studies have demonstrated the effectiveness of biopreservatives of microbial origin in fish preservation (Rodgers, 2001). Thus, the *Carnobacterium piscicola* (A10a), *Lactobacillus plantarum* and *Lactobacillus lactis. spp lactis*, used in smoked salmon allow a remarkable reduction of the levels of *Listeria monocytogenes* (Jeppesen & Huss, 1993; Leroi, Arbey, Joffraud, & Chevalier, 1996; Wessels & Huss, 1996). Other microorganisms such as *Leuconostoc* spp. and *L. plantarum* have successfully been used to inhibit *L. monocytogenes* and *Yersinia enterocolitica* in prawns (Jeppesen & Huss, 1993). The effect of *Enterococcus faecium* has been studied in vacuum-cooked fish controlling the development of *L.*

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monocytogenes, *Clostridium botulinum*, *Clostridium perfringens* and *Bacillus thermosphacta* (Rodgers, 2001). Also in salmon, it has been possible to control the development of SH_2 producing bacteria, as well as fungi and yeast by means of the use of *Carnobacterium piscicola* and *L. plantarum* (Leroi et al., 1996).

However, the use of bacteriocins produced by PC is often limited to its narrow activity. Bacteriocins are susceptible to degradation due to interactions with food ingredients or proteolytic enzymes action (Gálvez, Abriouel, López, & Omar, 2007). In addition, their limited diffusion in solid matrices occasionally generates loss of bactericidal power (Devlieghere et al., 2004). Nisin is a polypeptide produced by certain strains of the food grade *Lactococcus lactis*, has been used as an antimicrobial in foods.

Nevertheless, the use of natural biopreservatives in structured food matrices as fish, present several disadvantages associated to their inactivation and diffusion. For this reason, process that increase diffusional mechanisms can be useful to augment the effectiveness of these preservation techniques. One of these processes is vacuum impregnation.

Vacuum impregnation has been used in food processing being very useful in the modification of formulations and the development of new products by means of the ion incorporation (Na^+, Cl^-, Ca^{2+}) (Chiralt et al., 1999; González-Martínez, Cháfer, Fito, & Chiralt, 2002; González-Martínez, Fuentes, Chiralt, Andrés, & Fito, 1999), although its use toward the incorporation of physiologically active components (Betoret et al., 2003), has been scarcely studied. The structure of fish fillets is constituted of dot-matrix structures more or less compact responsible of many of its physical and mechanical properties (shape, size, texture). These structural characteristics allow modifying their composition by means of the use of suitable techniques (Fito, Andrés, Chiralt, & Pardo, 1996). Vacuum impregnation is a technique that allows controlling modification in the composition of the structural matrix of foodstuffs (Fito & Chiralt, 2000). As they do not need high temperatures, they allow preserving sensorial properties of the initial product (Chiralt et al., 1999; Fito & Chiralt, 2000). Therefore, the peculiar structural characteristics of fish permit the utilization of the same ones like support of components with biopreservative activity. Then, the combination of both process (biopreservation and vacuum impregnation) could be used as a tool to increase the efficiency of biopreservatives helping to their penetration and diffusion in the fillet. As a result, improvements in fish preservation and therefore a shelf-life extension of these products could be obtained.

In the current study, the main objectives were to evaluate the possible technological uses of biopreservation combined with vacuum impregnation techniques to extend shelf life of gilthead sea bream fillets; to determine the quantity of biopreservants that can be introduced in the fillets by this procedure; to determine the effect of the vacuum impregnation treatments on some physico-chemical properties as moisture, pH, water activity, color or TVBN content and finally to analyze and model the evolution of microbial population of sea bream fillets samples stored during 15 days under refrigeration.

2. Materials and methods

2.1. Preparation of samples

Cultured gilthead sea breams (*Sparus aurata*) used in this study were cultivated in net cages in a Spanish farm (GRAMASA) and harvested in Gandía (Valencia, Spain). Each fish had an approximate weight of 400 g (commercial size). After being caught, sea breams were covered in ice immediately, and maintained at −18 °C until required. In order to obtain the samples, the sea breams were defrosted (overnight, 4 °C), hand-filleted and vacuum packaged in polyethylene bags, and then stored at 4 °C.

2.2. Impregnation media

2.2.1. Lactic acid bacteria (LAB) impregnation solution

Lactococcus lactis spp lactis CECT 539 was grown in a customized culture broth (sucrose 13.33 g/L, peptone 13.33 g/L, yeast extract, 14 g/L, KH_2PO_4, 6.67 g/L, NaCl 3 g/L, $MgSO_4 \cdot 7H_2O$ 0.047 g/L). Fermentation was carried out at 37 °C for 8 h. At this point of the fermentation process, the microorganisms reached the stationary phase with a final concentration of 10^9 CFU/mL. Finally, the impregnation solution was obtained by diluting an aliquot (1:100) of the fermented broth in culture broth to a final concentration of 10^7 CFU/mL.

2.2.2. Nisin impregnation solution

Pure nisin powder (1 g = 10^6 IU) was kindly provided by Biostar S.A. (Valmojado, Spain). Nisin solutions (2000 IU/mL) were prepared by dissolving the appropriate amount of powder in a nisin diluent (0.1 N HCl until pH 5.3) solution. The solution was prepared with sterilized distilled water.

2.3. Impregnation treatments

Two vacuum impregnation treatments were performed in this work, depending on the biopreservative added. The first treatment was carried out with a solution containing lactic acid bacteria, while the second one consisted of a nisin solution.

Vacuum impregnation was carried out in a hermetic vessel connected to a vacuum pump. The sea bream fillets were impregnated at 4 °C. Fillets were immersed in the vessel with the impregnation solution (lactic acid bacteria or nisin solution). A vacuum pressure of 50 mbar was applied for 5 min, and then the atmospheric pressure was restored leaving samples under the liquid for 5 min more. After this period the fillets were drained during 5 min. Sample weight was monitored at the beginning and at the end of the process to evaluate the quantity of solution gained by the samples.

After the impregnation treatments, fillets were packaged in polyethylene bags, labeled, and stored at 4 °C for 15 days. Physical–chemical properties and color attributes were analyzed at 0, 5, 10 and 15 days. For microbial counts, samples were analyzed at 0, 3, 5, 8, 10, 12 and 15 days. Fillets without treatment were used as control.

2.4. Physico-chemical analysis

The moisture content was determined by oven drying at 105 °C for 20–24 h or until constant weight (AOAC, 1997).

For determination of pH, 10 g of fish sample was homogenized in 10 mL distilled water in the ratio 1:1 (w/v) using laboratory warring blender. The pH was measured using a Consort C830 pH-meter (Consort n.v. Parklaan, Turnhout, Belgium) by inserting the electrode into the homogenates (AOAC, 1995). The pH meter was calibrated using pH 4 and 7 buffer. Water activity was determined using an Aqualab GB-X model Fast-Lab water activity instrument (GBX, Romans-sur-Isère, France) (AOAC, 1998).

Color of fish samples before and after impregnation was determined using a Minolta CM3600d colorimeter (Minolta Co. Ltd, Tokyo, Japan) (Illuminant D 65, 10° viewing angle). The instrument was calibrated against a ceramic reference prior to use. Three random readings in different spots of the sample were taken and averaged. CIE Lab system, L^* (lightness), a^* (redness), and b^* (yellowness) were measured.