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Improving the Quality of Fermented Camel Sausage by Controlling Undesirable Microorganisms with Selected Lactic Acid Bacteria

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ABSTRACT

Main objectives of the present work was to study the possibility of preserving ground camel meat by a biological procedure using lactic acid bacteria to encourage an extended shelf-life of fresh meat in hot areas. Lactic acid bacteria isolated from natural fermented foodstuffs were selected for their antimicrobial activity, and used in sausage making from camel meat. Fresh meat purchased directly from market was deboned and fat separated. After freezing at -20°C, the meat and the fat were minced separately with a meat mincer. Meat and fat were mixed in the ratio of 80/20 and inoculated with the strain of *L. plantarum* from our collection. The mixture was stuffed in natural casing to make small pieces (weighing 50 g each) and exposed to drying at 15 to 18°C and 70 to 80% relative humidity. Sausages were analyzed for their Physico-chemical characteristics (pH, water activity & weight loss). Microbiological analyses were carried out to determine Standard Plate Count, Staphylococci, Coliforms, Enterococci, and Lactic Acid Bacteria (LAB), yeasts, lipolytic and proteolytic microorganisms. All analyses were made after 1, 3, 7, 14 and 21 days during the fermenting and ripening period. Results showed a significant decrease of coliforms, Enterococci and yeasts, which were completely eliminated after 21 days. Standard plate counts indicated a considerable decrease during the ripening period. Proteolytic microorganisms decreased from 10^6 cfu/g to less than 1 cfu/g at the end of the process. Lipolytic microorganisms were reduced to reach a number of 3×10^4 cfu/g. The pH decreased to 4.5 and the water activity reached also 0.7. The starter culture showed high counts during the drying period.

Key Words: Camel; Sausage; *Lactobacillus;* Microbiology; Preservation

INTRODUCTION

Several fermented meat products from various animals (pork, beef, sheep & chicken) had been studied to improve and modernize their processing. However, up till now no work had been done to determine the microbiological and chemical characteristics of products from camel meat. The meat of camels, 3 years old or less, had been reported to be comparable in taste and texture to beef meat (Khatami, 1970). Elgasim and Elhag (1992) concluded that the carcass of camel had the same characteristics as those of other red meat animals. The meat of camels is less tender than meat of other species, and the high protein content provides a good nutritional value product (El-Faer *et al.,* 1991; Elgasim & Elhag, 1992; Elgasim & Al-Kanhal, 1992; Dawood, 1995; Dawood & Alkhanhal, 1995).

During the past decade consumer requirements for a decrease of chemical additives in food was observed. Several studies were carried out on natural antimicrobial compounds secreted by Lactic Acid Bacteria for inhibiting hazardous microorganisms (Berry *et al.,* 1991; Foegeding *et al.,* 1992; Hugas *et al.,* 1995, 1996). These compounds could replace chemical preservatives such as sulphudioxide, benzoic and sorbic acids, nitrate and nitrite (Lloyd & Drake, 1975).

Lactic Acid bacteria are responsible of flavor development and preservation of fermented meat products by producing antimicrobials compounds such as lactic and acetic acids, hydrogen peroxide, diacetyle, and bacteriocins (Stiles & Hastings, 1991; Ray, 1992; Papamanoli *et al.,* 2003).

In the present study, new isolated strains of *Lactobacillus plantarum* from natural fermented foodstuffs were used in combination with natural additives (spices) for camel fermented sausage making.

MATERIALS AND METHODS

Sausage preparation. Camel meat pieces (1 kg each) purchased from the retail in Rabat (Morocco) was deboned, sliced and frozen at –20°C for 24 h and minced in a meat mincer. The fat was also frozen and minced in the same manner. The sausage mixture was done by mixing camel lean with hump fat in the proportion of 80/20. Other ingredients were added (g/kg) as follows NaCl (3), black pepper (2.5), cinnamon (2.5), glucose (2), garlic (5).

Fig. 1. Microbial profiles (SPC, Coliforms, Staphylococci and Enterococci) during ripening and drying process of camel sausage

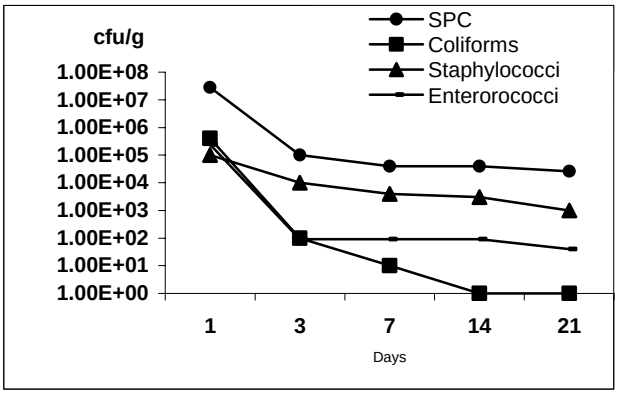


Fig. 2. Lactic acid bacteria profiles in camel sausage during camel sausage process

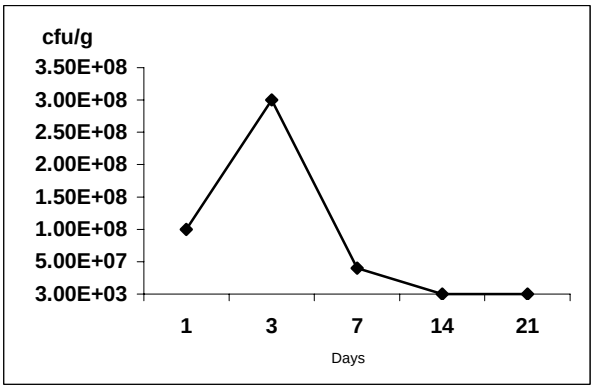


Fig. 3. Counts pattern of lipolytic and proteolitic microorganisms and yeasts during camel sausage process

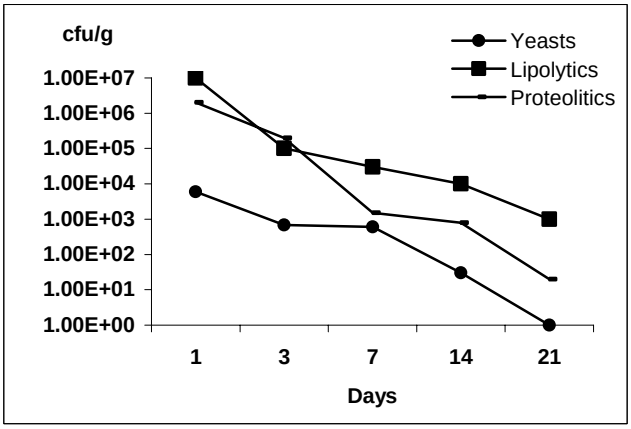


Fig. 4. Water activity and weight loss ratio decrease during camel sausage making

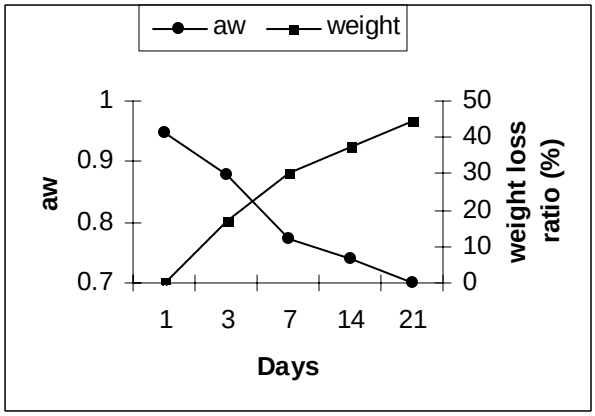
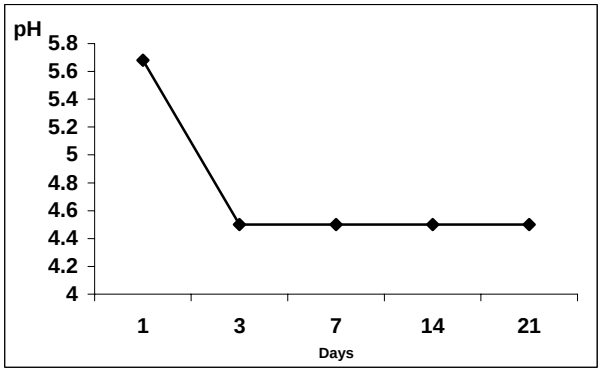


Fig. 5. pH decrease pattern during camel sausage making process



Ingredients were mixed in a bowl chopper and the appropriate starter culture was added during mixing. The mixture was then stuffed into natural casing with a diameter of 3 cm. Fermentation and ripening was carried out at 15 to 18°C and 70 to 80% relative humidity for 21 days. Sampling was done at 0, day, 3 days, 7 days, 14 days and 21 days after staffing.

Starter culture. The lactic acid bacterial strain was isolated from natural foodstuffs, and previously identified as *L. plantarum* according to the method described by Sharp (1975). The starter culture composed of *L. plantarum* was growth on MRS for 24 h at 30°C. From this culture dilutions up to 10^{-6} were plated on MRS to determine the cell concentration. Sausage was inoculated with a

concentration of 10^7 cfu/g as final concentration.

The antimicrobial activity of *L. plantarum* was studied using the spot test described by Harris *et al.* (1989) and by the agar diffusion test as described by Piddock method (1990). The antimicrobial activity was determined by measuring the clear zone around the colonies, considering a diameter of 1.5 mm or greater inhibitory activity.

Determinations of physico-chemical parameters pH. pH of the samples was measured by a pH meter apparatusmade Crison MicropH 2000.

A_w. The a_w was measured using Aqualab Cx2 instrument (Deacagon Devices, Pullman, Washington, USA). Three slices (5 g each) were used to determine.

Measurement of weight loss. The decrease in weight of three designated sausages was calculated by weighing the same sample in the first day and after 1, 3, 7, 14 and 21 days. The sample was kept at the same temperature as the sausages.

Microbiological determinations. Sausage samples (10 to 15 g) were cut into small pieces and blended in 90 mL of saline water (8.5 g/L) to make the initial dilution (10^{-1}). Serial dilutions up to 10^{-6} were then prepared.

Standard plate count (SPC) was determined by plating appropriate dilutions on plate count agar (Difco, USA). The plates were incubated at 30°C for 48 h. LAB were plated on MRS (De Man Rogosa Sharp medium; Difco, USA), incubated at 30°C for 48 h. Enterococci were determined on liquid media using 3 tubes per dilution. Dilution are inoculated into Azide dextrose broth (Difco, USA), incubated at 30°C for 24 h. Tubes that had shown growth were transferred on Ethyl Violet Azide Broth (Difco, USA) and incubated at 37°C for 24 h. Tubes that had shown growth and formation of a violet precipitation in the bottom were counted. The number of positive tubes is reported in the table for the most probable number. Staphylococci were determined on Mannitol Salt Agar (Merck, Germany); the plates were incubated at 37°C for 24 h.

Lipolytic microorganisms were determined on Victoria Blue Agar. Casein Soy Peptone Agar supplied with a Victoria Blue B buffer fat mixture according to the method described by Alford (1976). Plates were incubated at 30°C for 48 h to 72 h and blue colonies were counted. Proteolytics were determined according to the method described by Lee and Kraft (1984).

Yeasts were plated on Potato Dextrose Agar (Difco, USA), incubated at 30°C for 48 h. Total and fecal coliforms were plated on Deoxycholate Agar (Merck, Germany) at 37 and 44°C, respectively for 24 h.

RESULTS AND DISCUSSION

Sanitarian conditions were followed up during the sausage making process by the determination of Standard Plate Count (SPC), Coliforms, Enterococci and Staphylococci. The microbial profiles are plotted in (Fig. 1).

The SPC level in camel fermented sausage showed a high count during the first step of the process ($2.8\ 10^7$ cfu/g). Counts decreased drastically to reach levels around $2.6\ 10^4$ cfu/g after 21 days at 15 to 18°C.

The same decrease pattern was also observed for the enterococci and coliforms. The former was decreased from $2.4\ 10^5$ cfu/g to 40 cfu/g, and the later were reduced from $4\ 10^5$ to less than 1 cfu/g.

E. coli would be affected by pH and sodium in sausage making (Glass *et al.*, 1992). According to the same authors these microorganisms would be delayed rather than destroyed. Staphylococci counts were decreased by approximately 2 log units during the sausage fermentation. The initial staphylococci count of sausage mixture was around 10^5 cfu/g, which decreased to 10^3 cfu/g after 21 days.

Indicator microorganisms were eliminated in camel fermented sausage after 21 days of process. This may be due to subsequent actions of spicing. Spices such as garlic (Al-Delaimy & Barakat, 1971) and clove (Hao *et al.*, 1998) have antimicrobial activity in meats and meat products. It should be also emphasized that garlic is heavily used in Moroccan sausage. This may help in inhibiting the undesirable microorganisms (Mei-chin & Wen-shen, 2003). Lactic acid, hydrogen peroxide and bacteriocins by lactic acid bacteria starter (Stiles & Hasting, 1991; Ray 1992) and low water activity, as reported by Loncin *et al.* (1968), who stated that the bacterial growth is delayed or stopped when the a_w is reduced to below 0.9.

The determination of microorganisms involved in the biochemical processing of camel fermented sausage included proteolytics, lipolytics, lactic acid bacteria and yeasts. Counts of these microorganisms showed a high residual microbiota.

Lactic acid bacteria counts in the sample from the initial mixture (t_0) may predict about the level of the starter inoculation of starter culture (10^7 cfu/g) in the sample. The inoculation should be heavy and may be at least 10^6 cfu/g. LAB number remained approximately constant until the 14^{th} day of sausage fermentation (Fig. 2). This decrease can be explained by the water activity decrease in the product as well as the unfavorable conditions due to other factors.

The lipolytic and the proteolytic microorganisms may constitute a source of lipases and proteases, which can act during fermentation and also during storage, and which may lead to the characteristic flavor due to volatile free fatty acids. Oxidation of these fatty acids can occur since the a_w value of the product is in the range of lipid oxidation values (Labuza *et al.*, 1970).

The proteolytics and lipolytics growth pattern is plotted in (Fig. 3). Curves show a first phase with a rapid decrease to reach low levels after 21 days for proteolytic microorganisms (20 cfu/g), and after 7 days for lipolytics ($3\ 10^4$ cfu/g). Lipolytic counts remained constant during the last period of the processing. The low level reached during processing may indicate a regular stability at this level and may confirm the success of the preservation process of

sausage against undesirable biochemical breakdowns of the organic matter due to spoilage microorganisms.

Water activity, pH and weight loss. The water activity decreased markedly in the product during the period of sausage making (Fig. 4). This pattern remained with no change for 7 days and the ultimate value reached was close to 0.7. The decrease continued more slowly in coming days. This phenomenon is probably due to the free water driving into the product during the ripening period. The water activity is the most important factor to monitor during the dehydrating process. The a_w reduction to low levels would be related to microbial restriction. Biochemical reactions can occur, especially lipolytics and proteolytics (Leistner & Rodel, 1976). The phenomenon is suitable for obtaining a good product, since lipolytics and fat oxidation are the most important biochemical processes that lead to a typical flavour in camel sausage. According to Girard (1988) the low a_w may extend the lag phase of the growth of undesirable microorganisms, reduce the logarithmic phase and consequently lower the microorganism numbers in the stationary phase. Water activity must be reduced as quickly as possible to stop or to delay spoilage microorganisms in the product. Moreover, spicing is usually accompanied by a reduction in microorganisms and may help in the preservation of foods using a combination of spicing, drying and acidity.

The pH decreased drastically during the first 3 days of the fermenting process (Fig. 5), then remained at a constant value during the following days (4.5). It is assumed that the pH decreases in post-mortem meats to reach an ultimate value around 5.4 but this value can ran up to 6.5 and more after maturation. A drop in the pH from 6.5 to 4 may be due to lactic acid production by the *L. plantarum* starter, while a slight increase in pH was observed during ripening of spontaneously fermented sausages (Samelis *et al.,* 1988). According to Leistner and Roedel (1975) meat products are distinguished into "easily perishable", "perishable" and "shelf-stable" based on their pH and water activity values. The "easily perishable" meat products have a pH>5.2 and an a_w>0.95 and must be stored at or below +5 °C. The "perishable" meat products have either a pH of 5.2–5.0 (inclusive) or an a_w of 0.95–0.91 (inclusive) and must be stored at or below +10 °C. The "shelf-stable" meat products have a pH<5.2 and an a_w<0.95 or only pH<5.0 or a_w<0.91; these products need no refrigeration and their shelf-life is often not limited by bacteria but by chemical or physical spoilage, especially rancidity and discoloration.

The weight loss ratio may indicate the water-drying pattern during drying. As it could be seen the amount removed correspond to water and this is high approaching 44.76% after 21 days. Conditions affecting the weight loss ratio are the relative moisture of the environment in which the products is Stored. This is very low in Morocco since the temperature is higher than other countries. This factor is playing an important role in the drying process, which is favorable for the sausage making. Moreover, Elgasim and Alkanhal (1992) reported that camel meat moisture is highly comparable to other species. According to Forrest *et al*. (1975), high moisture is favorable for sausage making. It was reported by Kissingers and Zaika (1978) that spices used in the Lebanese bologna show stimulate the production of lactic acid by *L. plantarum*.

The acid production and drying may reduce the environmental conditions and stop the growth of gram-negative bacteria, which disappear completely. Some authors (Hernandez-Jouer *et al.,* 1997; Bover-Cid *et al.,* 1999, 2000), reported similar reduction of *Enterobacteriaceae* to non-detectable number during meat fermentation.

Parameters determined in this study may lead to a monitored process for the manufacture of camel meat products in arid areas for special dietary customs. It also brings out a first survey of the different parameters to be controlled during the fermenting and the repining process of camel sausage making. The traditional procedure used for camel meat preservation could be improved by a controlled process for extending it to a high scale production of camel sausages.

CONCLUSION

It is concluded that conditions of camel meat transformation into fermented sausage should be well monitored during processing for a best improvement of the organoleptic quality of the product.

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