# CLOSTRIDIUM SPOROGENES PA3679 SPORES GERMINATION IN LOW-COST SHELF-STABLE BOLOGNA-TYPE PRODUCT AS AFFECTED BY REPLACEMENT OF NaCl BY KCl

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## I. INTRODUCTION

Low-cost Bologna-type product stuffed in impermeable casings reaches high production volumes in Brazil. It is allowed to use up to 60% mechanically deboned chicken meat (MDCM), 10% edible offal and pork or poultry skin as raw materials. This product has been marketed at room temperature for a long time. In 2015, the Meat Technology Center of the Food Technology Institute established a Brazilian protocol for assessing the microbiological safety of Bologna marketed at room temperature in the country. The results showed that water activity was the most significant barrier in preventing the germination of spores of *Clostridium sporogenes* PA 3679, used as a surrogate for *Clostridium botulinum*, in pasteurised products containing 150ppm of nitrite and 400 ppm of sodium erythorbate. This study supported the Ministry of Agriculture, Livestock and Supply (MAPA) in establishing the maximum value of water activity (Aw) as 0.955 [1]. Partial replacement of sodium chloride by KCl is an usual strategy to decrease the sodium content of meat products [2] and the adjustment of the water activity is carried out taking into account the maintenance of the ionic strength in the system. It is unknown the impact of this replacement in preventing the germination of spores of the genus *Clostridium*, a microbiological hazard in this type of meat product. The aim of this work was to evaluate the impact caused by the replacement of sodium chloride by potassium chloride on the germination of *C. sporogenes* PA 3679 in Bologna-type product at 0.95 water activity.

## II. MATERIALS AND METHODS

*C. sporogenes* PA3679 (ATCC 11437) was used as a surrogate for *C. botulinum*. The spore suspension was prepared according to Mah et al. [3] and contained approximately $10^5$ spores/ml. In order to calculate the brine concentration corresponding to the different values of water activities, a standard batter containing MDCM (60%), pork skin (12%), pork kidney (1%), pork liver (1%), pork trimmings 70/30 (16.4%), texturized soy protein (3.5), tapioca starch (5.0%), sugar cane (0.6%), sodium tripolyphosphate (0.35), sodium acid pyrophosphate (0.15%), sodium nitrite (0.015%). The moisture of the standard batter was determined (58,97%) to calculate the amounts of KCl and NaCl to be added in order to reach the target water activities. The study comprised five treatments with different water activities (0,95, 0,96 and 0,97) adjusted with KCl (K95, K96, K97) or NaCl (N95, N97). The amounts of NaCl were calculated following Krispien et al., (1979) [4] and the corresponding amounts of KCl were calculated based on the molar concentration. Sodium erythorbate (0.04%) was added at the final comminution process in cutter. The data was submitted to one-way analysis of variance (ANOVA) and Tukey's test (V.9.1, SAS Institute, Cary, NC). Results were considered significant at $P<0.05$.

## III. RESULTS AND DISCUSSION

Spore germination was observed after 15 days of storage at 35ºC in treatments N97, K95, K96 and K97. At 30 days of storage, germination was confirmed in the K95 treatment. There was no germination in the N95 treatment during the entire period, confirming the previous results. After 30 days of storage, the counts reached 6.3 log CFU/g in the K95 treatment, but remained unchanged in the N95. In the other treatments (N97, K96 and K97) the counts were not performed after 30 days of storage since at 15 days they have already showed spores germination (Table 1).

Table1. *C. sporogenes* counts (log CFU/g) in inoculated Bologna-type product during storage at 35°C.

| | Storage time (days) | | | | | |
|---|---|---|---|---|---|---|
| | 1* | | 15 | | 30 | |
| | Spores | Spores and vegetative cells | Spores | Spores and vegetative cells | Spores | Spores and Vegetative cells |
| **N95** | 1.99 ± 0.07 h | 2.07 ± 0.10 gh | 2.57 ± 0.20 fgh | 2.53 ± 0.46 fgh | 2.65 ± 0.20 fgh | 2.44 ± 0.17 fgh |
| **N97** | 1.95 ± 0.08 h | 2.16 ± 0.23 gh | 7.46 ± 0.55 ab | 8.18 ± 0.18 a | ND | ND |
| **N96** | 2.02 ± 0.10 h | 2.00 ± 0.10h | 2.72 ± 0.22 fgh | 5.08 ± 0 .28 d | ND | ND |
| **K95** | 2.13 ± 0.38 gh | 2.17 ± 0.26 gh | 2.94 ± 0.54 fg | 4.06 ± 1.46 e | 3.20 ± 0.45 ef | 6.01 ± 0.86 c |
| **K96** | 1.99 ± 0.12 h | 2.16 ± 0.25 gh | 6.06 ± 0.29 c | 7.12 ± 0 .20 b | ND | ND |
| **K97** | 1.94 ± 0.08 h | 2.19 ± 0.18 gh | 7.54 ± 0.23 ab | 8.27 ± 0.40 a | ND | ND |

*Before incubation, 24 hours after processing; Mean and standard deviation (n=6); ND = non determined (germination occured on day 15); N95 (aw-0.95/NaCl); N97 (aw-0.97/NaCl); K95 (aw-0.95/KCl); K96 (aw- 0.96/KCl); K97(aw-0.97/KCl) Values with different superscripts are significantly different ($p < 0.05$).

## IV. CONCLUSION

The water activity value of 0.95 constitutes a barrier to the germination of spores of *C. sporogenes*, provided that the salt used is sodium chloride. However, when sodium chloride is completely replaced by potassium chloride, spores' germination occurs. Although the total replacement of sodium chloride is not practiced by meat processors due to sensory issues, it is necessary that the reformulation of this type of product includes a challenge test to assess microbiological safety.

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