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# Microbiological changes during the preparation steps of *Khliia Ezir*: a traditional cured meat product of Algeria

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## Abstract

*Khliia Ezir* is a traditional and popular meat product, which is produced from whole beef, camel, goat or lamb meat. It is an Algerian and a ready to eat meat product that is marinated, cooked and ripened. The product is for numerous months preserved in an earthenware jar at room temperature. Microbiological investigation on *Khliia Ezir* during preparation is a prerequisite and to our knowledge, this is the first study, which provide its preliminary microbiological characterization. Thus, the aim of the present work is to study the evolution of the microflora and microbiological safety of *Khliia Ezir* during the main traditional preparation steps. The microbiological counts indicated that Lactic Acid Bacteria (LAB) are the most abundant in the product, namely during the ripening and storage period. However, total Coliforms were very low in fresh beef, being eliminated after 3 days of curing. Yeasts and Molds were the highest in fresh beef, and then disappeared after cooking and during the ripening and storage step. None of the pathogenic flora during the whole preparation steps were detected. We think that the cooking temperature applied during thermal treatment (80°C) contributed to the high hygienic quality of *Khliia Ezir*. On another hand, a significant increase in pH was observed during the storage period to achieve a final pH of  $6.19 \pm 0.01$  at 30 days of storage.

## Introduction

*Khliia Ezir* is a typical ready-to-eat meat product prepared and consumed in the North East of Algeria. The preparation of *Khliia Ezir* is still basically a family art including three well-defined steps: marinade, cooking and ripening and ageing in an earthenware jar (*Ezir*). The origin of the word is thought to derive from old Arabic, “*Khliia*” referring to the storage step in olive oil and fat and “*Ezir*” to the earthenware jar; the utensil where it is preserved. The particularity of its traditional diagram process is the ripening step in *Ezir* for more than 1 year and at room temperature [1].

The marinade step is crucial because salt and spices, namely caraway, coriander and garlic, act in concert as bacteriostatic agents leading to the reduction of water content. This would also play an important role in i) the development of the sensory and textural properties and ii) the enhancement of the microbiological safety of the final product [2,3]. The microbial ecosystem of cured meat products, whether cooked or not, and simultaneously their quality and shelf life, are influenced by the environment, raw material characteristics, processing practices and storage conditions including packaging and temperature [4].

Investigation of specific spoilage organisms of several cured meat products have been reported in several studies [4-8]. However, to date no study is available on the microbiological characterization of *Khliia Ezir*. Thus, there is a need to characterize this traditional and popular meat product of Algeria during the main preparation steps by studying the dynamic microbiological changes that occur. Therefore, the objective of this study was to characterize and follow the evolution of natural microflora of *Khliia Ezir* at different steps of its preparation and assess its microbiological safety from raw ingredients to final product.

## Materials and methods

### *Khliia Ezir* preparation and sampling

*Khliia Ezir* was prepared using the traditional diagram described in Figure 1. Briefly, 9 preparations were conducted using beef *Semimembranosus* muscle of an average of 2 kg for each preparation. The fresh beef obtained from a local butcher was aged according to the standard conditions in Algeria. Fresh boneless lean meat cuts after salting and curing (5–8 cm of length, 4–6 cm thick) were marinated in a mixture of spices that contain caraway, coriander and garlic during 7 days before cooking at 80°C on water. Finally, the cooked meat was preserved in an earthenware jar (*Ezir*) and covered with a mixture of melted beef fat and olive oil. From each batch of *Khliia Ezir* preparation, samples of *fresh meat* (at 0 days), *marinade* samples (1, 3, 7 days), *cooked* samples, *immersed and stored* samples (at 10, 20, 30 days) were sampled for microbiological analysis (in triplicate).

### Microbiological analysis

At selected times during processing, 10g of each sample were aseptically homogenized with 90 mL buffered peptone water (AES Laboratories, Combourg, France) for 2 min using a Polytron homogenizer (Polytron® PT- MR 2100, Kinematica AG, Switzerland).

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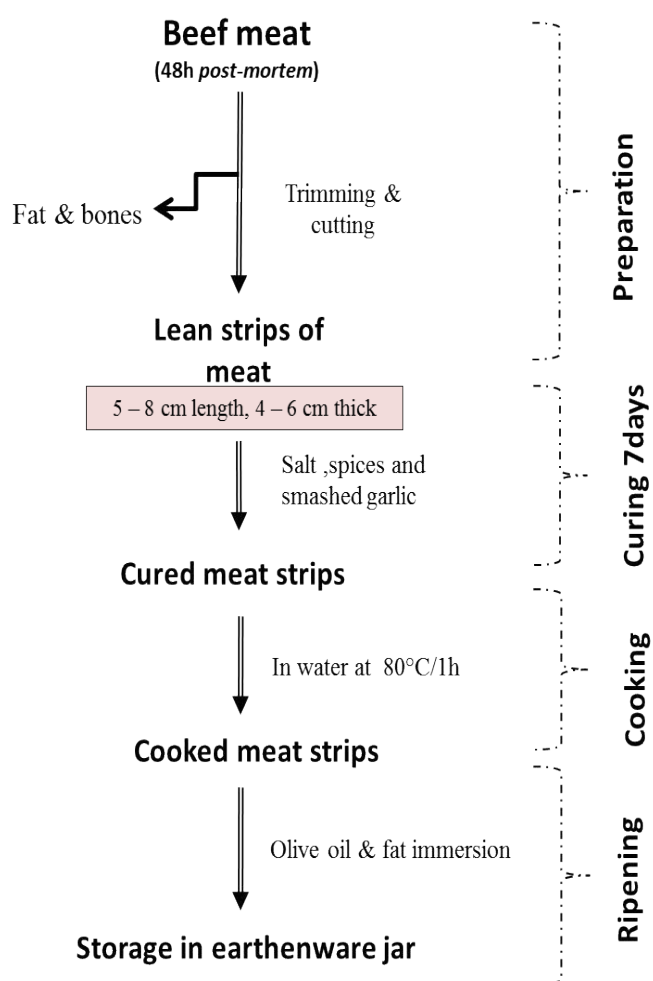


Figure 1. The traditional diagram of *Khliaa Ezir* preparation [1]

Appropriate decimal dilutions ( $10^{-1}$  to  $10^{-6}$ ) were prepared. From each sample and on each culture medium, 1 mL of each dilution was inoculated on different growth media. The microbial groups counted and the conditions of culture are summarized in Table 1. All the microbiological analyses were carried out in duplicate and the results were expressed as  $\log_{10}$  cfu/g.

### pH measurements

The pH was measured during the different preparation steps [fresh meat (at 0 days), marinade (1, 3, 7 days), cooking, immersion and storage (at 10, 20, 30 days)] in triplicate. The measurement was done using a pH meter (PHS-3CW microprocessor pH /mV meter, BANTE instrument) after mixing approximately 1 g of sample with 10 mL distilled water for 15s using a Polytron homogenizer [9].

### Statistical analysis

Data were analyzed using XLStat software (Version 2009.1.01, Addinsoft®). Analysis of variance and Tukey test were performed to investigate significant differences in microbial count at  $P < 0.05$  between the different preparation steps of *Khliaa Ezir*. The results of the statistical analysis are shown as mean values and standard deviation.

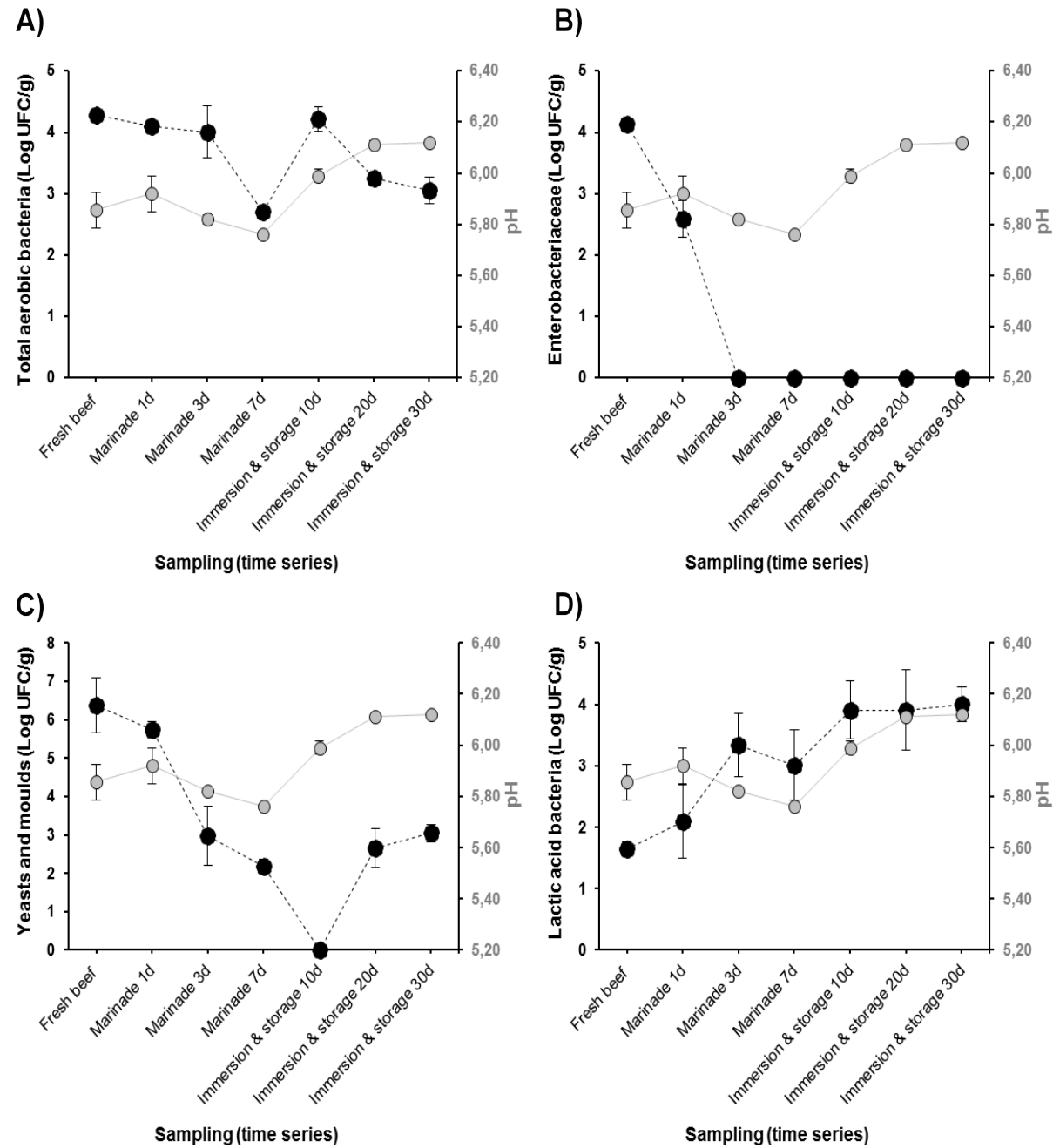
### Results and discussion

The evolution of both the microbial population changes and pH of *Khliaa Ezir* at the different preparation steps are shown together on the

same graph (Figure 2). From the results, total aerobic bacteria counts of fresh beef were relatively low ( $< 5 \log \text{cfu g}^{-1}$ ), indicating a good hygienic quality of raw materials. Several authors reported a positive correlation between slaughter conditions and initial contamination of meat [10,11]. However, microbiological spoilage and pathogen growth associated with fresh beef meat are directly related to several other factors including the transport conditions, handling practices and processing [12]. During marinade (from day 1 to 7), the development of total aerobic bacteria decreased gradually (Figure 2A). The decrease would be due to the synergic action of salt whose concentration exceeds 9% and spices. It is well known that salt has a bacteriostatic effect on bacteria [13]. For example, a sodium chloride concentration of 5% (w/v) inhibits the growth of many Gram-negative spoilage organisms including *Pseudomonas*. At the final step of *Khliaa Ezir* preparation, the total aerobic bacteria decrease progressively to reach a lower level after 30 day of ripening ( $3 \log \text{cfu g}^{-1}$ ). This would be assigned to the anaerobic conditions inside earthenware jar caused by melted fat and olive oil (sous-vide conditions). This phenomenon was reported by Cetin, *et al.* [14] during the preservation of *Kavurma*, a Turkish meat product. Also, the lack of  $\text{O}_2$  in the earthenware jar may delay the oxidative deteriorative reactions, and reduce aerobic bacteria growth as earlier reported [6].

The *Enterobacteria* population was found in low level in fresh beef ( $4 \log \text{cfu g}^{-1}$ ) before their disappearance after 3 days of curing (Figure 2B). In this case and as discussed above, the dual action of spicing and salting may be played a great role [15,16]. Among the spices used, garlic (*Allium sativum*) is highly appreciated for the taste it confers to *Khliaa Ezir*, which is usually added fresh and finely smashed [1,3]. Beside its contribution to the sensory properties of the final product, garlic also has a bactericidal effect, via *Allicin*, against contaminating flora (*Enterobacteriaceae*, *E.coli*, *Staphylococcus aureus*, Yeasts and Molds) [17]. The faecal *Enterobacteria* were totally absent during the different steps of *Khliaa Ezir* preparation. As reported by Castañ, *et al.* [18], faecal coliforms population is the main indicator of fecal contamination as they determine the hygienic quality of food processing. Furthermore, it has been well established that their growth leads to proteolytic activity that affect texture; also generating amines, ammonia, sulfides, alcohols, aldehydes, ketones, and organic acids that affect flavor [19,20]. The significant reduction or even disappearance of these groups of bacteria seems to clearly occur after the cooking step. In accordance, several studies characterizing traditional cooked meat products, reported the pivotal effect of heat treatment on growth of spoilage bacteria and pathogenic organisms [4,21]. However, the number and the type of the destroyed microorganisms depend on the internal time/temperature couple. Since thermal treatment of meat can have an impact on the growth of the meat bacteria, controlling temperature and time of cooking is one of the fundamental steps to provide the hygienic quality and extend the shelf life of *Khliaa Ezir*.

The counts of yeasts and molds showed a strong predominance of yeasts as compared to molds (Figure 2C). The ratio mold/yeast was found to be less than 10/100 in all the experiments. Moreover, the count of this flora is significantly higher than the other groups of microorganisms ( $P < 0.05$ ) in fresh beef. According yeasts is known to be the main causative agent of spoilage [22]. In *Khliaa Ezir*, their count was found to decrease during the marinade period before their disappearance after 10 days of storage (Figure 2C). The lower yeasts and molds counts is favored by the low water activity values (data not shown) and predominance of LAB (Figure 2D) that exerts an antagonistic action on contaminating flora in concert with pH [23,24]. Re-appearance of yeasts and molds was observed after 30 days of storage to reach  $3 \log \text{cfu g}^{-1}$ . We think that these bacteria groups might



**Figure 2.** Bacterial groups' evolution (Log CFU/g) and relation with pH during the main *Khliia Ezir* preparation steps for **A)** total aerobic bacteria; **B)** *Enterobacteriaceae*; **C)** Yeasts and Molds and **D)** Lactic acid bacteria

**Table 1.** Microbial groups and incubation conditions used in this study

Microbial groups	Selective media	Incubation conditions		Sampling time
		Temperature (°C)	Time (h)	
Total aerobic bacteria	PC agar	30	48	<ul style="list-style-type: none"><li>• Fresh meat</li><li>• Marinade (1, 3, 7 days)</li><li>• Cooking</li><li>• Storage and ripening (10, 20, 30days)</li></ul>
<i>Enterobacteriaceae</i>	VRBG agar	30	24	
Fecal <i>Enterococci</i>	VRBL agar	42	24	
Yeast and mold	Malt Extract Agar	25	48-72	
Lactic acid bacteria	MRS agar	30	72	
Sulphite reducer <i>Clostridium</i>	SPS Agar	37	48	
<i>Salmonella</i>	S-S agar	37	24	

**PCA:** Plate Count Agar; **VRBG:** Violet Red Bile Glucose; **VRBL:** Violet Red Bile Lactose; **MRS:** Man, Rogosa and Sharpe agar; **SPS:** Sulfite Polymyxin Sulfadiazine; **SS:** Salmonella-Shigella

play an important role in the definition of the organoleptic profile of the final product as reported for several traditional meat products [24-27].

The growth rate of Lactic Acid Bacteria (LAB) in *Khliia Ezir* during processing is consistent with the pH profile (Figure 2D). It has been well documented that high acidification rates are usually accompanied by fast and high LAB growth rates [28]. However, LAB in *Khliia Ezir* showed a load of  $1.64 \log \text{cfu g}^{-1}$  in fresh beef which increase during ripening to reach a maximum value of  $4 \log \text{cfu g}^{-1}$  after 30 days (Figure 2D). Despite the high rate of LAB, some ripened meat products do not undergo a fermentation period. As reported by Bover-Cid, *et al.* [29], pH may increase during the ripening time due to the liberation of peptides, amino acids and ammonia from proteolytic reactions, which limit the fermentation. The shelf life of these products is often not limited by bacteria but by physico-chemical condition, likely temperature and water activity [30].

Sulphite reducing *Clostridia* and *Salmonella* were not detected in none of the samples during the whole preparation steps of *Khliia Ezir*. We can suggest that the absence of these pathogenic bacteria in *Khliia Ezir* would be a consequence of i) the dual action of salt and spices; ii) the cooking temperature and iii) to the conditions of the ecosystem inside the earthenware jar (*Ezir*). A study performed by Pérez-Rodríguez, *et al.* [4] on traditional cooked cured meat from countries of West and South-east of Europe, revealed that the amount of nitrite and salt used are enough sufficient to inhibit the outgrowth of endospores, including those of *Clostridium* and *Botulinum*. Furthermore, Mayrhofer, *et al.* [31] observed that *Salmonella* were not detected in cooked beef at an end-point cooking temperature of  $71^{\circ}\text{C}$ .

The results of the variance analysis showed that the microbiological levels of the evaluated bacterial groups were statistically similar across the 9 preparations during the whole preparation steps. Thus, a consistency was observed which could validate the findings as the first preliminary microbiological results of *Khliia Ezir*. Among the chemical parameters reported in this work, pH values showed no significant differences ( $P > 0.05$ ) for all samples as a function of different preparation steps.

## Conclusion

This preliminary work showed that dynamic changes of the microbial profile of *Khliia Ezir* is related to specific its particular preparation steps which involve first, marinade/curing/salting and second, cooking/ripening steps. The results of the present study showed that *Khliia Ezir* could be considered as “shelf stable meat product”. The microbiological stability of the final product after 30 day of storage depends on the combination of several factors, mainly on the action of salt and spices, water activity reduction, temperature and time of cooking and the conditions of storage. Further studies are need for overall characterization of *Khliia Ezir* and its ecosystem using accurate techniques for an overall inventory of the microbiota.

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## References

- Boudechicha HR, Gagaoua M, Hafid K, Becila S, Boudjellal A, *et al.* (2015) *Khliia Ezir*, a traditional cured meat product of Algeria: Preparation and characterization In: Proceedings of the 61th International Congress of Meat Science and Meat Technology, pp. 1-4, Clermont-Ferrand, France.

- Benkerroum N (2013) Traditional fermented foods of North African countries: technology and food safety challenges with regard to microbiological risks. *Compr Rev Food Sci Food Saf* 12: 54-89.
- Boudechicha HR, Sellama M, Hafid K, Boudjellal A, Gagaoua M (2016) Adoption of proteomics in traditional meat products: the case of *Khliia Ezir*. In: Food futures: ethics, science and culture, pp: 600-606.
- Pérez-Rodríguez F, Castro R, Posada-Izquierdo GD, Valero A, Carrasco E, *et al.* (2010) Evaluation of hygiene practices and microbiological quality of cooked meat products during slicing and handling at retail. *Meat Sci* 86: 479-485. [[Crossref](#)]
- Bennani L, Zenati Y, Faid M, Ettayebi M (1995) Physico-chemical and microbiological characteristics of a dried salted meat product (Kaddid) in Morocco. *Z Lebensm Unters Forsch* 201: 528-532. [[Crossref](#)]
- Gök V, Obuz E, Akkaya L (2008) Effects of packaging method and storage time on the chemical, microbiological, and sensory properties of Turkish pastirma—A dry cured beef product. *Meat Sci* 80: 335-344. [[Crossref](#)]
- Soldatou N, Nerantzaki A, Kontominas MG, Savvaidis IN (2009) Physicochemical and microbiological changes of “Souvlaki”—A Greek delicacy lamb meat product: Evaluation of shelf-life using microbial, colour and lipid oxidation parameters. *Food Chem* 113: 36-42.
- Casquete R, Benito MJ, Martín A, Ruiz-Moyano S, Aranda E, *et al.* (2012) Microbiological quality of salchichón and chorizo, traditional Iberian dry-fermented sausages from two different industries, inoculated with autochthonous starter cultures. *Food Control* 24: 191-198.
- Lorenzo JM, García Fontán MC, Franco I, Carballo J (2008) Biochemical characteristics of dry-cured lacón (a Spanish traditional meat product) throughout the manufacture, and sensorial properties of the final product. Effect of some additives. *Food Control* 19: 1148-1158.
- Aksu MI, Kaya M (2005) Effect of storage temperatures and time on shelf-life of sliced and modified atmosphere packaged Pastirma, a dried meat product, produced from beef. *J Sci Food Agric* 85: 1305-1312.
- Ferreira V, Barbosa J, Silva J, Gibbs P, Hogg T, *et al.* (2009) Microbiological profile of Salpicão de Vinhais and Chouriça de Vinhais from raw materials to final products: Traditional dry sausages produced in the North of Portugal. *IFSET* 10: 279-283.
- Petit T, Caro Y, Petit AS, Santchurn SJ, Collignan A (2014) Physicochemical and microbiological characteristics of biltong, a traditional salted dried meat of South Africa. *Meat Sci* 96: 1313-1317.
- Papamanoli E, Kotzekidou P, Tzanetakis N, Litopoulou-Tzanetaki E (2002) Characterization of Micrococccaceae isolated from dry fermented sausage. *Food Microbiol* 19: 441-449.
- Cetin B, Sert S, Yetim H (2005) Microbiological quality of the kavurma samples marketed in Erzurum, Turkey. *Ann Microbiol* 55: 27-31.
- Dadalioglu I, Evrendilek GA (2004) Chemical compositions and antibacterial effects of essential oils of Turkish oregano (*Origanum minuti florum*), bay laurel (*Laurusnobilis*), Spanish lavender (*Lavan dulastoechas L.*), and fennel (*Foeniculum vulgare*) on common food borne. *Food Chemistry*.
- Chung JY, Choo JH, Lee MH, Hwang JK (2006) Anticariogenic activity of macelignan isolated from *Myristicafragrans* (nutmeg) against *Streptococcus mutans*. *Phytomedicine* 13: 261-266.
- González B, Díez V (2002) The effect of nitrite and starter culture on microbiological quality of “Chorizo”—a Spanish dry cured sausage. *Meat Science* 60: 295-298.
- Castañ oA, Fontán MCG, Fresno JM, Tornadijo ME, Carballo J (2002) Survival of Enterobacteriaceae during processing of Chorizo de cebolla, a Spanish fermented sausage. *Food Control* 13: 107-115.
- Fernandez-Fernandez E, Rozas-Barrero J, Romeo-Rodríguez MA, Vazque-Oderiz ML (1991) Changes in the physicochemical properties and organoleptic quality of Galician chorizos during curing and after vacuum packing. *Food Chemistry* 60: 555-558.
- Rubio B, Martínez B, González-Fernández C, Garci MD, Rovira J, *et al.* (2006) Influence of storage period and packaging method on sliced dry cured beef “Cecina de Leon”: Effects on microbiological, physicochemical and sensory quality. *Meat science* 74: 710-717.
- Little CL (1998) The microbiological quality of ready-to-eat dried and fermented meat and meat products. *Int J Environ Health Res* 8: 277-284.

22. Limsowtin GKY, Broome MC, Powell IB (2004) Lactic acid bacteria, taxonomy. In Encyclopedia of Dairy Sciences. Roginski H. Oxford, Elsevier, 1470-1478.
23. Lizaso G, Chasco J, Beriain MJ (1999) Microbiological and biochemical changes during ripening of Salchichón, a Spanish dry cured sausage. *Food Microbiology* 16: 219-228.
24. Ferreira V, Barbosa J, Silva J, Vendeiro S, Mota A, et al. (2007) Chemical and microbiological characterization of “Salpicão de Vinhais” and “Chouriço, a de Vinhais”: Traditional dry sausages produced in the north of Portugal. *Food Microbiology* 24: 618-623.
25. Geisen R, Lücke FK, Kröckel L (1992) Starter and protective cultures for meat and meat products. *Fleischwirtsch* 72: 894.
26. Encinas JP, López-Díaz TM, García-López ML, Otero A, Moreno B (2000) Yeast populations on Spanish fermented sausages. *Meat Sci* 54: 203-208. [[Crossref](#)]
27. Ferreira V, Barbosa J, Vendeiro S, Mota A, Silva F, et al. (2006) Chemical and microbiological characterization of alheira: a typical Portuguese fermented sausage with particular reference to factors relating to food safety. *Meat Sci* 73: 570-575.
28. Malti JE, Amarouch H (2008) Microbiological and physicochemical characterization of natural fermented camel meat sausage. *J Food Process Preserv* 32: 159-177.
29. Bover-Cid S, Izquierdo-Pulido M, Vidal-Carou MC (2001) Changes in biogenic amine and polyamine contents in slightly fermented sausages manufactured with and without sugar. *Meat Sci* 57: 215-221.
30. Leistner L, Roedel W (1975) The significance of water activity for microorganisms in meats. In R. B. Duckworth (Ed.), Water relations of foods (pp. 309-323). London: Academic Press.
31. Mayrhofer S, Paulsen P, Smulders FJM, Hilbert F (2004) Antimicrobial resistance profile of five major food-borne pathogens isolated from beef, pork and poultry. *Int J Food Microbiol* 97: 23-29.