

Charqui meats as fermented meat products: role of bacteria for some sensorial properties development

M.F. Pinto^{a,b,*}, E.H.G. Ponsano^a, B.D.G.M. Franco^b, M. Shimokomaki^{b,c}

^aPaulista State University—Campus Araçatuba, PO Box 341, CEP 16050-680, Araçatuba, SP, Brazil

^bPost-Graduate Course on Food Science, Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, São Paulo University, PO Box, 66083, CEP 05389-970, São Paulo, SP, Brazil

^cDepartment of Food and Drugs Technology, Agricultural Sciences Center, Londrina State University, PO Box 6001, CEP 86051-970, Londrina, PR, Brazil

Received 18 June 2001; received in revised form 24 August 2001; accepted 24 August 2001

Abstract

Jerked beef, a derivative of charqui meat, is a cured, salted and dried meat product. The presence of halotolerant bacteria, where *Staphylococcus* spp. (84.2%) were the predominant species, would act eventually as starter cultures and was followed throughout processing. Jerked beef prepared separately with exogenous *S. carnosus* and *S. xylosus* as starter cultures resulted in high proteolysis. Samples prepared with *S. xylosus* had the highest proteolysis and were preferred by the sensory panel. This research has suggested that jerked beef (and thus charqui meat) prepared under these conditions is a fermented meat product. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Fermented meat products; Starter cultures; Charqui; Jerked beef; Sensorial properties

1. Introduction

Charqui meats are Brazilian traditional salted and sun-dried beef products. A derivative popularly known as jerked beef (JB) has recently been introduced into the market. It differs from charqui by employing sodium nitrite in the brine during processing and by being vacuum packaged (Brasil, 2000; Shimokomaki, Franco, Biscontini, Pinto, Terra, & Zorn, 1998). Jerked beef is defined as intermediate moisture meat products preserved by hurdle technology principle (Torres, Shimokomaki, Franco, Landgraf, Carvalho, & Santos, 1994; Shimokomaki et al., 1998). An established A_w value between 0.70 and 0.75 should be recommended as a parameter for official inspection which would be needed for commercialization. In fact, this concept has recently been imposed for jerked beef by the Brazilian Ministry of Agriculture in which this product should have a maximum of 55% moisture, 18.3% ash, 50 ppm sodium nitrite, 0.78 A_w and must be vacuum packaged (Brasil,

2000). These related A_w values would confer microbiological stability to these meat products stored at room temperature for several months (Pinto, Ponsano, Franco, & Shimokomaki, 1998). Investigations on diverse products have been reported including chemical and physicochemical properties (Torres, Pearson, Gray, Ku, & Shimokomaki, 1989; Torres et al., 1994), as well as microbiological (Franco, Landgraf, Shimokomaki, & Azevedo, 1987; Pinto, Ponsano, Franco & Shimokomaki, 1993, 1998) and ultra-structural studies (Biscontini, Shimokomaki, Ferreira, & Zorn, 1996). More recently, this laboratory has reported the possibility of these products to be fermented meat products (Shimokomaki et al., 1998). This current research gives further evidence of jerked beef to be derived from meat fermentation.

2. Materials and methods

2.1. Samples

Jerked beef samples were collected from 10 different points from a processing plant that produces JB in São Paulo State (Indústrias Allyson, Santana de Parnaíba).

* Corresponding author. Tel.: +55-18-620-3270; fax: +55-18-622-6487.

E-mail address: mfpinto@fmva.unesp.br (M.F. Pinto).

Fig. 1 shows JB processing flow chart (Pinto et al., 1998; Shimokomaki, Franco, & Carvalho Jr., 1987; Shimokomaki et al., 1998) and the points from where samples were collected for analysis.

2.2. Microbial counts throughout processing

Twenty-five grams samples were homogenized with 225 ml of sterile phosphate buffer (pH 7.2) for 1 min in a Stomacher 400 as described in Franco et al. (1987). Homogenates were submitted to decimal dilutions in the same diluent. Total aerobic colony counts were determined in triplicate by pour plate methodology using Standard Plate Count Agar (PCA). Plates were incubated at 37 °C for 48 h. Results in CFU/g corresponded to the average of three counts.

2.3. Identification of microorganisms

Characterization of microorganisms in the raw meat, brine and processed product was performed according

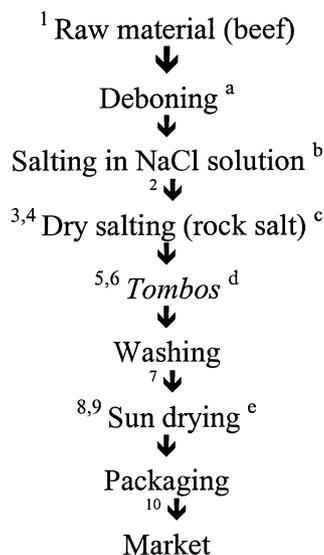


Fig. 1. Flow chart of JB processing showing the actual location of samples collection for microbial analysis. a. After deboning, the meat is cut in 3 to 5 cm thick layers known as *mantas*. b. Brine containing 25% NaCl and 200 ppm NaNO₂ was automatically injected at 20% (v/w) into the raw meat. c. The *mantas* were submitted to dry salting as described in Torres et al. (1994). Essentially, the pieces of meat were submitted to dry salting and stacked into piles separated from each other by layers of coarse marine salt. d. After approx. 48 h the meat was restacked and the uppermost manta was repositioned on the bottom of the piles. This maneuver is known as *tombo* and was repeated for three to five times. e. After washing to remove the excess crystalline salt from the surface, the *mantas* were exposed to sun drying on a stainless steel rail for 4 to 8 h. The *mantas* were collected at night and piled on a concrete floor and covered with a tarpaulin. This session was repeated several times. The samples were collected in the points listed below: 1. Raw material. 2. After wet salting. 3. Salting pile after 24 hr 4. Salting pile after 48 h. 5. 1st *tombo*. 6. End of *tombos*. 7. After washing. 8. Tarpaulin covered pile, after 1st sun exposition period. 9. 2nd sun exposition period. 10. Finished manufactured product.

to Silla, Molina, Flores, and Silvestre (1989). Colonies randomly selected from total count plates were submitted to Gram staining and tested for catalase activity, endospore formation, motility, lactose fermentation and anaerobic growth ability (Banwart, 1989; Vanderzant & Splittstoesser, 1992). Bacteria classified as Micrococcaceae were also submitted to dextrose fermentation and motility tests for genus identification. Mannitol fermentation, coagulase and thermolysin activity were tested to characterize *Staphylococcus aureus* (Buchanan & Gibbons, 1974).

2.4. Starter cultures

Cultures of *Staphylococcus carnosus* — Floracarn S[®] and *Staphylococcus xylosum* — Floracarn SX[®], donated by Ha-La of Brazil—Chr. Hansen Industries were used. These cultures were maintained on Tryptic Soy Agar slants (TSA, Difco) under refrigeration (<5 °C). Inoculum was prepared by transferring two loops of each culture to 10 ml tubes containing Tryptic Soy Broth (TSB, Difco). After incubation at 35 °C for 48 h, each starter culture was transferred to tubes containing sterile meat broth (defatted meat finely ground homogenized and boiled in 0.85% NaCl solution in the ratio of 1:2, filtered and sterilized at 121 °C for 15 min) and incubated at 35 °C for 48 h.

2.5. Starter cultures proteolytic activity

Supraspinosus muscle samples were submitted to the sterilization process as described by Jackson, Acuff, Sharp, and Savell (1992). Meat pieces were briefly dipped into 70% ethanol and flamed. The burned layer (5 mm thick) was removed with a sterile knife. The remaining meat was submitted to the JB processing in a model system. Sterile brine was injected into one group of meat samples (control) and the starter cultures were individually inoculated into the other two groups, mixed with the sterilized brine. In these salt solutions, the viable cell counts of *Staphylococcus xylosum* and *S. carnosus* were 1.2×10^8 CFU/ml and 9.3×10^7 CFU/ml, respectively.

In order to evaluate the starter culture proteolysis, a sarcoplasmic and myofibrillar protein KCl 0.6 M extract was divided into two fractions: trichloroacetic acid (10% final concentration) was added to one of them to cause protein fraction precipitation, while the same volume of KCl 0.6 M was added to the other fraction to make a blank. Protein analysis was performed by Lowry method (AOAC, 1995). The acid treatment caused precipitation of the protein fraction and larger molecular weight polypeptides. Thus, the ratio between acid treatment and blank was considered to be the proteolysis index (Flores, Bermell, Nieto, & Costell, 1984).

2.6. Sensory analysis

Approximately 5.0×5.0×3.0 cm samples were desalted in fresh water for 24 h, cooked in boiling water for 30 min, allowed to cool to 40 °C and tested by Paired Preference (IFT, 1981). Each fermented inoculated sample was tasted by a group of 25 randomly selected untrained panelists, usual consumers of the product, and compared to the control. Inoculated samples were also compared to each other. The consumers were asked to make a choice, considering the general characteristics of the product. The significance of samples difference

was determined based on the number of coincident replies (Roessler, Pangborn, Sidel, & Stone, 1978).

2.7. Statistical analysis

Descriptive statistic analysis was performed with average results and standard deviation for each variable in all selected processing phases. Pearson linear correlation coefficient among the variables in all processing phases was calculated. Inferential statistic was performed through regression and variance analysis. Tukey test was used for means comparison (Zar, 1984).

Table 1
Total counts in samples from selected points at JB processing plant^a

Point	Microbial count (log CFU/g)
1.	4.93±1.40 ab
2.	4.71±0.55 b
3.	4.59±0.29 b
4.	5.36±0.36 ab
5.	4.79±0.22 ab
6.	5.15±0.60 ab
7.	5.36±0.66 ab
8.	4.86±0.15 ab
9.	6.37±0.28 a
10.	4.49±0.17 b

^a Means with different letters are significantly different—5% significance level ($P < 0.05$).

Table 2
Microbiota composition in JB, raw meat and saline solution

	Microorganisms	%
JB	<i>Staphylococcus</i> spp. ^a	84.2
	<i>Micrococcus</i> spp.	15.8
Raw meat	<i>Staphylococcus</i> spp. ^a	47.2
	<i>Micrococcus</i> spp.	17.6
	<i>Lactobacillus</i> spp.	17.6
	Coryneforms	17.6
Saline solution	<i>Staphylococcus</i> spp. ^a	63.0
	<i>Micrococcus</i> spp.	14.8
	<i>Bacillus</i> spp.	7.4
	Coryneforms	14.8

^a Coagulase-negative species.

3. Results and discussion

As shown in Table 1, no consistent alteration in the microbial counts throughout JB processing was observed.

Conversely, a significant change in the composition of microbiota was detected (Table 2). Brine is repeatedly employed for several days during JB processing by automatic injection into the meat. This fact is probably the reason for the high amount of microorganisms present in brine at the starting phase of JB processing. As preliminarily described elsewhere, the processing conditions select the microbiota (Shimokomaki et al., 1998). This assumption is corroborated by the results presented in Table 2. *Staphylococcus* spp. (84.2%) and *Micrococcus* spp. (15.8%) are the predominant microorganisms in the processed product, but coagulase positive *Staphylococcus* species were not detected. The described microbiota composition in the processed product is very similar to that found in basturma, a fermented dry cured salted meat product, typical in Eastern Mediterranean countries (Kotzekidou & Lazarides, 1991), ham (Silla et al., 1989) and dried sausages (Selgas, Garcia, Fernando, & Ordoñez, 1993). Many reports have demonstrated the influence of both of the bacteria on the sensorial properties of those products (Barbieri et al., 1992; Lücke & Hechelmann, 1988).

Data presented in Table 3 shows that both starter cultures induced proteolysis, which was higher than that observed in the control. Among the added cultures, *S. xylosus* caused more intense proteolysis. Several authors

Table 3
Hydrolysis of sarcoplasmic and myofibrillar proteins in JB with and without addition of starter cultures

Sample	KCl 0.6 M solubilized proteins (A)	Amino acids and small peptides not precipitated by TCA (B)	(B) in (A) percentage
Control	3.700 mg/ml	0.103 mg/ml	2.78 ^a
<i>Staphylococcus carnosus</i>	1.370 mg/ml	0.083 mg/ml	6.03 ^a
<i>Staphylococcus xylosus</i>	1.710 mg/ml	0.112 mg/ml	6.55 ^a

^a Significance level: $P < 0.01$.

Table 4
Paired-Preference Test among samples of JB with and without addition of starter cultures

Sample	Number of judges	Number of indications for each sample	Significance level
<i>Staphylococcus xylosum</i>	25	19	0.02
Control		6	
<i>Staphylococcus carnosus</i>	25	18	0.05
Control		7	
<i>Staphylococcus xylosum</i>	25	18	0.05
<i>Staphylococcus carnosus</i>		7	

have reported the proteolytic role of *Staphylococcus* spp. and their influence on the sensory characteristics of meat products (Hammes & Knauf, 1994; Liepe, 1982; Lücke, 1994; Talon et al., 1996). Many compounds that contribute to the flavor of the products are formed through microbial enzymes activity on the protein fraction (Barbieri et al., 1992; Lücke, 1994). As shown in Table 4, samples of JB elaborated with starter cultures presented better acceptability when compared to the control and among all of them, those inoculated with *S. xylosum* were preferred by the panelists.

As previously reported, the harmless strains of staphylococci play an important role in the control of *Staphylococcus aureus* development (Pinto et al., 1998). These results suggest that microbiota are involved in JB processing to influence its sensory characteristics. Consequently, variations in the microbiota composition may lead to changes in the product. According to Geisen, Lücke, and Kröckel (1992), a meat processing mediated by microorganisms whose metabolic activity causes essential changes to the product sensorial characteristics can be considered a fermentative process. The discovery of the occurrence of meat fermentation during processing brings consequences for the charqui meat industries. It is feasible to change its production technology considering the fermentation step, with the consequence of improvement in the standardizing of quality, particularly of taste and flavor.

4. Conclusion

JB is an intermediate moisture fermented meat product in which the inoculation of starter cultures can substantially improve its sensorial quality.

References

AOAC—Association of Official Analytical Chemists. (1995). *Official methods of analysis* (16th ed.). Washington, DC: AOAC.
Banwart, G. J. (1989). *Basic food microbiology*. New York: Avi.

Barbieri, G., Bolzoni, L., Parolari, G., Virgili, R., Buttini, R., Careri, M., & Mangia, A. (1992). Flavor compounds of dry-cured ham. *Journal Agricultural Food Chemistry*, 40(12), 2389–2394.
Biscontini, T. M. B., Shimokomaki, M., Oliveira, S. O., & Zorn, T. M. T. (1996). Ultrastructural observation on charquis, salted and intermediate moisture meat products. *Meat Science*, 43(3), 351–358.
Brasil. (2000). Portaria n. 3, 17 jan. 2000. Diário Oficial, (Seção 1). *Brasília*, 16, 16–27.
Buchanan, R. E., & Gibbons, N. E. (1974). *Bergey's manual of determinative bacteriology*. Baltimore: Williams & Wilkins.
Flores, J., Bermell, S., Nieto, P., & Costell, E. (1984). Cambios químicos en las proteínas del jamón durante los procesos de curado, lento y rápido, y su relación con la calidad. *Revista de Agroquímica y Tecnología de Alimentos*, 24(4), 503–509.
Franco, B. D. G. M., Landgraf, M., Shimokomaki, M., & Azevedo, C. H. M. (1987). Condições higiênicas-sanitarias do charque comercializado em São Paulo, Brasil. *Revista de Microbiologia*, 18(1), 98–102.
Geisen, R., Lücke, F. K., & Kröckel, L. (1992). Starter and protective cultures for meat and meat products. *Fleischwirtschaft*, 72(6), 894–898.
Hammes, W. P., & Knauf, H. J. (1994). Starters in the processing of meat products. *Meat Science*, 36, 155–168.
IFT—Institute of Food Technologists. (1981). Sensory evaluation guide for testing food and beverage products. *Food Technology*, 35(11), 50–59.
Jackson, T. C., Acuff, G. R., Sharp, T. R., & Savell, J. W. (1992). Volatile compounds on sterile pork loin inoculated with *Lactobacillus plantarum* and *Lactobacillus fermentum*. *Journal of Food Science*, 57(3), 783–784.
Kotzekidou, P., & Lazarides, H. N. (1991). Microbial stability and survival of pathogens in an intermediate moisture meat product. *Lebensmittel- Wiss.- Technologie*, 24, 419–423.
Liepe, H. U. (1982). Starter cultures in meat production. In H. J. Rehm, & G. Redd (Eds.), *Biotechnology*. Vol. 5 (pp. 400–424). Basel: Verlag Chemie.
Lücke, F. K. (1994). Fermented meat products. *Food Research International*, 27, 299–307.
Lücke, F. K., & Hechelmann, H. (1988). Cultivos starter para embutido seco y jamón crudo. *Fleischwirtschaft, español*, 1, 38–48.
Pinto, M. F., Ponsano, E. H. G., Franco, B. D. G. M., & Shimokomaki, M. (1993). Charque e sucedâneos são produtos cárneos obtidos por processos combinados (Hurdle Technology). *Revista Nacional da Carne*, 17(195), 66–68.
Pinto, M. F., Ponsano, E. H. G., Franco, B. D. G. M., & Shimokomaki, M. (1998). Controle de *Staphylococcus aureus* em charques (jerked beef) por culturas iniciadoras. *Ciência e Tecnologia de Alimentos*, 12(2), 200–204.
Roessler, E. B., Pangborn, R. M., Sidel, J. L., & Stone, H. (1978). Expanded statistical tables for estimating significance in paired-preference, paired-difference, duo-trio and triangle tests. *Journal of Food Science*, 43(3), 940–943, 947.
Selgas, D., Garcia, L., Fernando, G. G., & Ordoñez, J. (1993). Lipolytic and proteolytic activities of Micrococci isolated from dry sausage. *Fleischwirtschaft*, 73(10), 1175–1176, 1179.
Shimokomaki, M., Franco, B. D. G. M., & Carvalho, B. C. Jr. (1987). Charque e produtos afins: tecnologia e conservação—uma revisão. *Boletim da Sociedade Brasileira de Ciência e Tecnologia de Alimentos*, 21(1), 25–35.
Shimokomaki, M., Franco, B. D. G. M., Biscontini, T. M., Pinto, M. F., Terra, N. N., & Zorn, T. M. T. (1998). Charqui meats are hurdle technology meat products. *Food Reviews International*, 14(4), 339–349.
Silla, H., Molina, I., Flores, J., & Silvestre, D. (1989). A study of the microbial flora of dry-cured ham. I. Isolation and growth. *Fleischwirtschaft*, 69(7), 1128–1131.

- Talon, R., Berdagué, J. L., Dauneau, P., Hinrichsen, L., Masson, F., Montel, M. C., Pedersen, S. B., & Pérez-Martinez, G. (1996). Bacterial production of volatiles in meat fermentation. In *Proceedings 42nd International Congress of Meat Science and Technology* (pp. 33-36), 1-6 September 1996, Lillehammer, Norway.
- Torres, E., Pearson, A. M., Gray, J. I., Ku, P. K., & Shimokomaki, M. (1989). Lipid oxidation in charqui (salted and dried beef). *Food Chemistry*, 32, 257–268.
- Torres, E. A. S., Shimokomaki, M., Franco, B. D. G. M., Landgraf, M., Carvalho, B. C., & Santos, J. C. (1994). Quality parameters determination of charqui, an intermediate moisture meat product. *Meat Science*, 38, 229–234.
- Vanderzant, C. C., & Splittstoesser, D. F. (1992). *Compendium of methods for the microbiological examination of foods* (3rd ed). Washington: Apha.
- Zar, J. H. (1984). *Biostatistical analysis* (2nd ed). Englewood Cliffs: Prentice Hall International.