INTERMEDIATE MOISTURE MEAT PRODUCT. EVALUATION OF BOTULINAL TOXIN PRODUCTION IN CHARQUI MEAT

J. A. F. LARA[1], I. S. DUTRA[2], M. F. PINTO[2] AND M. SHIMOKOMAKI[1,3*]

[1]Graduate Course on Food Science, Department of Food and Drugs Technology, Agricultural Sciences Center, Londrina State University, Londrina, Pr, Brazil, *P. O. Box, 6001, CEP-86051-970 Londrina, Pr, Brazil.* [2]Department of Support, Production and Animal Health, Paulista State University, Araçatuba, São Paulo, Brazil. e-mail: mshimo@uel.br

BACKGROUND

Intermediate moisture (IM) meat products are processed almost in every country in the world and each product has its own characteristic. Since there has been an increase in refrigeration cost, IM meat products have gained a further interest (Chang et al., 1996). They have been classified as IM because after drying processing the product reaches the value of Aw 0.6-0.90 (Leistner, 1985). Charqui meat is a tropical intermediate moisture meat product obtained by salting and sun-drying beef samples. The official definition is "a beef product which should not contain more than 45% moisture within intramuscular portion and not more than 15% of mineral residue" (Brazil, 1962). Charqui meat is a very important source of animal protein in particular for Brazilian rural people and studies related with its safety are very scarce and they are restricted to the development of *S. aureus* (Senigalia et al. in preparation). The toxin botulinal production in charqui by *C. botulinum*, is unlikely to growth because of its harsh processing conditions. However, the recent introduction of vacuum packaging at the final processing step associated with the continuous epizootic report of botulism and environmental contamination in Brazilian farm cattle and the lack of hygiene frequently observed in small charqui processing plants makes this possibility feasible (Lara et al., 1999).

OBJECTIVES

To establish the processing parameters conditions for inhibiting botulism toxin by *C. botulinum* in charqui meat during its processing.

METHODS

Clostridium botulinum spores. *C. botulinum* proteolytic type B spores (STUA-88-Cb) inoculated in charqui meat were obtained from collection cultures kept at Infections Disease Laboratory at Paulista State University, Araçatuba, Brazil. Charqui meat samples preparation. Fifteen samples of *semitendinous m.* acquired from a commercial butchery were sterilized as described by Jackson et al. (1992). The was submitted to charqui processing as described elsewhere (Pinto et al., 1998). Three aliquots of 5g were taken from the remaining meat (± 300g) were cut in cubes of approximately 1.0 cm^3 from each sample. 100 spores/g of charqui samples were inoculated into the test tubes and set under anaerobiosis condition at 35°C for 96 h. Three samples were analyzed each time from different processing steps at dry salting, (day 1), tumbling, (day 2-6), and sun-drying (day 7-13). Samples stored for 30 days (day 43) and 60 days (day 73)were also evaluated (Fig. 1). *C. botulinum* growth. Enumeration of *C. botulinum* type B spores was carried by spreading onto the plate 0.2 ml of 1:5 dilutions of homogenized charqui samples, in duplicate, on trypticase-peptone-glucose-yeast extract mixtured with 0.8% egg yolk 1:1 with bromocresol purple (TPGY-EY) (Hauschild and Hishelmer, 1977). After inoculation, a layer of solution containing ditiothreitol and agar (0.01% and 2.0%, respectively) was added to the plate surface (Hauschild and Hishelmer, 1977) and incubated at 35°C for 48 h, in anaerobiosis. Typical *C. botulinum* colonies were then counted. Toxin B assay. Botulism toxin B was detected by mice bioassay as described by Smith (1977) and microplate complement fixation (Weiss and Weiss, 1988). Other microbiological analyses. Anaerobic mesophyllic spores counts and aerobic mesophyllic plate counts were measured according to Vanderzant and Splittstoesser (1992).Physicochemical evaluation. Sodium chloride was quantitatively measured according to AOAC (1980). Aw was monitored using a water activity analyzer (Aqualab CX-2), USA, and pH and Eh using Sentron 1001 equipment, USA. All measurements were performed in triplicate.

RESULTS AND DISCUSSIONS

Results for moisture, chloride residues, Aw, pH, Eh and also total counts for aerobic and facultative anaerobic mesophyllic bacteria throughout charqui meat processing and during storage are summarized in Table 1. Samples taken at phase day 2-6, already reached the official values of 40–50.0% moisture and 10–20.0% mineral residue (Brasil, 1962). However, the expected Aw values between 0.70–0.75 for charqui (Torres et al, 1994) were only reached at phase day 2-6 (Fig. 1). pH and Eh values remained constant at 5.30-5.60 and + 69-80, respectively. These values are within the minimum growth requirements for the bacteria (Hauschild and Dodds, 1993). The survival of halophylic bacteria throughout charqui processing and storage showed that some of them were aerobic facultative. The results obtained for growth viable cells in TPGY-EY in charqui samples were 1.22 $x10^2$ for day 1, 1.24 $x10^2$ for day 2-6, 1.21 $x10^2$ for day 7-13, 1.06 $x10^2$ for day 43 and 1.18 $x10^2$ for day 73. The spore cell counts remained constant at the rate of *C. botulinum* 100 viable cells/g of charqui sample. These results demonstrated the inability of *C. botulinum* spores to germinate during charqui processing or even after 30 and 60 days of storage. The bioassay and microplates complement fixation presented negative results for botulinum toxin B production. The bacteria spores did not germinate after transference onto an appropriate culture such as TPGY-EY hence they could not be counted. These negative results were expected although there were possibilities of *C. botulinum* growth based on the NaCl maximum concentration for the bacteria growth requirements (Baird-Parker and Freame, 1967).

CONCLUSIONS

Our results allowed us to state that under our experimental conditions, the resulting Aw values of 0.70-0.75 should be considered as the official parameter for charqui meat since it was the main factor that inhibited *C. botulinum* growth.

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TABLE 1 – Physico-chemical and microbiological analysis throughout charqui meat processing.

PROCESSING STEPS	*Moisture (%)*	*Chloride (%)*	*Aw*	*pH*	*Eh (mV)*	*APC[1] (CFU/g)*	*ASC[2] (CFU/g)*
Day 1	67.00	10.10	0.821	5.39	+81	$3.96x10^2$	$4.00x10^2$
Day 2-6	54.13	13.46	0.777	5.47	+77	$4.00x10^1$	0
Day 7-13	44.13	15.53	0.742	5.58	+72	$1.33x10^1$	0
Day 43	47.00	17.15	0.748	5.57	+72	$2.00x10^1$	0
Day 73	52.30	14.00	0.760	5.46	+77	$3.33x10^1$	0

[1] APC: Aerobic mesophilic plate count. [2] ASC: Anaerobic mesophilic spores count.

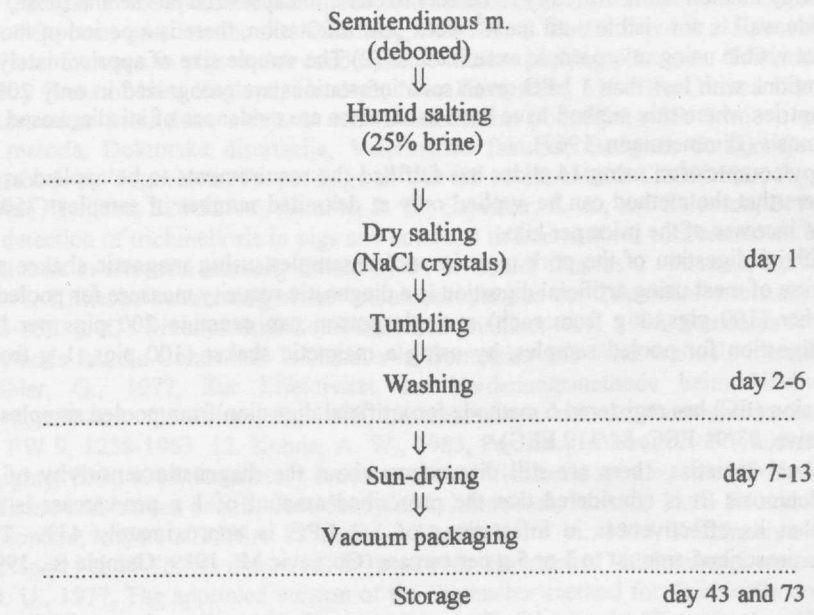

FIGURE 1 – Flow chart of charqui meat processing indicating the steps were the samples were taken for analysis (Shimokomaki *et al.*, 1998)