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

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Abstract

Khliaa 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 *Khliaa 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 *Khliaa 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 repining 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 *Khliaa Ezir*. On another hand, a significant increase in pH was observed during the storage period to achieve a final pH of 6.19 ± 0.01 at 30 days of storage.

Introduction

Khliaa Ezir is a typical ready-to-eat meat product prepared and consumed in the North East of Algeria. The preparation of *Khliaa 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, "*Khliaa*" 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 *Khliaa 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 *Khliaa Ezir* at different steps of its preparation and assess its microbiological safety from raw ingredients to final product.

Materials and methods

Khliaa Ezir preparation and sampling

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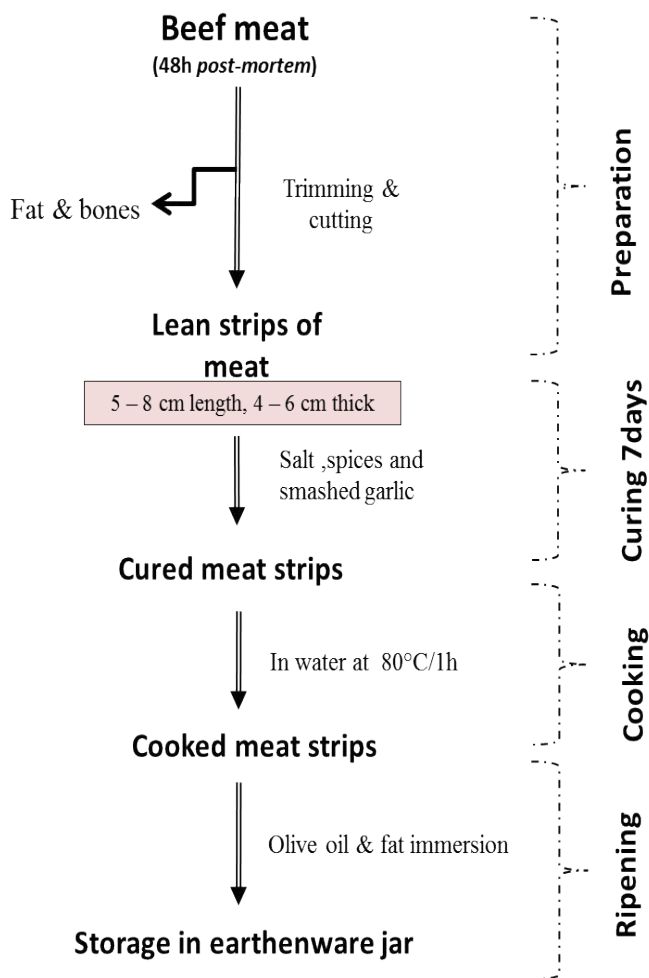
Figure 1. The traditional diagram of *Khliaa Ezir* preparation [1]

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 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 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 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 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 feacal *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 pathogen 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 3log cfu g^{-1}. We think that these bacteria groups might
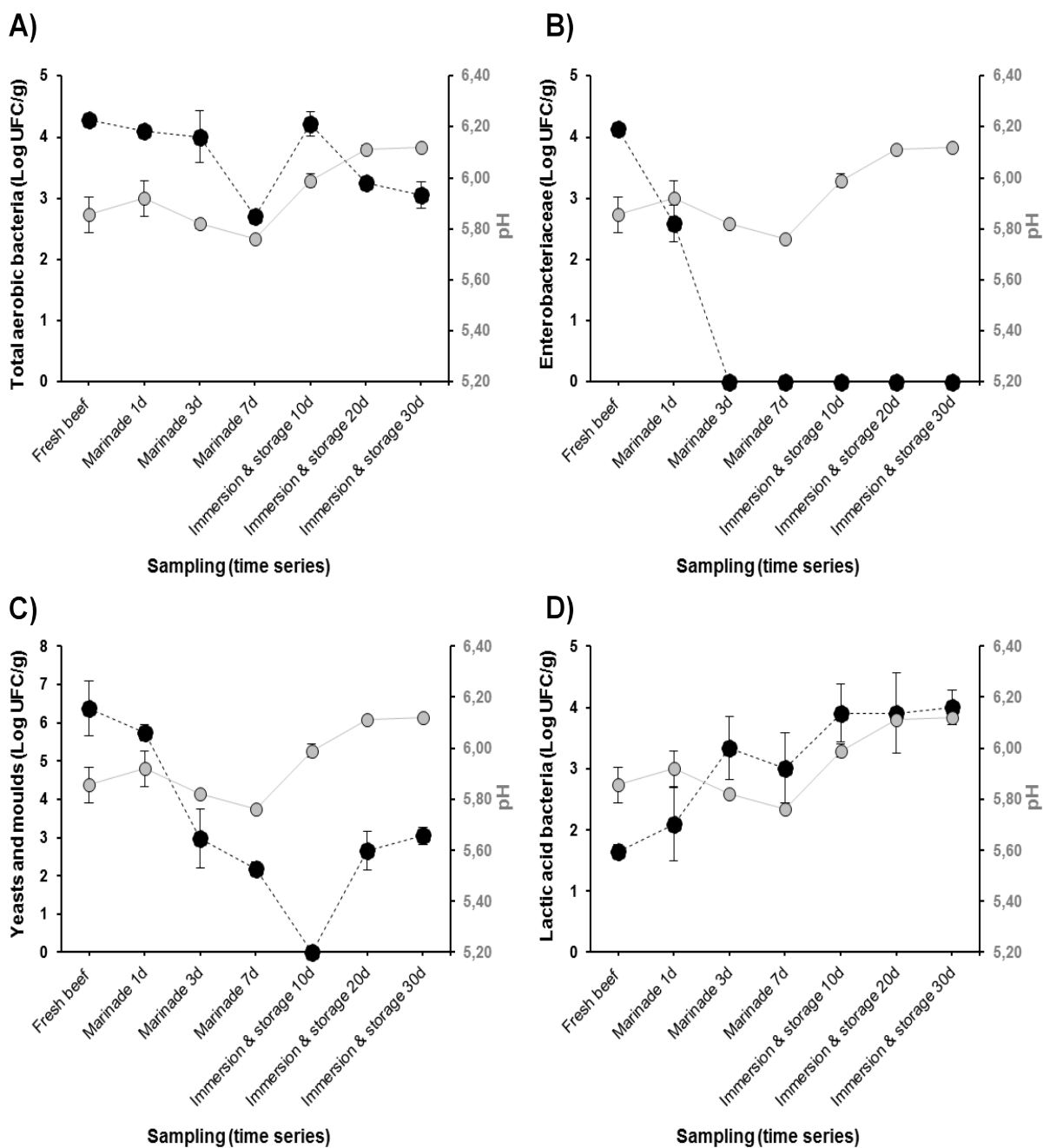
Figure 2. Bacterial groups' evolution (Log CFU/g) and relation with pH during the main *Khliaa Ezir* preparation steps for **A**) total aerobic bacteria; **B**) *Enterobacteriaceae*; **C**) Yeasts and Molds and **D**) Lactic acid bacteria

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	• Fresh meat • Marinade (1, 3, 7 days) • Cooking • Storage and ripening (10, 20, 30days)
Enterobacteriaceae	VRBG agar	30	24	
Fecal *Enterococci*	VRBL agar	42	24	
Yeast and mold	Malt Extract Agar	25	48-72	
Lactic acid bacteria	MRS agar	30	72	
Sulphite reducer Clostridium	SPS Agar	37	48	
Salmonella	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 *Khliaa 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 *Khliaa Ezir* showed a load of 1.64 log cfu g^{-1} in fresh beef which increase during ripening to reach a maximum value of 4log 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 physic-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 *Khliaa Ezir*. We can suggest that the absence of these pathogenic bacteria in *Khliaa 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°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 *Khliaa* 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 *Khliaa 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 *Khliaa 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 *Khliaa Ezir* and its ecosystem using accurate techniques for an overall inventory of the microbiota.

Acknowledgements

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