

ANIMAL SCIENCE

Microbiological characterization of camel and sheep meat preserved by refrigeration and lactic acid

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Abstract

The microbial growth of certain bacteria contaminating camel and sheep meat, kept under refrigeration was evaluated. The samples were collected at the Ouargla slaughterhouse. The selected compartment for monitoring was the thigh (most demanded by consumers of the region). The shelf life of the two types of meats studied was five days against the total mesophilic aerobic flora, yeasts, enterobacteria and fecal coliform contamination whose percentages were respectively 30.26%, 26.55% , 22.74% and 20.44% for camel meat; 28.91%, 28.21%, 22.87% and 20% for sheep meat. The lactic acid concentration that ensures better conservation, was 4% for sheep meat while a concentration of 2% was sufficient for camel meat. The duration of cold preservation of both meats (treated and untreated) was nine days except for yeasts whose duration was seven days.

Key words: Meat, Dromedary, Sheep, Lactic acid, Conservation

Introduction

The richness of meat in water, proteins of high biological value makes it an essential food for a balanced diet. However, these virtues make it a favourable breeding ground for most microbes (Clinquart et al., 1999). Most of the germs contaminating carcasses after different stages of slaughter (skinning and evisceration) are saprophytes. These bacteria, yeasts and molds, are germs that cause alteration or putrefaction of meat. In addition, the presence of pathogens in food is often responsible for borne illness (Cottin et al., 1985). Food preservation is conservation of its edibility, taste and nutritional properties. This requires the prevention of microbial growth and retarding the oxidation of fats which cause rancidity (Bourgeois et al., 1991). Conservation at low temperature retards the growth of microorganisms. The majority of germs such as coliforms have limited metabolic activities at temperatures below 5°C (Craplet, 1966). It is the preferred method of preserving meat, and the best currently known

(Laurent, 1974).

Refrigeration is the storage of food at low positive temperatures. In general, the temperature is around 0°C to +4 °C. Refrigeration should be applied initially to fresh healthy foods. During this type of storage, water maintains liquid constitution (Bourgeois et al., 1996). Most bacteria grow rapidly in fresh non-acidic meat, fish and vegetables causing deterioration. Other forms of spores make them resistant to preservation techniques and resume their multiplication upon return to ambient conditions (Multon, 1984). The use of chemical additives to acidify the meat can preserve them in the best conditions. The addition of these agents is designed to optimize the preservation of food while conserving the organoleptic and nutritional qualities (Multon, 1984). Organic acids, such as lactic acid are widely used as condiments in food preparations. Lactic acid bacteria inhibit the growth of pathogenic microorganisms especially *E. coli* (Huxley, 1969; Houtma et al., 1986; Miller, 1994).

The present work aims to characterize the microbiological properties of meats from two species (camel and sheep) kept under refrigeration after undergoing treatment with lactic acid solutions at concentrations of 2% and 4% through daily monitoring of the proliferation of aerobic mesophilic total flora, yeasts, Enterobacteriaceae and coliforms. This is initiated to evaluate preservative effect of organic acid (lactic acid).

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Materials and Methods

Biological material

The samples used in this study were taken immediately after slaughtering at the Ouargla slaughterhouse. The samples were collected using a sterile knife, (thigh) for both types of meat (sheep and camel). In total, 20 samples of meat (coming from thighs) investigated 10 samples for each studied species (camel and sheep). Because sample types are perishable, transportation of the samples was carried out in a cooling system (isothermal cooler). In the laboratory, the meat was cut aseptically into 10g pieces, using a chisel and sterile forceps. The weighing was determined using an analytical balance. Manipulations were carried out with a maximum asepsis (Bunsen burner lit bench for 15 minutes and washed with bleach).

Series of samples (for both meat origins camel and sheep) were received or in a solution of lactic acid 2%, 4% or sterile distilled water. A series of samples was left untreated (control). Each sample was placed (10 g) individually in a sterile bag and stored in a refrigerator at a temperature between 0 and 4°C.

Preparation of the initial suspension and decimal dilutions

The procedure was performed according to the French standard NF V-057-2. Ten gram of meat was introduced aseptically into a sterile "Stomacher" bag containing 90 ml of diluent (Water Buffered Peptone). After grinding and homogenization of the solution, the obtained initial suspension was subsequently diluted 1/100 (10^{-2}), and 1/1000 (10^{-3}) and one ten thousandth (10^{-4}).

Microbiological characterization of camel meat

The culture medium used for the enumeration of total aerobic mesophilic flora was the *plate count agar* (PCA), according to the ISO 4833 standard. Fecal coliforms were counted on VRBL environment, according to NF V 08-017. For the Enterobacteriaceae, the selective medium used was VRBG with incubation of Petri dishes seeded depth for 24 h at 37°C. Enumeration of yeasts was performed by counting colonies on OGA (Glucose agar with oxytetracycline) medium after seeding the surface with 0.1ml of the stock solution and serial dilutions and incubation Petrie plates at 25°C for 2 to 5 days (NF V 03-454).

Statistical analyses

Statistical analyses included two stages, (i) analysis of internal variability in each species of meat (camel and sheep) and (ii) comparative analysis to determine the variability between germs sought.

-The analysis of internal variability included- the description of the mean and standard deviation for each of the germ,

-Correlations between rates for each meat contamination (Pearson correlation).

Establishing a contingency table between germs sought in each species of meat. These analyzes were performed using the software XLStat (Addinsoft®)

Results and Discussion

Effect of the nature and treatment of meat on the evolution of the mesophilic aerobic flora total enterobacteria, coliforms and yeasts

The results for the microbiological quality of meat, camel and sheep kept under refrigeration, namely the total mesophilic aerobic flora, fecal coliforms, Enterobacteriaceae and yeasts are summarized in (Figure 1).

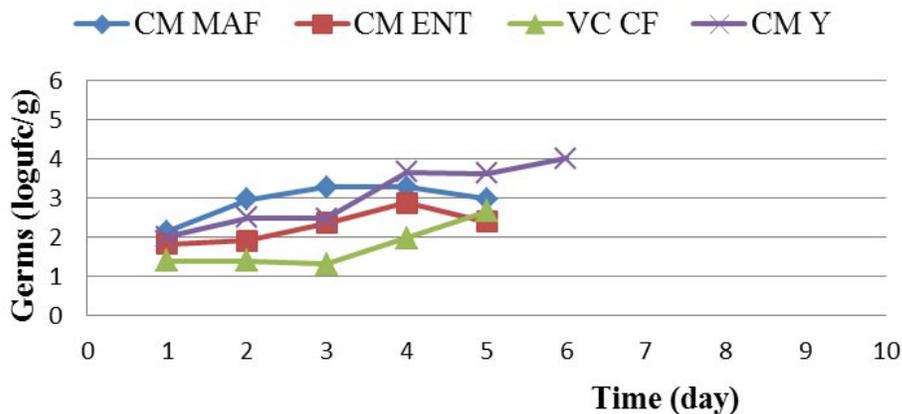


Figure 1. Changes in germs over time in the control camel meat. CM: Camel Meat, MAF: mesophilic aerobic flora total, ENT: Enterobacteriaceae, FC: fecal coliforms, Y: yeast, d: day.

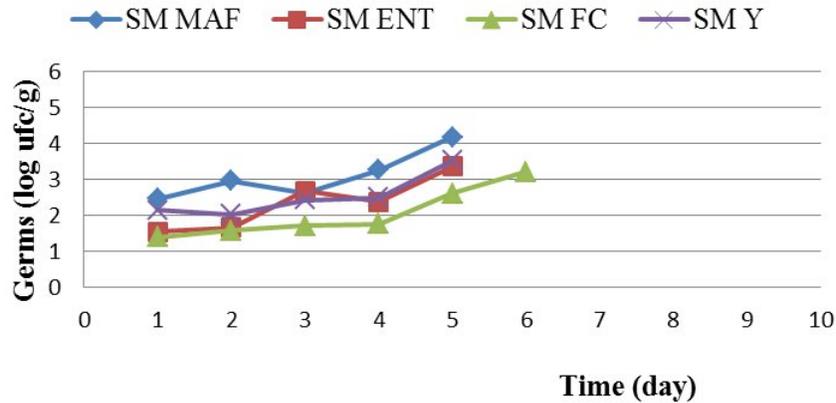


Figure 2. Changes in germs sought over time in the control sheep meat
 SM: Sheep Meat, MAF: mesophilic aerobic flora total, ENT: Enterobacteriaceae, FC: fecal coliforms, Y: yeast, d: day.

Overall, the rate of contamination maximum total mesophilic aerobic flora samples of camel meat is 3.17 ± 0.62 log₁₀ ufc / g (Figure 1), while that of sheep meat samples is about 3.27 ± 0.57 log₁₀ ufc / g (Figure 2). Values of the flora that we obtained in this study are consistent with those reported by Hamad (2009). According to this author, contamination rates were of the order of 1.79 log₁₀ ufc/cm² for camel meat and 3.08 log₁₀ ufc/cm² for the sheep meat. Camel meat has a maximum rate of contamination by enterobacteria 2.88 ± 0.71 log₁₀ ufc / g (Figure 1), relatively lower than that of the (3.35 ± 0.05 log₁₀ ufc / g). Our results were within the range of values reported by Hamad, (2009) whose results were 2.60 log₁₀ ufc/cm² and 3.38 log₁₀ ufc/cm² respectively for camel and sheep meat.

Fecal coliforms samples of sheep and camel meat reached maximum contamination levels of

3.21 ± 0.21 log₁₀ ufc / g and 3.65 ± 0.46 log₁₀ ufc / g, respectively. So, as the rates for yeast was 3.52 ± 0.62 log₁₀ ufc / g and 3.66 ± 0.49 log₁₀ ufc / g (Figure 1 and Figure 2).

The comparison of the maximum contamination by germs counted recorded on both studied meat left out that camel meat was less contaminated than the sheep. This could be explained by the difference in skinning techniques for animal skinning, despite the fact that these meats were processed in the same slaughterhouse.

The predominant flora of contamination for both meats was constituted by the total mesophilic aerobic flora reflecting their hygienic quality. The presence of fecal coliforms was indicative of poor hygiene and in particular defects that occur during evisceration because coliforms are saprophytes of the digestive tract of man (Basel et al., 1983).

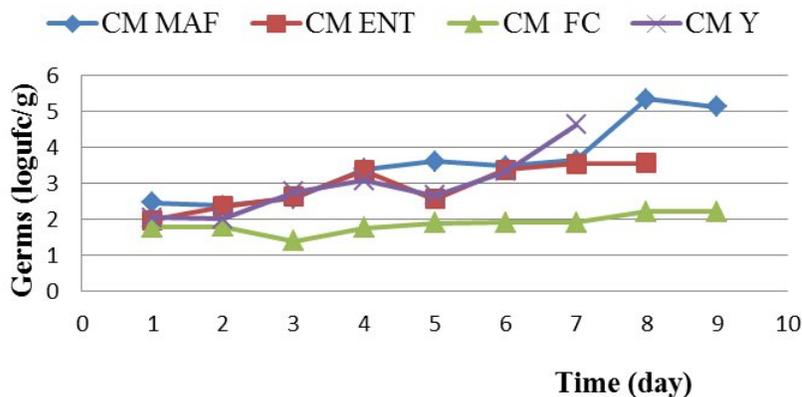


Figure 3. Changes in germs sought over time in processed camel meat by lactic acid 2%
 CM: Camel Meat, MAF: mesophilic aerobic flora total, ENT: Enterobacteriaceae, FC: fecal coliforms, Y: yeast, d: day.

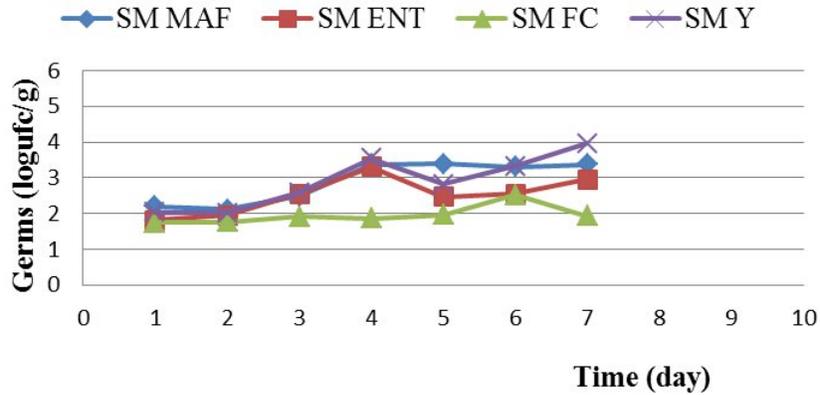


Figure 4. Changes in germs sought over time in processed sheep meat by lactic acid 2%
 SM: Sheep Meat, MAF: mesophilic aerobic flora total, ENT: Enterobacteriaceae, FC: fecal coliforms, Y: yeast, d: day.

Immersion of meat before refrigeration in a solution of lactic acid 2% or 4% appeared to slow the multiplication rate of bacteria. The maximum rate of contamination of the camel meat rinsed with a solution of 2% lactic acid were respectively 5.12 ± 0.14 logufc/g, 3.54 ± 0.21 logufc/g, 2.20 ± 0.28 logufc/g and 4.63 ± 0.38 logufc/g. The duration of its conservation was seven days for yeast, eight days for Enterobacteriaceae and nine days for the

total mesophilic aerobic flora and coliforms (Figure 3).

Sheep meat was pretreated with a solution of 2% lactic acid storage before refrigeration, presented maximum contamination rate, total mesophilic aerobic flora of 3.38 ± 0.21 logufc/g of Enterobacteriaceae logufc/g 3.31 ± 0.32 of fecal coliforms logufc/g 2.52 ± 0.66 logufc/g and 3.97 ± 0.30 logufc/g of yeast (Figure 3). The shelf life of the meat was seven days for these germs (Figure 4).

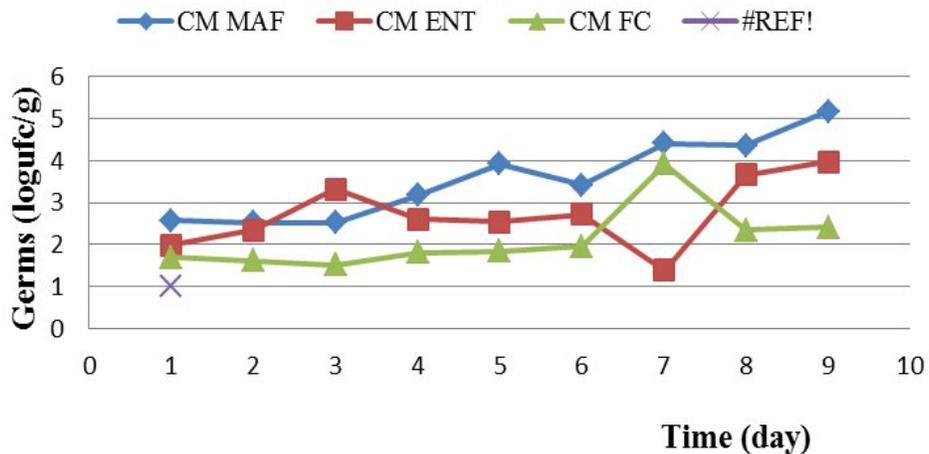


Figure 5. Changes in germs sought over time in processed camel meat by lactic acid 4%.
 CM: Camel Meat, MAF: mesophilic aerobic flora total, ENT: Enterobacteriaceae, FC: fecal coliforms: yeast, d: day.

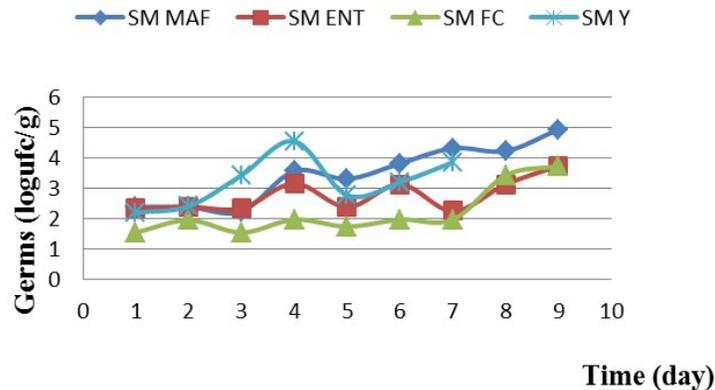


Figure 6. Changes in germs sought over time in processed sheep meat by lactic acid 4%.
 SM: Sheep Meat, MAF: mesophilic aerobic flora total, ENT: Enterobacteriaceae, FC: fecal coliforms, Y: yeast, d: day.

A lactic acid concentration of 4% did not reduce the rate of contamination of camel meat by the total mesophilic aerobic, Enterobacteriaceae, coliforms and yeasts whose respective values were 5.17 ± 0.3 logufc / g, 3.98 ± 0.50 logufc / g, 3.93 ± 0.26 logufc / g and 4.65 ± 0.70 logufc/g, nor the duration of its conservation compared to the results obtained with concentration of 2%, except the shelf-life against enterobacteria which was extended to nine days (Figure 5).

Whereas in the presence of a lactic acid concentration of 4%, the maximum rate of contamination of sheep meat were of the order of, 4.94 ± 0.10 logufc/g for mesophilic aerobic flora total, 3.74 ± 0.67 logufc/g for Enterobacteriaceae, 3.73 ± 0.84 logufc/g for fecal coliforms and 4.55 ± 0.27 logufc/g for yeasts (Figure 5). The shelf life of the sheep meat was nine days against the majority of organisms investigated (Figure 6).

The lower infection rates of the total flora, Enterobacteriaceae and coliforms in processed meat by lactic acid could be explained by the fact that organic acids inhibit pathogenic microorganisms. The latter cannot grow in foods at acid pH (below 4.5). The decrease in pH affects even the heat resistant spores (Bourgeois et al., 1996; Lyreal and Vierling, 1997).

The high rate of yeast in the treated meat could be explained by the selective effect exerted by organic acids on the microbial population. They inhibit pathogenic microorganisms but stimulate yeasts. Yeasts are extremely tolerant to changes of pH; they can grow at pH 4 to 6. Moreover, those germs can locally adapt their optimum pH. The pH is not a good indicator to control their development (Lyreal and Vierling, 1997).

Table 4. Correlation matrix between the rates of infection in camel and sheep meat.

Sheep meat	FAMT ST	FAMT 2%	FAMT 4%	ENT ST	ENT 2%	ENT 4%	CF ST	CF 2%	CF 4%	L ST	L 2%	L 4%
FAMTST	1	0,442	0,162	0,572	0,694	0,212	0,136	0,206	0,082	0,659	0,445	0,683
FAMT2%	0,442	1	0,650	0,648	0,839	0,323	0,716	0,498	0,116	0,842	0,889	0,699
FAMT4%	0,162	0,650	1	0,238	0,536	0,700	0,502	0,374	0,765	0,421	0,681	0,437
ENTST	0,572	0,648	0,238	1	0,528	0,029	0,449	0,301	0,012	0,723	0,401	0,338
ENT2%	0,694	0,839	0,536	0,528	1	0,281	0,335	0,286	0,103	0,698	0,918	0,961
ENT4%	0,212	0,323	0,700	0,029	0,281	1	0,368	0,376	0,791	0,301	0,261	0,324
CFST	0,136	0,716	0,502	0,449	0,335	0,368	1	0,887	0,103	0,510	0,521	0,159
CF2%	0,206	0,498	0,374	0,301	0,286	0,376	0,887	1	0,078	0,215	0,439	0,166
CF4%	0,082	0,116	0,765	0,012	0,103	0,791	0,103	0,078	1	0,104	0,130	0,097
LST	0,613	0,842	0,421	0,723	0,698	0,301	0,510	0,215	0,104	1	0,591	0,594
L2%	0,445	0,889	0,681	0,401	0,918	0,261	0,521	0,439	0,130	0,591	1	0,830
L4%	0,683	0,699	0,437	0,338	0,961	0,324	0,159	0,166	0,097	0,594	0,830	1

The characters in bold are significant at $P < 0.05$.

Table 5. Mean \pm standard deviation of rates of infection in camel and sheep meat.

	Camel meat	Sheep meat	Significant level P <0.05
FAMTST	3,036 \pm 0,697	2,922 \pm 0,463	N Sign
FAMT2%	3,073 \pm 0,573	2,910 \pm 0,614	N Sign
FAMT4%	3,365 \pm 0,801	3,283 \pm 0,849	N Sign
ENTST	2,927 \pm 0,897	2,318 \pm 0,749	N Sign
ENT2%	2,636 \pm 0,545	2,484 \pm 0,479	N Sign
ENT4%	2,730 \pm 0,672	2,894 \pm 0,861	N Sign
CFST	2,027 \pm 0,727	2,025 \pm 0,867	N Sign
CF2%	1,826 \pm 0,211	1,960 \pm 0,261	N Sign
CF4%	2,126 \pm 0,742	2,206 \pm 0,805	N Sign
LST	2,927 \pm 0,776	2,852 \pm 0,751	N Sign
L2%	2,927 \pm 0,897	2,899 \pm 0,748	N Sign
L4%	2,966 \pm 0,905	3,269 \pm 0,739	N Sign

Infection rates by total mesophilic aerobic flora, enterobacteria, fecal coliforms and yeasts were not significantly different in both species of meat and when these meat were treated by lactic acid (Table 4). The correlations were generally not significantly in the two meats (Table 5).

Conclusion

The present study regarding meat from two different species (camel and sheep) aimed to highlight the preservative effect of a physical technique (refrigeration). The presence of certain flora from the first day was indicator of contamination of the carcasses during slaughtering operations and contamination of meat during cutting operations. The daily counts of these organisms were highlighting important points: (i) the difference in sensitivity of germs to refrigeration as well as treatment with lactic acid. (ii) the preservation of meat at a temperature between 0 and 4°C to slow the multiplication of germs without being able to destroy them. (iii) the storage temperature of meat which presented an undeniable effect in prolonging the shelf life of meat. (iv) the combination of two methods of conservation for improving the shelf life of meat, whatever the species (camel and sheep), and finally which could help more families to keep their surplus product directly without freezing. (v) a concentration of 2% sufficient to increase the shelf life of camel meat to nine days for the majority of bacteria sought, while a concentration of 4% was needed to achieve this length for sheep meat.

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