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INTRODUCTION

Aerobic sporulated bacilli represent an important flora in the fermented sausage chorizo, mainly because of the incorporation of contaminated spices in the product. The physicochemical characteristics of chorizo during the fermentation and drying process (low pH and low water activity) don't allow the germination and growth of *Bacilli*. However, the actual trend to reduce the salt content in food products could have an impact on the behavior of sporulated bacteria during the manufacturing process of the chorizo sausage. The aims of this study were to quantify and to describe the biodiversity of sporulated aerobic bacilli in chorizo and to assess the impact of different salt concentrations on the behavior of the dominant species.

BIODIVERSITY OF SPORULATED AEROBIC BACTERIA IN SPICES AND CHORIZOS

Spices, fresh minced pork meat, fermented sausage after drying and chorizo at the end of the shelf life after storage at room temperature (from the same batch) were supplied by four industrial companies (three batches per company).

Microbial analysis were performed. The enumeration were realized on PCA agar at 30°C for the aerobic mesophilic flora and on TSA with yeast extract (2.5 g/l) and starch (2g/l) at 30°C for the aerobic sporulated flora. After heat treatment of the sample at 80°C during 10 min. *Bacillus cereus* were enumerated on Mossel agar. pH and a_w of the ground pork meat and chorizos were also measured.

180 strains were isolated and genotyped by M13-PCR and representative strains were subsequently identified by 16S rDNA sequencing.

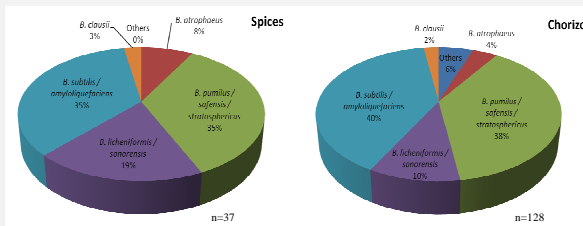
Table 1: Physicochemical characteristics of the analyzed chorizos

Company	Ingredients	Size	Drying time	Shelf life	a_w after drying (mean (sd))
2	Spices mix	small (Ø 38 mm)	1.5 week	8 months	0.844 (0.026)
3	Spices and Cayenne pepper	small (Ø 38 mm)	3 weeks	14 weeks	0.816 (0.032)
1	Spices mix	large (Ø 84 mm)	7 to 8 weeks	4 months	0.913 (0.005)
4	Spices and Cayenne pepper	large (Ø 84 mm)	7 to 8 weeks	2 months	0.891 (0.006)

pH of the fermented sausages = 4.9

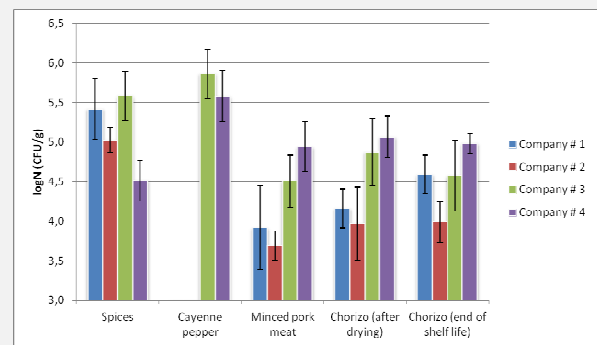
a_w remained stable during the storage except for the company 1 (0.89 at the end of shelf life).

Figure 3: Biodiversity of *Bacilli* in spices and chorizos



Sixteen genetic clusters were obtained by M13-PCR. 1-4 representative strains from each cluster were selected for species identification by 16S gene sequencing. The dominant species were *Bacillus subtilis* / *amyloliquefaciens*, *B. pumilus*, and *B.licheniformis*, and, to a lesser extent *Bacillus atrophaeus* and *Bacillus clausii*. These species are ubiquitous and have already been described in spices (Giaccone *et al.*, 1996). *B. cereus* was detected in 28% of chorizos at the end of shelf life with contamination levels below 50 CFU.g⁻¹.

Figure 2: Contamination levels of spices and chorizos products by sporulated aerobic flora

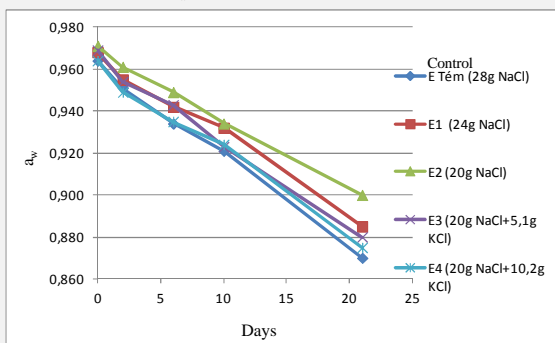


Aerobic sporulated flora is abundant in chorizo and its ingredients. Average contamination levels range from 3.6 to 5 log CFU.g⁻¹. Adding spices and Cayenne pepper during the transformation process of chorizo is likely responsible for (at least) a large part of the contamination of the end product with *Bacilli*. Indeed the aerobic sporulated flora reaches 4.5 to 5.5 log CFU.g⁻¹ in spices mix and 5.5 to 5.8 log CFU.g⁻¹ in Cayenne pepper.

IMPACT OF NaCl ON THE BEHAVIOR OF BACILLUS IN CHORIZO

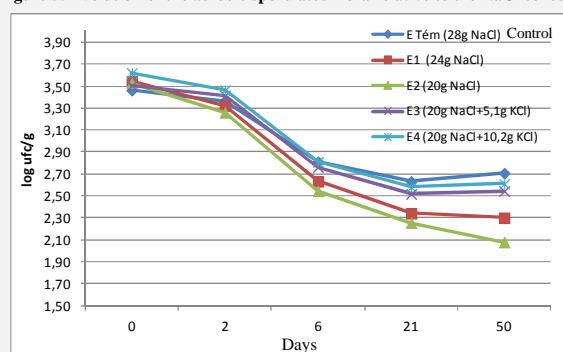
Challenge-tests were performed with a two strains cocktail (*B. pumilus* and *B. subtilis*) in different chorizo recipes containing not contaminated spices. NaCl concentrations ranging from 20 to 28g.kg⁻¹ were tested with or without KCl substitute. Fresh meat was artificially contaminated with spores (3 log spores.g⁻¹). pH, a_w , weight loss measurements and microbiological analysis were performed during the fermentation (22/24°C), the drying process (13°C) and during the storage at 18°C.

Figure 4: Evolution of a_w during the process relative to the NaCl concentration



Physicochemical characteristics (pH, weight loss) and evolution of the technological flora (lactic acid bacteria and *Micrococaceae*) were similar in every experimentation. However, a_w increased when NaCl concentration decreased (figure 4).

Figure 5: Evolution of the aerobic sporulated flora relative to the NaCl concentration



Initial inoculum of sporulated flora was 3.5 log spores/g. 14% and 28% reductions in NaCl concentration both induced a stronger decrease in the spores number (due to their germination) than in the control condition (figure 5). The substitution of NaCl with KCl in the low-salt products (-28.5% NaCl) reduced the germination rate of spores and provided results similar to the control condition (28g/kg NaCl). The presence of K⁺ may quantitatively compensate the lack of Na⁺ ions.

CONCLUSION

This study has shown that the aerobic sporulated flora is present at high levels in chorizo (3.6 to 5 log CFU/g) and the contaminating *Bacilli* originate from spices and Cayenne pepper where they reach 4.5 to 5.8 log CFU/g. This flora remains stable during drying and shelf life of the end product. Physicochemical parameters in chorizo after drying (pH 4.9 ; a_w 0.82-0.84 and 0.89-0.91 for small and large chorizos, respectively) don't allow the *Bacillus* species to grow, even at 20°C.

Biodiversity in the analyzed fermented sausage and ingredients has been shown to be quite wide even though two species are predominant: *Bacillus subtilis*/ *amyloliquefaciens* and *Bacillus pumilus*/ *safensis*. Decreasing the NaCl concentration by 14 or 28% triggers an increase in the germination rate of spores compared to the control condition. Adding KCl in low-salt samples seems to reduce the germination of spores.

ACKNOWLEDGMENTS

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