Sous-vide cooking of traditional meat products: effect on the microbiology of dry-cured pork foreleg

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The aim of this work was to assess the effect of *sous-vide* cooking and storage at different temperatures in the microbiology of a traditional meat product (dry-cured pork foreleg, *Lacón gallego*) that is usually consumed after it is desalted and boiled. Microbial parameters (total plate counts, *Micrococcaceae*, *Staphylococcus aureus*, lactic acid bacteria, *Enterobacteriaceae*, Clostridia hydrogen sulphide producers and the presence or absence of *Salmonella*) were determined in raw, desalted and packed and unpacked samples after cooking and at 14 and 28 days of storage at 2, 10 and 20ºC. Chemical and physicochemical parameters that affect microbial growth (pH, water activity and chloride, nitrate and nitrite contents) were also determined. The *sous-vide* cooking of dry-cured pork foreleg pieces significantly reduces microbial growth at storage temperatures of 2ºC, 10ºC and 20ºC. Moreover, it is able to extend the product shelf life to 28 days even when stored at 20ºC.

Keywords *sous-vide* cooking; dry-cured meat; ready-to-eat meat products; shelf life; chilled storage

1. Introduction

Sous-vide or vacuum cooking technology began in the early 1960s; it is used in restaurants, catering and industrial processing (particularly for meat and meat products). Nowadays, it is becoming increasingly popular because it affords convenient, ready-to-eat foods of high organoleptic quality, prevents evaporative losses of water and flavour volatiles during heat treatment, at the same time as it also maintains nutritional quality by reducing leaching and oxidative losses of nutrients during preparation and chilled storage [1, 2].

In *sous-vide* processing, the ingredients are precooked, packaged under vacuum in heat-stable, high gas barrier materials, and then cooked by hot air, steam or water at 70-100ºC. Heating is followed by rapid cooling. The products are then stored, distributed and retailed under refrigeration so as to inhibit the growth of aerobic microorganisms. The shelf-life of *sous-vide* products ranges from 7 to 45 days and depends on the food composition, processing and legal regulations [2, 3].

However, on the other hand *sous-vide* processing might permit the survival of heat resistant and psychrotolerant obligate and facultative anaerobes due to the low oxygen tension produced in foods. Moreover, the vacuum inhibits the aerobic spoilage microorganisms; thus it reduces the population of microbial competitors. For this reason, cook-chilled vacuum packaged food might exhibit health risks before noticeable spoilage [1]. Hence, *sous-vide* cooked foods have received a lot of attention by researchers over the past two decades.

Dry-cured pork foreleg is a traditional meat product produced in the northwest of Spain (Galicia) where it is called *Lacón gallego*. The product is elaborated by cutting the fore extremity of the pig at the shoulder blade–humerus joint. The processing is very similar to that used in the elaboration of dry-cured ham: salting, washing, standing or post-salting, and drying or curing. The length of these stages is shorter in dry-cured forelegs (approximately 35 days) than in dry cured ham due to the smaller size of the pieces (approximately 4 kg). Dry cured pork foreleg is a shelf-stable meat product due to its low water activity (< 0.92) and its high sodium chloride content (> 7.8 g/100g meat) [4].

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Lacón gallego is generally eaten after it is desalted and cooked (normally boiled). Generally, the desalting period takes 48-72 hours for whole forelegs or approximately 24-30 hours for smaller pieces. It is done in order to reduce the salt content and to improve the taste of the product. Cooking takes from 60 minutes to several hours, depending on the size of the piece. Afterwards, the cooked product must be consumed in a short period of time.

This *sous-vide* technology could afford a cooked product that satisfies the consumer demands for foods with reduced or simple culinary preparation and extended shelf-life. Although dry cured pork forelegs are very stable and safe, the culinary treatment includes a desalting step, which involves soaking the meat in water for a long period of time; in this stage the product loses sodium chloride and increases its water content. This fact might permit good enough conditions for the rapid growth of the microorganisms already present in the raw product. The heat treatment is usually carried out at 100ºC, so it is probably not enough to guarantee the destruction of some heat resistant organisms, which could grow during storage.

The aim of this work was to assess the effect of *sous-vide* cooking and storage at different temperatures on the microbiology of a traditional meat product (dry-cured pork foreleg, *Lacón gallego*) that is usually consumed after it is desalted and boiled.

2. Methods

2.1   Processing of forelegs and sampling

Nine dry cured pork forelegs were used for the analysis. The manufacture process was carried out as follows: the fore extremities were removed from the carcass post-slaughter at the shoulder blade-humerus joint. The raw forelegs (about 4 kg each one) were rubbed with salt containing about 0.1% potassium nitrate, and placed in piles of salt at a low temperature (2-5ºC) and high relative humidity (80-90%) for 4-5 days (1 day per kg of weight). After washing to remove the salt from the surface, the forelegs were hung in a post-salting or standing room at 2-5ºC and relative humidity of 85% for 15 days. Once the post-salting stage had finished, the forelegs were transferred to a drying-ripening room at 12ºC and 60% relative humidity for 15 days.

Eight portions (200 g approximately) were taken from each foreleg. The first was analysed without any treatment (dry cured sample). The other portions were desalted in water at 2ºC for 24 hours (4 litres of water/kg piece); the water was changed after 10 hours. Afterwards, one of the desalted portions was analysed as a desalted sample, three of them were vacuum-packed in a polypropylene/polyamide laminated pouches using a Stephan Alval vacuum packer (mod. 190), while the other three remained unpacked. The six portions were inserted into boiling water and cooked for 1 hour. The temperature at the thermal centre of the pieces was taken. Afterwards they were cooled rapidly. Two portions, one vacuum-packed and the other unpacked, were analysed as packed and unpacked cooked samples (0 days) respectively. The other two unpacked ones were introduced in clean plastic boxes after boiling. Then, the remaining four (two packed and two unpacked) portions were stored for 14 or 28 days (one packed and one unpacked for each sampling period). The experiments were carried out in triplicate for each storage temperature: 2ºC (an appropriate storage temperature for *sous-vide* cooked products), 10ºC (temperature in domestic refrigerators and in some retail display cabinets [5]), and 20ºC (corresponding to abuse conditions, room temperature).

2.2   Microbial analyses

Microbial analyses were performed on raw, desalted and packed and unpacked pieces after cooking and at 14 and 28 days of storage at 2, 10 and 20ºC. Meat samples of 10 g were collected aseptically in a Stomacher bag containing 90 ml of sterile buffered peptone water (2% w/v) and were blended for five

minutes using a Colworth 400 Stomacher. Subsequent dilutions were obtained by mixing 1 ml aliquots with 9 ml of buffered peptone water.

The microbial determinations were selected according to the Spanish laws for cooked ham and for ready-to-eat foods, due to the lack of other specific legislation for the product studied. In addition, *Micrococcaceae* and lactic acid bacteria were also counted since they are usually present in meat products and in some cases might cause deterioration of cooked products. Total plate counts were determined on plate count agar pour plates and *Micrococcaceae* on mannitol salt agar, and both were enumerated after an incubation period of 72 hours at 31ºC. Lactic acid bacteria were determined on double-layer Man-Rogosa-Sharp agar adjusted at pH 5.6 also incubated at 31ºC for 72 hours. *Enterobacteriaceae* determination was based in ISO7402 standard [6]; they were enumerated on double-layer violet red bile glucose agar (incubated at 37ºC, 24 hours). The confirmation of colonies was carried out with the cytochromo oxidase test (Biofix oxidase strips) and the innoculation of triple sugar iron agar (37ºC, 24 hours). *Staphylococcus aureus* was determined with the Most Probable Number (MPN) technique according to the UNE standard [7] using Giolitti-Cantoni broth as an enrichment medium. The positive broths were streaked on Baird-Parker agar and incubated at 37ºC, 24 hours. The presence of enterotoxigenic *Staphylococcus aureus* was confirmed with the inoculation of DNAse agar plates (37ºC, 24 hours) and flooded with 1N HCl. Approximately 90-100% of the enterotoxigenic strains produce desoxirribonuclease [8]. After determining the positive broths, a standardized MPN table was consulted to determine the most probable number of the microorganism.

Clostridia hydrogen sulphide producers (*Cl. perfringens, Cl. botulinum, Cl. sporogenes,* etc.) were enumerated on sulfite polymixin sulfadiazine agar plates and were incubated at 37ºC for 48 hours in anaerobic jars. The number of vegetative forms was determined by direct inoculation of the corresponding decimal dilutions; the number of spores was determined by inoculation of aliquots of peptone water dilutions previously heated at 80ºC for 5 min to kill vegetative bacteria. The determination of presence or absence of *Salmonella* was carried out following the ISO 6579 standard method [6]. 25 g of meat were aseptically added to 225 ml of buffered peptone water and incubated at 37ºC for 18-20 hours. Afterwards, pre-enrichment cultures were made in Rappaport-Vassiliadis enrichment broth (42ºC, 24 hours) and selenite-cystine broth (37ºC, 24 hours). Finally, plates of brilliant green agar and Hektoen agar were inoculated and incubated at 37ºC, 24 hours. Presumptive *Salmonella* colonies were transferred to triple sugar iron and lysine iron agar slants for confirmation.

Nutrient media were obtained from Oxoid and Cultimed. All the determinations were made in duplicate.

2.3   Physicochemical analyses

Some physical and chemical parameters that can influence the growth of the microorganisms were also studied in all sample types. After microbial sampling, the pH was determined by introducing a pH electrode into the meat sample. The measurements were carried out in triplicate with a pHmeter GLP21 (Crison Instruments S.A., Barcelona, Spain). Then, the meat samples were finely minced in a blender (Polytron PT 10-35). AOAC methods [9] were used for moisture and nitrite (colorimetric method) determinations. NaCl (Carpentier-Volhard method) and nitrates were determined according to the Official Standards for Spain [10]. The sodium chloride content was expressed as g/100g meat and also as a percentage of NaCl in the aqueous phase of the product using the following equation:

NaCl (% aqueous phase) = 100 x NaCl (g) / [NaCl (g) + moisture content (g)].

The water activity (a_w) was measured with an Aqualab CX-2 Water Activity System apparatus (Decago Devices, Pullman, WA.).

All the determinations were made in duplicate.

3. Results

Several intrinsic (water activity, pH, presence of sodium chloride, preservatives, etc.) and extrinsic (temperature, nutrient and oxygen availability, atmosphere composition, etc.) factors—and also the

competitiveness and interactions among microorganisms growing in the same substratum—can influence microbial growth in foods. In fact, there are ecological factors that define the stability and safety of foods. In dry-cured meat products such as dry-cured ham, the main ecological factor is its low water activity (0.81-0.87, [11]), due to the dehydration during processing and to the high sodium chloride content (3.8-8.6 g/100g meat, [12]). The addition of nitrates to the salt in the rubber step of the processing (approximately 100 ppm in meat) constitutes a minor hurdle [13]. Nitrates are transformed into nitrites, the active antimicrobial component. Dry cured pork foreleg also shows the same stability factors: low water activity (0.77-0.90), high sodium chloride content (7.31 g/100g meat) and nitrates and nitrites (79 and 8 ppm in meat, respectively) [14, 15]. These parameters, corresponding to the foreleg pieces used in our experiment, are shown in Table 1. Water activity values and NaCl content showed similar values, while nitrate and nitrite values were lower than those described above.

Table. 1 Effect of culinary treatment (desalting and unpacked or *sous vide* cooking) on some chemical and physicochemical parameters of dry cured pork foreleg pieces.

	Dry cured	Desalted	Desalted and unpacked cooking	Desalted and sous vide cooking
Moisture (g/100g meat)	55.77 ± 1.86	63.92 ± 1.19	56.11 ± 4.59	54.87 ± 4.50
NaCl (g/100g meat)	7.83 ± 1.73	4.47 ± 0.13	3.20 ± 0.97	4.19 ± 0.60
NaCl (% in the aqueous phase)	12.33 ± 2.73	6.54 ± 0.69	5.43 ± 1.82	7.14 ± 1.26
Nitrates (ppm)	44.16 ± 18.00	23.75 ± 1.65	20.80 ± 1.08	27.88 ± 4.36
Nitrites (ppm)	5.06 ± 1.40	3.30 ± 1.47	2.47 ± 0.35	2.47 ± 1.36
pH	6.07 ± 0.14	6.01 ± 0.16	6.11 ± 0.24	6.07 ± 0.19
Water activity	0.907 ± 0.009	0.963 ± 0.002	0.957 ± 0.012	0.945 ± 0.011

In these conditions, dry cured meat products do not constitute a risk for health and can be stored at room temperature for months. The only microorganisms that can grow are *Micrococcaceae*, moulds and yeasts; other bacteria such as *Enterobacteriaceae* disappear in the first stages of processing, and certain pathogenic bacteria (*Listeria, Yersinia, Salmonella* or *Clostridium*) are not usually detected [11, 16]. Lactic acid bacteria can be present at low levels in dry cured pork forelegs, while *Staphyloccus aureus* has not been detected [17]. In our study, most of the mesophilic microorganisms are *Micrococcaceae*. Low counts of lactic acid bacteria ($< 10^3$ cfu/g) were found; *Enterobacteriaceae* were detected only in some samples (Fig 2); the presence of these bacteria in *Lacón gallego* might be due to the shorter length of the elaboration process when compared with that of dry cured hams and dry cured forelegs studied in other experiments [16, 17], which permits these microorganisms to be found in the final product. Pathogens were not detected in our samples.

Nonetheless, this "safe" situation changes when dry-cured *Lacón gallego* is submitted to culinary treatment. The first step is to desalt it in water for several hours or days, depending on the size of the piece and the amount of salt that one wishes to eliminate from the meat. Table 1 shows the changes that occurred after this period in some biochemical parameters which are important for microbial growth and for food preservation. The immersion in water affords a decrease in the sodium chloride content to 4.47 g/100g meat and, consequently, an increase of a_w values to 0.96; nitrate and nitrite contents also decrease to 23.8 and 3.3 ppm respectively. As pH values are not a microbial growth limiting factor (the average was around 6.0), the new situation might also provide for the multiplication of many bacteria, including some pathogenic microorganisms (*Clostridium botulinum, Staphyloccus aureus, Salmonella*, etc) [18]. However, the low temperature used (2ºC) during desalting prevents high increases in microbial counts (Fig. 1); only the *Enterobacteriaceae* population increased in comparison to that of raw pieces. During the desalting step, higher desalting temperatures (such as 10 and 20ºC) cause important increases in the microbial counts, as was observed by our group in a previous study [19]. In any case, pathogenic bacteria were not found in desalted samples.
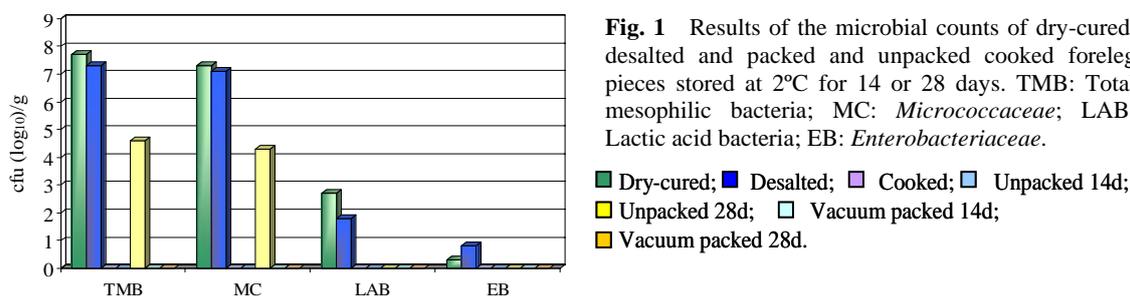
Fig. 1 Results of the microbial counts of dry-cured, desalted and packed and unpacked cooked foreleg pieces stored at 2ºC for 14 or 28 days. TMB: Total mesophilic bacteria; MC: *Micrococcaceae*; LAB: Lactic acid bacteria; EB: *Enterobacteriaceae*.

■ Dry-cured; ■ Desalted; ■ Cooked; ■ Unpacked 14d;
■ Unpacked 28d; □ Vacuum packed 14d;
■ Vacuum packed 28d.

When pieces were cooked, microbial growth was not detected (Fig. 1). The heat treatment, in which the pieces remained at a temperature higher than 90ºC in the thermal centre for about 30 min destroyed most of the microorganisms studied (or at least made them undetectable with the techniques used) in both packed and unpacked pieces. However, this is usually considered to be a mild thermal treatment [13] and complete bacterial inactivation cannot be expected. Thus, some spore-forming and non spore-forming microorganisms can survive mild stress conditions which produce changes in them called sublethal injuries; this damage prevents their growth in appropriate media immediately, but there also exist certain mechanisms with which they can repair the damage, recover their capacities, and become even more resistant to homologous or heterologous stresses [20].

The adaptation to mild stresses takes some time and it is influenced by environmental conditions. The water activity and pH of the pieces were not factors that limited the recovery of the bacteria determined in this study; nevertheless, the concentration of NaCl in the aqueous phase (Table 1) and the small amounts of nitrates and nitrites could slow this process down. In the packed samples, in which there was another preservation factor (anaerobic conditions), the microbial development was null or very small. The storage temperature of the product was another important preservation factor. The microorganisms analysed were not detected when the samples were stored at 2ºC after 28 days (Fig. 1) and at 10ºC after 14 days (Fig. 2a). However, small counts were obtained at 10ºC and 28 days of storage and in all pieces stored at 20ºC (Fig. 2b). These temperatures, particularly 20ºC, probably permitted the microorganisms to repair their sublethal damage and they were able to grow after a lag period; in our study they were detected in plate count agar for both temperatures and also in mannitol salt agar at 20ºC. In order to evaluate if the results at 2ºC and 10ºC storage temperatures could change after the sublethal stressed bacteria repaired their damage, another trial was carried out, using the liquid medium resuscitation method described by Mossel et al. [21]. Dry-cured foreleg pieces were desalted, vacuum packed, subjected to culinary treatment and stored at 2ºC and 10ºC for 14 and 28 days. Meat samples of 10 g were collected aseptically in a Stomacher bag containing 90 ml of triptone soya broth, were blended and incubated 2 hours at 25ºC. The length of time was controlled to help prevent their multiplication before plate counts. Afterwards the microbiological determinations described above were carried out. The results were similar to those found before; the sublethal damage probably needs a longer period of time to be repaired.

In unpacked pieces, the counts were variable depending on the storage temperature. Thus, counts below 10^5 cfu/g were found after 28 days at 2ºC; however, at 10ºC and 20ºC total mesophilic bacteria and *Micrococcaceae* counts reached values above 10^9 cfu/g (Figs. 1 and 2); these pieces were completely spoiled. According to the standards for the microbiological quality of ready-to-eat foods produced by the UK Public Health Laboratory service, cooked meats are considered unsatisfactory when their total counts surpass 10^6 cfu/g [22]. Lactic acid bacteria and *Enterobacteriaceae* also accounted values ranging from 5 x 10^4 to 1.5 x 10^5 cfu/g. Due to the pronounced spoilage observed, the pieces stored 28 days at 10ºC and 20ºC were discarded and not analysed. Although the unpacked cooked pieces were carefully handled and introduced into clean plastic boxes just after boiling, the environmental microorganisms might have contaminated them. At low storage temperature many of them could not grow, but when the temperature was higher, the conditions were good enough to allow for their multiplication. The aerobic conditions also favoured the rapid development of spoilage microorganisms.
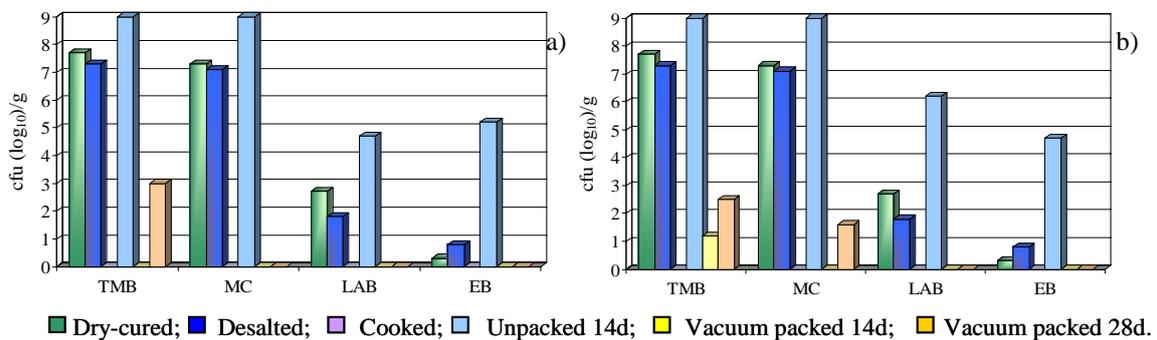
■ Dry-cured; ■ Desalted; ◻ Cooked; ◻ Unpacked 14d; ◻ Vacuum packed 14d; ◻ Vacuum packed 28d.

Fig. 2 Results of the microbial counts of dry-cured, desalted, and packed and unpacked cooked foreleg pieces stored at 10ºC (a) and stored at 20ºC (b) for 14 or 28 days. TMB: Total mesophilic bacteria; MC: *Micrococcaceae*; LAB: Lactic acid bacteria; EB: *Enterobacteriaceae*.

The pathogens studied (*Staphyloccus aureus, Salmonella,* hydrogen sulphide reducers Clostridia) were not detected in the samples no matter what storage temperature or packaging was used. The characteristics of the raw material, a shelf-stable dry-cured meat product, probably affected the viability of these bacteria and might even allow us to obtain a cooked product without any health risks.

Several controlling factors have been proposed to ensure the microbial safety of *sous-vide* cooked foods with a shelf life greater than 10 days at chilled temperatures (< 8ºC); they are based on the potential for *Clostridium botulinum* to grow in the anaerobic conditions inside the packages and at low temperatures [23]; it may even be able to produce a powerful neurotoxin and to survive the mild heat treatments applied to these products [5]. The safety of these products should be controlled by one or more of the following factors [24]: a) minimum heat treatment of 90ºC for 10 min or a different treatment of equivalent lethality; b) pH of 5 or less throughout the product; c) salt level in the aqueous phase of 3.5% throughout the food; d) water activity of 0.97 throughout the product; e) a combination of heat and preservation factors than can consistently prevent the growth and the toxin production of *Cl. botulinum*. In the *sous-vide* cooked foreleg pieces analysed in our study, factors a), c) and d) have been achieved, and a low level of preservatives (nitrites) is also present. Hence, this product is likely to be microbiologically safe. However, this study is the first stage in the development of a ready-to-eat cooked pork foreleg product. Before its commercialization, it will be necessary to perform challenge tests with pathogenic and spoilage microorganisms to determine the safety and the shelf life of this product, as has been suggested in the guidelines for the development of *sous-vide* products [20].

4. Conclusions

The *sous-vide* cooking of dry-cured pork foreleg pieces significantly reduces the microbial growth at storage temperatures of 2ºC, 10ºC and 20ºC, and it is able to extend the product shelf life to 28 days even at 20ºC. On the contrary, unpacked pieces showed considerable spoilage and became unsatisfactory after only 14 days of storage at either 10 or 20ºC.

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