Physicochemical and microbiological characteristics of diverse Spanish cured meat products

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ABSTRACT

A study about the influence of commercial packaging on physicochemical and microbiological characteristics of Spanish cured meat products was performed. For this purpose, 100 samples of fermented sausages (FS) and pieces of cured meat (P) were analyzed. Results for mean pH values were 5.12 (FS) and between 5.79–5.83 (P). All mean values for \(a_w\) were under 0.90. Mean counts for mesophilic aerobic bacteria were more than 8 log cfu/g (FS) and they were between 4.47 and 7.61 log cfu/g (P). Microbiological groups of Enterobacteriaceae were not detected in more than two-thirds of the samples. The presence of Staphylococcus aureus was frequent. Salmonella spp. and Listeria monocytogenes were detected, after enrichment, in 4 and 5 samples, respectively. Clostridium botulinum was not detected. A statistically significant \((p < 0.05)\) absence of enterobacteria was observed in packaged samples.

Características fisicoquímicas y microbiológicas de diversos productos cárnicos curados españoles

RESUMEN

Se ha llevado a cabo un estudio sobre la influencia que puedan tener las distintas presentaciones comerciales de productos cárnicos españoles sobre las características fisicoquímicas y microbiológicas de los mismos. Para conseguir este objetivo, 100 muestras de embutidos (FS) y piezas curadas (P) fueron analizadas. Los resultados para los valores medios de pH fueron 5,12 (FS) y entre 5,79–5,83 (P). Todos los valores de \(a_w\) estuvieron por debajo de 0,90. Las medias de los recuentos para la flora aerobia mesófila fueron de más de 8 log ufc/g (FS) y entre 4,47-7,61 log ufc/g (P). Distintos componentes de la familia Enterobacteriaceae no fueron detectados en más de dos tercios de las muestras. La presencia de Staphylococcus aureus fue habitual. Salmonella spp. y Listeria monocytogenes fueron detectados, tras enriquecimiento, en 4 y 5 muestras, respectivamente. Clostridium botulinum no fue detectado. Fue observada una ausencia, estadísticamente significativa \((p < 0.05)\), de enterobacteria en las muestras envasadas.

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own microbiota. However, although rare, meat products have been identified as the focus of some foodborne outbreaks. In Spain, meat and meat products represented 8% of the total of foods implied in foodborne outbreaks, excluding waterborne outbreaks, between the years 2008 and 2011 (Espinosa, Varela, Martínez, & Cano, 2014). This percentage identifies meat products as the second cause of foodborne outbreaks. Alternatively, foodborne outbreaks, or the likelihood of occurrence, produced by pathogens such as Staphylococcus aureus, Listeria monocytogenes, Salmonella spp. and Clostridium botulinum, specifically related to fermented sausages, hams and cecinas, have been reported many times by numerous authors (CDC, 2010; Lyytiäinen et al., 2000; Sofos, 2008).

With regard to this article, the main aspect of interest is the effect that the changes of consumer preferences toward convenience foods could have on the microbiological quality and safety of traditional cured meat products. Presently, convenience foods are highly demanded, and meat products not only follow this trend, they are at the heart of it (Leroy & Degreef, 2015). Specifically, very popular and highly demanded products are the trays of mechanically sliced cured meat products (del Olmo, Calzada, & Núñez, 2014). However, steps such as cutting, slicing and packaging that the post-manufacturing processing of this kind of product requires may cause recontamination with microorganism pathogens (del Olmo et al., 2014).

There are few studies that have revised the risks related to commercial presentations with the physicochemical and microbiological characteristics of Spanish meat products. This survey has been proposed as an overview of a combination of all of these aspects. The first step was to evaluate the physicochemical and microbiological characteristics of various typical cured meat products produced and commercialized in Spain. Subsequently, it was studied if the commercial presentations of cured meat products could have some relationship to either of these parameters, or to an increase in the microbiological risk.

2. Materials and methods

2.1. Materials

Commercial cured meat products were purchased from different local stores, such as supermarkets and grocery stores. A total of 100 samples were taken, which were distributed between the most popular cured meat products: CH and SA as representatives of fermented sausages, and samples of dry cured loin, LO, CE and cured ham, JA as samples of cured meat pieces. A few samples of other special products were taken to complete an overview of the most frequent cured meat products present in Spanish food markets. Furthermore, different commercial presentations and types of packaging were taken as representative of the most common products of cured meat products available in food markets. All the samples taken for this experiment with the type of product, commercial presentation and type of packaging are described in Table 1.

2.2. Methods

2.2.1. Physicochemical analyses

The samples purchased for this study were analyzed in order to measure water activity, pH and NaCl and nitrate/nitrite content. A water activity (a_w) measurement was carried out and duplicated for each sample, at 25°C with a LabMaster (Novasina AG, Switzerland) instrument. The pH of the samples was measured using a pH meter (model PH 25, Crison Instruments, S.A., Spain) with a penetration electrode (model 52–32, Crison Instruments, S.A., Spain). Each value represents the mean of two different puncture points. The extraction procedure for the measurement of NaCl and nitrate/nitrite content is the same for two parameters (Spanish Ministry of Health, 1985). The Volhard method was used to determine the NaCl content as chloride concentration. The nitrate/nitrite content was directly valued as anions by capillary electrophoresis, as based on Friedberg, Hinsdale, and Shihabi (1997).

2.2.2. Microbiological analyses

To evaluate the microbiological quality of the cured meat products, several microorganism groups, habitually considered as indicator microorganisms of the quality of food products (Busta et al., 2003), were evaluated in the samples. Microbial groups used as indicators were: mesophilic aerobic bacteria, psychrotrophic bacteria, Enterobacteriaceae and Escherichia coli with other fecal coliforms. For this purpose, subsequent microbiological analyses of the samples were carried out according to the following steps. An amount of 25 g of each meat product piece was aseptically taken and homogenized with 225 ml of buffered peptone water (Oxoid Ltd., Hampshire, U.K.) in a sterile plastic bag in a Silver Panoramic Masticator (IUL, Spain) for two minutes. Serial decimal dilutions of the homogenized samples were made in sterile Bacteriological Peptone 0.1 % (Oxoid Ltd., U.K.) and duplicate aliquots were plated onto appropriate culture media (all from Oxoid Ltd., U.K.) in order to determine the next indicator microorganisms under the conditions described as the following: mesophilic.

Table 1. Summary of the type cured meat products, the commercial presentations and kind of packaging of the samples used in this study.

| Cured meat product type | Number of sample taken, presentation, packaging |
|-------------------------|-----------------------------------------------|
| FS                      | CH 5 P; 8 P MAP; 4 SS; 8 PS MAP               |
| FS                      | SA 5 P; 4 P MAP; 4 SS; 5 PS MAP               |
| CM                      | JA 1 P; 1 P MAP; 7 SS; 10 PS MAP              |
| CM                      | CE 2 P MAP; 3 SS; 8 PS MAP; 3 PS OP           |
| CM                      | LO 2 P; 1 P MAP; 6 SS; 9 PS MAP               |
| 0                       | Salami 1PS MAP; Rabbit jamón 1 P MAP; Horse cecina 1 PS MAP; Cow cured tongue 1 PS MAP |

FS: fermented sausages; CM: cured meat; O: other cured meat products; P: not sliced piece of cured meat product; SS: sliced in food store; PS: trays of cured meat product sliced in food manufacturing factory; MAP: modified atmosphere packaging; OP: packaging in olive oil.

Presentaciones: FS, embutidos fermentados; CM, carne curada; O, otras carnes curadas; P, pieza de producto curado no loncheada; SS, loncheada en tienda; PS, bandejas de producto curado envasadas en fábrica de origen; -, no packaging, MAP, envasado en atmósfera modificada; OP, envasado en aceite de oliva.
aerobic bacteria and psychrotrophic bacteria in Standard Plate Count Agar, incubated at 30°C for 48 h for mesophilic and at 10°C for 7 days for psychrotrophic; Enterobacteriaceae in Violet Red Bile Glucose Agar, double-layered and incubated at 37°C for 24 h; *E. coli* and other fecal coliforms in BrillianceTM E. coli/ Coliform Selective Medium incubated at 37°C for 24 h.

In order to assess the safety of the cured meat product, the following microbiological analysis was performed to detect and identify *S. aureus*, *Salmonella* spp., *L. monocytogenes* and *C. botulinum*. From the homogenized samples prepared to evaluate the microbiological quality of the samples, decimal dilutions were made in Buffer Peptone Water (Oxoid Ltd., U.K.) and 0.1 ml was plated onto Baird Parker Agar (Oxoid Ltd., U.K.) to detect the presence of *S. aureus* (SA). The plates were incubated at 37°C for 48 h. If gray-black colonies with a clear zone around them were found, they were suspected of being SA. These colonies were confirmed to be SA with the Staphyloase Test Kit (Oxoid Ltd., UK). Results were considered positive when suspect colonies showed agglutination. For the detection of *Salmonella* spp. (S), the ISO 6579:2002 method was applied (Anonymous, 2002). These colonies were confirmed as being SS with a *Salmonella* Test Kit (Oxoid Ltd., U.K.). To detect the presence of *L. monocytogenes* (LM) in samples, specific microbiological analysis, based in UNE-EN ISO 11290–2 and UNE-EN ISO 11290–1 methodology (Anonymous, 1996, 1998), were made. To confirm these colonies as being LM, a PCR technique was used based on Furrer, Candrian, Hoefelein, and Luethi (1991), which uses primers that amplify a fragment of the gene coding for α-haemolysin of *L. monocytogenes*. For detection and identification of *C. botulinum* (CB), a PCR technique was used with primers U1 and U2 that identified genes encoding for botulinum neurotoxins (Fach, Micheau, Mazuet, Perell, & Popoff, 2009; Fenia et al., 2011). As a previous step, based on Solomon and Lilly (2001), an enrichment was done as well.

### 2.2.3. Statistical study

Data were analyzed using the statistical program SPSS for Windows (Version 21; SPSS INC., U.S.A.). Measures of central tendency and dispersion were calculated for the data samples when the values of the parameters to be measured, physicochemical values or microbiological counts, were over the detection limit. Values under the detection limit were expressed as a percentage of “not-detected.” The Kolmogorov–Smirnov test was used to test the normality of the sample distribution and Levene’s test was performed to assess the equality of variances. The Student’s t-test for independent samples was applied individually to each product, for statistical determination of the association between the slicing and the packaging of the samples with their physicochemical characteristics and with their counts for mesophilic aerobic bacteria and psychrotrophic bacteria. In order to evaluate the influence of slicing or packaging on the presence of enterobacteria, total coliforms, *E. coli* and all evaluated pathogens, a Pearson’s chi-squared test was done with all samples. Differences were considered significant at the level of *p* < 0.05.

### 3. Results and discussion

#### 3.1. Physicochemical characteristics of analyzed cured meat products

The results of physicochemical analysis for pH, water activity and salt and nitrate content are shown in Table 2. Residual nitrate/nitrite levels for all meat products were below the detection limit for the technique applied, 0.30 mg/kg, except for one sample of cured loin where nitrite content was 0.89 mg/kg.

With respect to mean values of pH obtained for CH and SA, 5.12 for both, they are similar to those reported by other authors, such as Aymerich, Martín, Garriga, and Hugas (2003), and Marco, Navarro, and Flores (2008). These pH values represent the type of fermented sausages (FS) consumed in Spain, which can be considered fermented meat products with low acidity, which are typical of Mediterranean countries. The mean pH values for pieces of cured meat products (P) were higher than the values observed in FS (Table 2). These values were approximately 5.7–5.8 and they were consistent with expected values for this type of product, especially in the case of JA and CE (Rubio et al., 2007; Sánchez-Moliner, García-Regueiro, & Arnau, 2010). For LO, Ruiz-Ramírez, Serra, Arnau, and Gou (2005), reported a pH value of approximately 6.

With regards to the *a*<sub>w</sub> mean values for cured meat products obtained, all of them were low, between 0.85 and 0.90, which was to be expected from this type of product, according to the literature (Rubio et al., 2006; Ruiz-Ramírez et al., 2005; Sánchez-Moliner et al., 2010).

Another physicochemical aspect of importance was the salt content of the studied cured meat products. Our values for percentages of NaCl were near 3% in most products, except for CH, whose mean value was 1.86%. At this point, we have to point out that our results were lower, especially for CE samples,

| Table 2. Mean values and desviaciones estándar del pH, actividad de agua y contenido de NaCl y nitratos de las muestras de productos cárnicos curados. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| CH | SA | JA | CE | LO |
| pH | 5.12 ± 0.28 | 5.12 ± 0.43 | 5.73 ± 0.22 | 5.83 ± 0.29 | 5.71 ± 0.37 |
| *a*<sub>w</sub> | 0.86 ± 0.05 | 0.85 ± 0.05 | 0.89 ± 0.03 | 0.90 ± 0.03 | 0.90 ± 0.03 |
| NaCl (%) | 1.89 ± 0.78 | 2.66 ± 0.85 | 2.95 ± 1.17 | 3.04 ± 0.88 | 2.53 ± 1.00 |
| Nitrates (mg/kg) | 2.28 ± 3.06 | 2.20 ± 2.46 | 1.64 ± 0.80 | 1.52 ± 1.23 | 2.76 ± 2.84 |
| NND (%) | 32 | 27.78 | 21.05 | 50 | 16.67 |

| MP | Salami | Rabbit jamón | Horse cecina | Cow cured tongue |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| pH | 4.25 | 6.05 | 5.35 | 5.24 |
| *a*<sub>w</sub> | 0.87 | 0.90 | 0.91 | 0.83 |
| NaCl (%) | 1.75 | 2.30 | 3.49 | 1.52 |
| Nitrates (mg/kg) | 0.89 | ND | 0.32 | 0.53 |

*a*<sub>w</sub>: water activity; % NND: % de muestras donde el nivel de nitratos estaba por debajo del límite de detección de la técnica, 0.3 mg/kg; CH: chorizo; SA: salchichón; JA: jamón; CE: cecina; LO: lomo.
Table 3. Valores medios (log cfu/g) y desviaciones estándar de microorganismos indicadores en muestras de productos cárnicos curados. Porcentajes de muestras donde no fueron detectados.

| MP  | CH     | SA     | JA     | CE     | LO     |
|-----|--------|--------|--------|--------|--------|
| MB  | 8.79 ± 1.22 | 8.35 ± 0.99 | 4.47 ± 0.87 | 6.70 ± 1.46 | 7.61 ± 1.06 |
| PB  | 8.37 ± 1.04     | 8.19 ± 0.95     | 4.58 ± 0.93     | 6.25 ± 1.81     | 7.35 ± 1.57     |
| E   | 2.35 ± 1.55     | 1.94 ± 0.73     | 1.56 ± 0.45     | 1.41 ± 0.89     | 1.95 ± 0.89     |
| TC  | 2.84 ± 1.39     | 1.92 ± 1.06     | 2.28*          | 1.85 ± 1.08     | 0.70*          |
| EC  | 2.15 ± 0.77     | 1.26 ± 0.43     | –              | –              | –              |
| SA  | 4.93 ± 0.66     | 4.42 ± 1.32     | 4.05 ± 1.13     | 3.91 ± 1.41     | 4.57 ± 1.24     |
| E nd (%) | 68       | 72.28      | 84.20      | 68.75      | 83.40      |
| TC nd (%) | 76       | 66.72      | 94.70      | 81.25      | 94.52      |
| EC nd (%) | 84       | 77.84      | 100       | 100       | 100       |
| STA (d%) | 32       | 33.33      | 42.11      | 18.75      | 72.22      |

MP: Mesophilic aerobic bacteria (MB), psychrotrophic bacteria (PB), enterobacteria (E), total coliforms (TC), Escherichia coli (EC), Staphylococcus aureus (STA).

Table 3. Mean values (log cfu/g) and standard deviation of indicator microorganisms in samples of cured meat products and Staphylococcus aureus. Percentages of samples where some of them were not detected.

For dry-fermented sausages, CH and SA, the mean values for mesophilic aerobic bacteria (MB), are between the range of 8 and 9 log cfu/g, as reported by other authors in most of the studies that have been consulted (Casquete et al., 2012; Lorenzo, Temperán, Bermúdez, Cobas, & Purriños, 2012; Rubio et al., 2007). These high counts can be considered normal characteristics of this type of fermented sausage, and they do not represent microbial alteration. With respect to enterobacteria (E), total coliforms (TC) and E. coli (EC), were not detected in more than two thirds of the total number of analyzed samples (Table 3). The mean count values, in log cfu/g, of the samples where they were present were: 2.35 (E), 2.84 (TC) and 2.15 (EC) for CH and 1.94 (E), 1.92 (TC) and 1.26 (EC). In both aspects, the large number of samples where these microorganisms were not detected, and the low counts when they were present, indicate a good hygienic quality of the samples, and they are consistent with the observations published by other authors, such as Casquete et al. (2012) and Rubio et al. (2007).

The pathogen S. aureus was detected in 32% of the samples of CH and in 33.33% of the samples of SA, with mean counts of 4.93 log cfu/g for CH and 4.41 log cfu/g for SA (Table 3). S. aureus can be considered a common microorganism found in chopped meat mixes and in processing plants, and its presence in both types of fermented sausages, CH and SA, can be considered habitual (Casquete et al., 2012; Martin, Colin, Aranda, Benito, & Córdoba, 2007).

For pieces of cured meat products, JA, CE and LO, the obtained results for counts of MB and PB, expressed as log cfu/g, were 4.47, 6.70 and 7.61, respectively. For PB, which was expressed in the same way as for MB, the values were: 4.58, 6.25 and 7.35. All these values can be considered representative of these types of cured meat products, expect for cured loin, LO, for which our values could be considered higher with respect to what other authors found. For JA, other authors reported values very similar to ours (Blesa et al., 2008; Parra et al., 2010). With respect to CE, our values are in the range reported by Rubio et al. (2006), in their studies about the influence of different types of packaging over some characteristics of CE. In the case of cured loin, LO, our results were higher than the values observed by other authors in the few publications about this product. For
instance, Aliño et al. (2009) and Aliño et al. (2010), obtained mean count values between 2.77 and 3.48 log cfu/g. Campus, Flores, Martínez, and Toldrá (2008), reported higher values for control samples of cured loin after 45 days of storage. With this data, it cannot be concluded which counts, for MB and PB, are the most habitual for cured loin. For more than 80% of the analyzed cured meat pieces, enterobacteria and total coliforms were not found (Table 3). There was a total absence of E. coli in all the P samples. The mean values of the counts where E and CT were present were slightly lower than the same values for fermented sausages (Table 3) These results are in agreement with those found by Parra et al. (2010), Rubio et al. (2007), and Aliño et al. (2010).

*S. aureus* was detected in all types of pieces of cured meat products with these percentages: 42.11% for the JA, 18.75% for CE and 72.22% for LO samples. These counts were 4.05 log cfu/g for JA, 3.91 log cfu/g for CE, and 4.57 log cfu/g for LO. In the bibliography consulted, SA was not found except for Blesa et al. (2008), who detected counts of SA of about 3 log cfu/g in cured hams. In the case of CE, the presence of this pathogen in high counts has been detected as well in products similar to CE, such as biltong (Mhambi, 2008) and charqui (Pinto, Ponsano, Franco, & Shimokomaki, 1998). Finally, for LO, a high prevalence of SA with high counts has been found by Aliño et al. (2010). We can conclude that the presence of *S. aureus* in this type of cured meat product is frequent, as it is present in fermented sausages.

The levels of *S. aureus* are another discussion point of interest. From a safety point of view, in relation with the capacity of the microorganism to produce enough amounts of the SE toxins as if to cause foodborne illness it is firmly established that it is needed more than $10^{5}$ cfu/g of *S. aureus* to cause staphylococcal intoxication (Huong et al., 2010; Vestergaard, 2001). Values higher than $10^{5}$ cfu/g have not been observed in the meat products microbiological tested. With respect to other pathogen microorganisms, it was considered suitable to discuss the results for all types of cured meat product conjointly. After enrichment, *Salmonella* spp. was detected and confirmed in only two samples of CH and two samples of SA, which were all from the same manufacturer. *L. monocytogenes* were detected after enrichment in five samples, one sample of CH, one of SA, two of CE and one of LO. In JA, this microorganism was not detected. *C. botulinum* was not detected after enrichment in any sample.

Regarding *Salmonella* spp., based on our results and on the results found in other studies, the presence of this pathogen can be considered infrequent in cured meat products (Casquete et al., 2012; Gormley, Little, Grant, de Pinna, & McLauchlin, 2010). However, there are documented outbreaks of salmonellosis associated with cured meat products, especially with fermented sausages such as salami (CDC, 2010). Incidentally, in our investigation, *Salmonella* spp. was only detected in fermented sausages. *L. monocytogenes* was found in many studies previously cited, such as Aliño et al. (2010), Gormley et al. (2010), Casquete et al. (2012). Based on these publications, we can consider our results about LM to be normal for this type of product.

Disregard to the threshold values for tolerance of these pathogens, related to *Salmonella* spp. It is commonly accepted that values above $10^{2}$–$10^{5}$ cfu/g are necessary to cause infection (Jay, 2002) but there are evidences that values about 10 cfu/g can cause the illness (D’Aoust & Maurer, 2007). In relation with *L. monocytogenes*, published data about the observed microbial load in documented outbreaks are regularly higher than 100 cfu/g (Swaminathan, Cabanes, Zhang, & Cossart, 2007). These levels had not been observed in the study, these pathogens only had been detected in a few samples after enrichment.

Finally, *C. botulinum* has not been detected in any analyzed sample, which is consistent with observations made by experts on this microorganism (Bell & Kyriakides, 2005), although food outbreaks related to this pathogens and cured meat products has been documented often over time (Bell & Kyriakides, 2005).

To finish this overview of cured meat products, the effects of slicing and packaging on the microbiological findings of meat products was studied statistically. The results showed that there was no association between the slicing of the samples with the microbiological counts for indicator microorganisms or pathogens (*p* > 0.05). A statistically significant influence of the packaging on E, TC, EC and S in samples was detected (*p* < 0.05).

Contrary to what could be expected – that the intense handling of the meat products during packaging could enhance the risk of microbial contamination – a significant absence of these groups of microorganisms in packaged samples was found. For other studied microorganisms, the packaging of the products appeared to have no significant effects.

**4. Conclusions**

The mean values for physicochemical characteristics of cured meat products can be considered normal for this type of product. With regards to microbiological characteristics, our results can be considered representative of cured meat products. A statistically significant influence of the packaging on the absence of enterobacteria, total coliforms, *E. coli* and *Salmonella* spp. in analyzed cured meat products was found.

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