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Food safety and microbiological quality aspects of QDS process® and high pressure treatment of fermented fish sausages

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ABSTRACT

A new fermented fish sausage product, based on monkfish, was developed by using an accelerated drying process, the QDS process®. To evaluate food safety, a challenge test was performed, in which the raw materials were inoculated with low levels of *Listeria monocytogenes* and *Salmonella enterica* (≤150 CFU/g). The product was manufactured, fermented, QDS dried, and half of the samples were pressurized (600 MPa, 5 min, 13 °C). Pathogens, technological microbiota, spoilage indicator bacteria from fish (hydrogen sulphite producing bacteria, coliforms and *Escherichia coli*) and physicochemical parameters were monitored during manufacturing and after 6, 13, 20 and 27 days of refrigerated storage at 4 and 8 °C. Results showed that in the finished product, pathogens and spoilage indicator bacteria could not grow but decreased and *E. coli* was not detected during storage. Pressurization had an important reducing effect on technological microbiota, and eliminated *L. monocytogenes, S. enterica*, hydrogen sulphite producing bacteria and coliforms immediately after production and during refrigerated storage.

1. Introduction

Fish is a good protein source, contains iodine, fatty acids with high nutritional value, is easy to digest (Gelman, Drabkin, & Glatman, 2000; Nordvi, Egelandsdal, Langsrud, Ofstad, & Slinde, 2007) and has therefore a wholesome and healthy image. The development of stable fish products would widen the range of health-giving fish based food products and could represent an intelligent option to upgrade low-value species and waste generated by the fish processing industry (Gelman et al., 2000).

Fermentation of fish is one of the oldest methods to prevent fish spoilage and to extend its shelf-life (Gelman et al., 2000; Ghaly, Dave, Budge, & Brooks, 2010). Nowadays, in Northern Europe various fermented fish products such as *Rakorret*, *Tidbits* and *Surströmming* are produced in general by spontaneous fermentation (Nordvi et al., 2007). Aryanta, Fleet, and Buckle (1991) studied the occurrence and growth of inoculated pathogens (*Salmonella* Thyphimurium, *Salmonella* Sofia, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens* and *Vibrio parahaemolyticus*) during the fermentation period of fish sausages and observed that the growth of these bacteria was significantly inhibited in the product fermented with the starter *Pediococcus acidilactici*. The benefits of using lactic acid bacteria (LAB) and *Staphylococcus xylosus* as starter cultures in fish sausages have also been reported on chemical, microbiological and technological aspects (Hu, Xia, & Ge, 2008).

Food safety of fermented fish sausages is based, as in fermented meat sausages, on the hurdle technology, where acidification, addition of NaCl, nitrite/nitrate, drying and sometimes smoking are used together for preservation (Leistner, 2007). In the present study, drying was performed by the Quick-dry-slice process® (QDS process®), an innovative technology for the accelerated production of dried sliced food products (Comaposada, Arnau, Gou, & Monfort, 2004). During the process, sausages are fermented, frozen and cut into slices, which are subsequently dried in a continuous system with convective air. The fast achievement of the finished products with this new system makes it suitable for the development of new ready-to-eat (RTE) products. Various studies confirm the applicability of the QDS system on different meat products (Comaposada et al., 2008; Ferrini, Comaposada, Arnau, & Gou, 2012; Stollewerk, Jofré, Comaposada, Ferrini, & Garriga, 2011), however, up to the present day QDS processing has not been applied on products made out of fish.

High pressure (HP) processing can be applied as an additional hurdle in the framework of the hurdle technology. It is an alternative technology which allows the production of safer dry-cured meat products without introducing important modifications in the

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sensory characteristics (Garriga & Aymerich, 2009). HP is nowadays commercially applied on different fish products such as salted squid, fish sausages and shellfish (Cheftel, 1995; Garriga & Aymerich, 2009; Ohshima, Ushio, & Koizumi, 1993) but there is a lack on studies focused on pathogenic microorganisms inactivation.

Fish and fish products have been associated with outbreaks of food-borne diseases and the behavior and/or survival of pathogenic bacteria in fermented fish during production and throughout shelf-life has been investigated (Aryanta et al., 1991; EFSA, 2013a; Nieto & Toledo, 1989). Several studies have shown that *Listeria monocytogenes* occurs in fish and fishery products (Kuzmanovic et al., 2011; Lambertz et al., 2012) and EFSA reported that in 2011, 6.7% of RTE single food samples of fishery products at processing did not comply with the European Commission Regulation EC N°2073/2005 microbiological criteria (EFSA, 2013b). Further, it is also worth mentioning that *L. monocytogenes* was reported to consistently reappear at processing plants even after the application of strict sanitary and disinfection practices and could be detected at very low levels on final fish products even when strict hygienic practices were applied (Eklund et al., 1995; Garland, 1995). For *Salmonella* spp., EFSA reported that 0.6% of the tested samples (fish and fishery products) resulted positive in 2010 (EFSA, 2012).

With the aim to evaluate the food safety of QDS dry fermented fish sausages a challenge test was performed, where the fish batter was spiked with low levels of *L. monocytogenes* and *Salmonella enterica*. During manufacturing and the following storage period of 27 days under refrigeration at 4 and 8 °C, pathogens, spoilage indicator bacteria (hydrogen sulphite producing bacteria (SPB), coliforms and *Escherichia coli*) and technological microbiota were monitored. The effect of an HP treatment at 600 MPa was also evaluated.

2. Materials and methods

2.1. Production, inoculation and fermentation

Fish sausages were produced from ultra-deep frozen monkfish meat (*Lophius piscatorius*) without bones and skin. Frozen blocks of 5 kg were cut with a guillotine and coarsely chopped to reduce the size of fish pieces to *ca.* 1–2 cm^2 (to simulate the pieces of fat in a "chorizo" type product). Additionally, a part of this fish was comminuted to a lower size in a cutter. Coarsely and finely chopped fish were mixed separately with the following ingredients (in g/kg): NaCl, 20; sucrose, 10; sweet red pepper, 10; dextrose, 3.5; white pepper, 2; paprika oleoresin 20.000 U.C. (Collelldevall, Banyoles, Spain), 2; garlic powder, 1.4; ascorbate, 0.5; α-tocoferol, 0.2; Rosmanox (Sensient Technologies, Milwaukee, WI, USA), 0.15; and sodium nitrite, 0.1. Four kg of coarsely chopped and 6 kg of finely chopped fish were mixed with dextrose (3.5 g/kg) and a commercial starter culture mixture specially useful for short processing (Lyocarni VHI-41, ClericiSacco, Cadorago, Italy) containing the fast acidifying cultures *Pediococcus pentosaceus, Lactobacillus plantarum* (inoculated at 6×10^6 and 4×10^6 CFU/g, respectively) and *S. xylosus*, a gram positive catalase positive cocci (GCC+, inoculated at 1×10^7 CFU/g). The pathogens *L. monocytogenes* (a mixture of the strains CTC1011, CTC1034 and CECT4031^T at a final level of 150 CFU/g) and *S. enterica* (serovars Typhimurium GN6, London CTC1003 and Derby CTC1022 at 30 CFU/g) coming from −80 °C stocks and diluted in 10 ml of water, were also added to the fish batter. Subsequently, the mixture was stuffed in 80 mm diameter collagen casings. Fish sausages were fermented for 32 h at 20 °C and 90% relative humidity (RH), until the pH reached 4.8. Subsequently they were frozen at −18 °C. Two independent batches were manufactured.

2.2. Slicing, QDS drying and HP treatment

Fermented sausages were sliced and placed on gratings (48 slices per grate). Subsequently, QDS drying was performed by applying convective air (velocity 3.5 m/s, at 30 °C and 35–40% RH) for *ca.* 45 min until slices reached a weight loss of 40%, comparable to other dry-cured meat products. Dried slices were vacuum-packed in PET/PE pouches (oxygen permeability of 50 cc/m^2 (24 h 23 °C) and water vapor permeability of 2.6 g/m^2 (24 h, 23 °C, 85% RH), Sacoliva, S.L., Castellar del Vallès, Spain) and stored at 4 °C. After 24 h half of the samples were submitted to an HP treatment of 600 MPa for 5 min at 13 °C in a Wave 6000 equipment (Hiperbaric, Burgos, Spain). The chamber volume was 120 l, the come up time was 3.83 ± 0.13 min and the pressure release was immediate (<10 s). All samples were stored under refrigeration (9 days at 4 °C and 18 days at 8 °C (AFNOR, 2004; CRL/AFSSA, 2008)).

2.3. Microbiological analysis

Microbiological counts were analyzed in duplicate during production (raw fish, fish batter, after fermentation and after drying), after pressurization and periodically during refrigerated storage (after 6, 13, 20 and 27 days) for both pressurized and non-pressurized slices.

For each sampling, a quantity of 25 g was aseptically minced, diluted 1/10 in Brain Heart Infusion (BHI, BD Becton, Dickinson and Company, NJ, USA) and homogenized for 1 min in a Masticator Classic (IUL S.A., Barcelona, Spain). After doing the appropriate dilutions in 0.1% Bacto Peptone (Difco Laboratories, Detroit, MI, USA) with 0.85% NaCl, *S. enterica* was counted in CHROMagar™*Salmonella* plus (Sharlab, Barcelona, Spain) by spread plating and incubation at 37 °C for 48 h; *L. monocytogenes* was counted in Chromogenic *Listeria* agar (Oxoid Ltd., Basingstoke, UK) by spread plating and incubation at 37 °C for 48 h; GCC+ were counted in Mannitol salt phenol-red agar (MSA, Merck, Darmstadt, Germany) by spread plating and incubation at 30 °C for 48–72 h; LAB were counted in de Man, Rogosa and Sharpe agar (MRS, Merck) by pour plating (non-diluted homogenate) and spread plating (diluted homogenate) and incubation at 30 °C for 48–72 h in anaerobiosis; SPB were counted in Lyngby agar (Gram, Trolle, & Huss, 1987) by pour and spread plating and incubation at 25 °C for 72 h; *E. coli* and coliforms were counted in Coli ID agar (Biomerieux® SA, Marcy-l'Etoile, France) by pour plating and incubation at 37 °C for 48 h. When counts of *L. monocytogenes* and *S. enterica* were under the detection limit of 135 mm ∅ plates (LOD, 10 CFU/g), presence or absence of viable cells was investigated in the enriched homogenates (48 h at 37 °C) by seeding dots on selective media. For every *L. monocytogenes* enrichment two 20-μl dots were seeded onto Chromogenic *Listeria* agar. For *S. enterica* 200 μl of the enriched homogenate were transferred onto 10 ml of Rappaport-Vassiliadis Enrichment Broth (Oxoid). After incubation at 41.5 °C for 48 h, 10 μl were seeded onto CHROMagar™*Salmonella* Plus. Presumptive colonies of both *L. monocytogenes* and *S. enterica* were confirmed by real time PCR using primers amplifying the *hly* and *ttrBCA* genes for *L. monocytogenes* and *Salmonella* spp., respectively (Malorny et al., 2004; Rodríguez-Lázaro, Jofré, Aymerich, Hugas, & Pla, 2004).

2.4. Physico-chemical analysis

The pH was measured with a portable Crison penetration electrode connected to a Crison pH metre PH25 (Crison Instruments S.A., Alella, Spain). Water activity (a_w) values were determined using an Aqualab S3TE dew point water activity meter (Decagon Devices, Inc. Pullman, Washington, USA).