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Short communication

Listeria monocytogenes and ready-to-eat seafood in Spain: Study of prevalence and temperatures at retail

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ABSTRACT

The aim of this study was to obtain data from refrigerated ready-to-eat seafood products at retail in Spain (young eels, crabstick and smoked salmon), regarding prevalence and levels of *Listeria monocytogenes*, storage temperatures and the impact of transport conditions (type of bag) on the temperature of the product. The one-year surveillance period was carried out according to the EC Regulation No. 2073/2005, taking 5 units/batch and analyzing 250 samples following ISO 11290-1/A1 and ISO 11290-2/A methodologies. Low prevalence of *L. monocytogenes* was observed in surimi products, while 4.8% of smoked salmon samples were positive for Listeria with low levels (<10 cfu/g) and uneven pathogen distribution. A single company was responsible for 80% of the positive lots. All purchased products showed values higher than 4 °C at retail and an average increase of 2.5 °C or up to 6.2 °C was recorded when isothermal or plastic shopping bags were used for transport, respectively. To avoid noncompliance of the Food Safety Objective for *L. monocytogenes* in seafood RTE products more efforts from all stakeholders are needed, with special attention so as to improve control and maintenance of refrigerators at retail and to enhance consumer education regarding food safety practices.

1. Introduction

The number of reported listeriosis cases in European countries has increased in recent years (EFSA, 2012), and this illness is the most important cause of mortality from foodborne infections. The extended consumption of ready-to-eat (RTE) products (Lianou and Sofos, 2007; Uyttendaele et al., 2009) and the increase of risky population (i.e, the elderly and immunocompromised individuals) could be some of the reasons for the observed increase of illness in Europe (Garrido et al., 2012).

Listeria monocytogenes is widely extended throughout the environment (Fenlon et al., 1996) because this pathogen has the ability to survive in stress conditions such as low temperature, high salt concentration and low pH (Ryser and Marth, 1999; Warriner and Namvar, 2009). Taking into account that *L. monocytogenes* is a psycotrophic microorganism, refrigerated RTE foods must be kept at safety temperatures to minimize the growth of the pathogen (Ben Embarek, 1994; Brackett, 1999; Cox et al., 1999; Farber and Peterkin, 1999), especially in the case of products with a long shelf-life (Huss et al., 2000). However, reported prevalence of *Listeria* in RTE products ranges from 3% to 25% depending on the type of product (Cabedo et al., 2008; Garrido et al., 2009; Lianou and Sofos, 2007; Meyer et al., 2012).

According to the reported infective dose of *L. monocytogenes* and taking into account the possible pathogen growth during storage and distribution, the European Commission Regulation EC No. 2073/2005 established a limit of 100 cfu/g for "RTE foods able to support the growth of *L. monocytogenes* placed on the market and during their shelf-life" (Food Safety Objective). The latest European Zoonoses Summary Report (EFSA, 2012) shows that the safety limit is exceeded in different RTE products at retail level, but especially in fishery products (1% of non-compliance). However, the number of samples with levels higher than 100 cfu/g at the time of consumption could be underestimated due to deficient sampling procedures (for example, analyses of only 1 unit per batch) and because analyses of retail products not always are performed at the expiry time. In addition, reported information concerning storage conditions of RTE products until consumption is very limited (Carrasco et al., 2007; Garrido et al., 2010; Morelli et al., 2012).

The main goal of this study was to obtain data regarding the occurrence of *L. monocytogenes* at retail in the most consumed RTE seafood products in Navarra (Spain), as well as to acquire information regarding refrigeration temperatures recorded at market and after transport to home by using the standard procedures of

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consumers. Specific objectives were: (i) to estimate the prevalence and levels of *L. monocytogenes* in smoked salmon and surimi (crabstick and young eels) at the time of purchase and at the end of shelf-life; (ii) to determine the average temperature of storage products at retail; and (iii) to study the temperature increase when products are transported to home using either common or isothermal bags.

The work performed in this study is allocated within the large-scale integrating project "BASELINE: Selection and improving of fit-for-purpose sampling procedures for specific foods and risks" (Seven Framework Programme, grant agreement *n*° 222738). Data provided within the BASELINE project will be helpful for the harmonization and validation of sampling strategies suitable for food producers and food authorities in order to improve food safety management.

2. Materials and methods

2.1. Sample collection

The study was conducted on RTE seafood that was selected based on the following features held in common: widespread consumption in Navarra (northern Spain), packaged (sliced or unsliced), marketed at refrigeration temperatures and with extended shelf-life (>15 days). We carried out surveillance of *L. monocytogenes* in smoked salmon and surimi (crabstick and young eels) from October 2010 to September 2011. For each product category, samples were purchased at retail ($n = 8$ supermarkets) from all available brands with a sampling frequency of 4 batches per month (2 smoked salmon and 2 surimi). In accordance with European Regulation (EC) No 2073/2005, 5 samples per batch were purchased (one sample = one package), with packaging sizes ranging from 100 to 200 g in the case of smoked salmon and from 100 to 400 g in the case of surimi. The number of analyzed lots in each category was different depending on the brands that were available in the market.

A total of 250 seafood samples were purchased and transported to the laboratory within 30 min, using common bags or isothermal bags simulating consumer transport conditions. Information regarding each product was recorded: storage temperature conditions indicated on the label, batch number and expiry date.

2.2. Temperature recording (storage and transport)

The food temperature of a test-product subject was taken from the same cabinet area where samples are purchased. Product temperature was measured twice by using a calibrated thermometer (Checktemp1, Hanna Instruments): at the point of sale and immediately after arrival to the laboratory. At both moments, the probe was placed in the core of the test-food (prior disinfection with ethanol) and temperature was recorded. In addition, display temperatures of open refrigerated cabinets were also recorded (if available).

2.3. Analytical methods (bacteriological and serological)

Samples were examined for the detection and enumeration of *L. monocytogenes* according to ISO 11290-1/A1 (ISO, 2005a) and ISO 11290-2/A1 (ISO, 2005b), respectively. The laboratory has implemented a quality system based on the standard ISO/IEC 17025 and is accredited by ENAC (the official Spanish organism to asses technical competence of laboratories) for performing both microbiological assays. Each sample was tested twice, resealing the package after the first analysis: day of shopping (immediately after arrival to the laboratory) and at the end of the shelf life (storage at 4 °C ± 1 °C).

Twenty-five grams of each sample were analyzed for the investigation and enumeration of pathogen. Briefly, selective enrichments Fraser and Half Fraser (Biomerieux, Marcy L'Etoile, France) were used for the investigation and colonies were isolated on Palcam and ALOA agar (AES, Cedex, France). For the enumeration, Buffered Peptone Water (Merck, Darmstadt, Germany) was used at 20 °C ± 2 °C during 1 h ± 5 min in order to recover stressed microorganisms. Next, 1 ml of this suspension was plated on three ALOA plates. Following incubation of ALOA and Palcam plates at 37 °C ± 1 °C during 24–48 h, five suspicious *Listeria* colonies were isolated on Trypcase Soy Yeast extract agar plates (TSAY, Biomerieux) in order to perform biochemical confirmation (gram stain, catalase, Henry illumination test, xylose and ramnose fermentation, β-hemolysis and CAMP test).

Serotyping was carried out using specific antisera (Denka Seiken, Tokyo, Japan) following the manufacturer's instructions. Both polyclonal anti-O antisera (O-I/II, O-V/VI, O-I, O-II, O-VI, O-VII, O-VIII, O-IX) and anti-H (H-A, H-AB, H-C, H-D) were used in the determination of somatic and flagellar antigens, respectively. Results were interpreted according to the serotyping scheme established by Seeliger and Höhne (Seeliger and Höhne, 1979).

2.4. Statistical analysis

Factorial ANOVA were used to test for differences in the increase of product temperatures during transport and season. The statistical analyses were performed using SPSS 15 software.

3. Results and discussion

3.1. Prevalence and levels of L. monocytogenes

The prevalence of *L. monocytogenes* in the analyzed samples on the day of purchase is shown in Table 1 (similar data were obtained at the expiry time). None of the 125 surimi tested samples were contaminated with the pathogen. These results coincide with those obtained by other authors (Farber et al., 2000; Farber and Peterkin, 1991), showing a very low prevalence of the pathogen and a high quality product from a safe point of view.

A higher occurrence was found in smoked salmon. However, prevalence was different depending on the item. If we consider results obtained from the batches, the incidence of the pathogen was 20% (5 out 25), whereas 4.8% of incidence was detected among analyzed samples. Prevalence ranging from 5% to 15% has been reported in the latest years in different countries, in studies in which only one sample per batch was analyzed (Cabedo et al., 2008; EFSA, 2011; Inoue et al., 2000; Kovacevic et al., 2011; Latorre et al., 2007). In a similar way, previous surveys regarding smoked salmon purchased in Navarra, testing a single sample per lot, reported prevalence ranging from 28.2% during the period

Table 1
Listeria monocytogenes in RTE seafood products: occurrence by lots, samples and brands from October 2010 to September 2011.

Product	No. of			No. (%) of lots/samples/brands positives for *L. monocytogenes*[a]		
	Lots	Samples	Brands	Lots	Samples	Brands
S. salmon	25	125	12	5 (20%)	6 (4.8%)	2 (16.7%)
Young eels	13	65	6	0	0	0
Crabstick	12	60	6	0	0	0

[a] Analyzed samples at the day of purchase. Similar results were obtained at the expiry time.