SPANISH FERMENTED SAUSAGES (SALCHICHÓNES) OBTAINED WITH THE ADDITION OF TWO ANTI-LISTERIAL AUTOCHTHONOUS STARTER STRAINS

Federica Barbieri[1*], David García-López[2], Alberto Baños[2], Josè M. Garcia Madero[2], Fausto Gardini[1], Chiara Montanari[1] and Giulia Tabanelli[1]

[1]Department of Agricultural and Food Sciences, University of Bologna, Italy

[2]Department of Microbiology, DOMCA S.A.U., Spain

*Corresponding author email: federica.barbieri16@unibo.it

I. INTRODUCTION

Nowadays, starter cultures are commonly used in industrial production of fermented sausages to obtain reproducible final product characterised by desired sensorial properties and safety. In particular, lactic acid bacteria (LAB) are used to guide the fermentation process, contributing to pH decrease by lactate production, important to reduce the growth potential of undesired bacteria (spoilage or pathogens) [1]. Regarding the selection of LAB starter cultures, safety and technological aspects are considered. In the first case, the inability to produce biogenic amines (BA) and the absence of antibiotic-resistance horizontally transmissible are considered [2]. Moreover, the acidification rate and growth performances in relation to process conditions (*i.e.* temperature and presence of salt) are evaluated. Recently, bioprotective aspects are also considered, in particular the possible production of specific molecules characterised by bacteriostatic or bactericidal effects (*i.e.* bacteriocins and other antimicrobial peptides) [3]. Despite the great diffusion of starter cultures in fermented sausage industry, in Europe several artisanal products obtained through spontaneous fermentations are still present [4]. These productions represent an important biodiversity reservoir of strains to be exploited as new potential starter cultures, that could also favour the differentiation of traditional products [5]. Within the framework of the European project BioProMedFood (PRIMA – Section 2 Programme), in this work two LAB strains, isolated from spontaneously fermented sausages (Andalusia, Spain) and characterised by a promising anti-listerial activity, were tested as starter cultures to produce salchichónes in pilot plants. These samples were compared to a product obtained with a commercial starter culture and a control sample (without starter culture). Finally, a challenge test against *Listeria monocytogenes* was also performed.

II. MATERIALS AND METHODS

Fermented sausages were produced by DOMCA (Granada, Spain), according to their manufacturing protocol. The different LAB cultures, *Latiplanctibacillus paraplantarum* BPF2, *Pediococcus acidilactici* ST6 and a commercial starter culture (RAP; Biovitec, France), were inoculated separately starting from the same meat batter at a final concentration of about 6.3 log cfu/g, in order to obtain 15 samples for each recipe. In addition, a control sample (spontaneously fermented) was considered. Each sample was monitored during ripening regarding physico-chemical parameters (pH and weight loss) and the principal microbial groups (LAB, staphylococci/micrococci, yeasts and *Enterobacteriaceae*), detected through plate counting onto selective media, provided by Oxoid (Basingstoke, UK), and plates were incubated according to the manufacturer's recommendations. Volatile organic compounds of products at the end of ripening were detected through a gas-chromatography-mass spectrometry coupled with the solid-phase microextraction technique (GC-MS-SPME) [4]. Moreover, biogenic amines (BA) content was also assessed through HPLC analysis [4]. Each analysis was performed in triplicate. Finally, a challenge test against *List. monocytogenes* was performed, in order to evaluate the anti-listerial activity of the tested strains. In particular, the target microorganism was inoculated into the meat batter before stuffing at a final concentration of about 3 log cfu/g and its growth was monitored over time by sampling onto LSO plates (Oxoid). Data were analysed through a one-way ANOVA model with Statistica 8.0 software (StatSoft Inc., Tulsa, USA), according to Tukey test ($P \leq 0.05$).

III. RESULTS AND DISCUSSION

Technological parameters of fermented sausages during ripening showed differences concerning pH, whose decrease was more relevant in the sausages RAP and *Ltp. paraplantarum* BPF2, reaching a pH value of 5.0 ± 0.03, compared to other samples with final pH 5.2 ± 0.02. Conversely, *P. acidilactici* ST6 failed its action as starter culture, resulting in lower concentration if compared with other added LAB (8 *vs.* 8.6 log CFU/g). In the control sample, the initial lactobacilli count on MRS was 3.3 log CFU/g. This microbial population gradually increased during ripening, without exceed a final concentration value of 6.3 log CFU/g. Moreover, samples presented interesting differences in relation to the volatile profile. Furthermore, relevant differences were observed in BA content. In particular, the total BA concentration reached its maximum in the control (539.5 mg/kg), while RAP sample was characterised by the lower accumulation of these compounds (189.2 mg/kg), mainly due to a very low concentration of putrescine and cadaverine. RAP and ST6 samples were characterised by a reduced concentration of tyramine (122.4 ± 9.9 mg/kg), with respect to the control (150.9 ± 7.3 mg/kg). However, the presence of BPF2 determined the lowest accumulation of this compound (93.1 ± 2.1 mg/kg). In addition, histamine was always under the detection limit in all samples. Finally, challenge test results are reported in Table 1, underlining the anti-listerial activity of BPF2 and ST6 strains. In fact, after a small initial increase, the viable counts of *List. monocytogenes* progressively decreased and reached a final concentration of about 2.0 log CFU/g. Conversely, RAP showed a similar behaviour than the control sample, in which *List. monocytogenes* constantly grew and reached a final concentration of 5.7 and 6.4 log CFU/g, respectively.

Table 1 *List. monocytogenes* growth kinetics in the challenge test performed on Spanish fermented sausages. Standard error is also reported.

Time (days)	Control	RAP	BPF2	ST6	SE	P-values
0	3.18	3.18	3.18	3.18	0.01	0.2368954
1	3.56	3.56	3.55	3.53	0.01	0.6890816
7	4.81^{a*}	4.69^{a}	3.03^{b}	3.13^{b}	0.32	0.0001489
15	5.64^{a}	4.99^{b}	2.86^{c}	2.76^{c}	0.48	0.0000065
21	5.95^{a}	5.07^{b}	2.57^{c}	2.62^{c}	0.56	0.0000068
40	6.37^{a}	5.73^{b}	2.08^{c}	2.40^{d}	0.73	0.0000004

* for each sampling time different letters indicate significant differences (P ≤ 0.05) according to ANOVA

IV. CONCLUSION

In conclusion, the BPF2 and ST6 strains demonstrated a relevant inhibiting activity against *List. monocytogenes*, both compared to the spontaneously fermented control and the commercial starter culture (RAP). These results confirmed their bioprotective features and their ability to produce bacteriocins in a real system. Moreover, the use of these new strains as starter cultures significantly affected the aroma profile of the salchichón, thus favouring product differentiation and increasing the possibility for tailor made fermentations aimed to improve the recognisability and the peculiar traits of fermented sausages.

REFERENCES

1. Laranjo, M., Potes, & Elias, M. (2019) Role of starter cultures on the safety of fermented meat products. Frontiers in Microbiology 10: 853.
2. Laulund, S., Wind, A., Derkx, & Zuliani, V. (2017). Regulatory and safety requirements for food cultures. Microorganisms, 5: 28.
3. Woraprayote, W., Malila, Y., Sorapukdee, S., Swetwiwathana, A., Benjakul, S., & Visessanguan, W. (2016). Bacteriocins from lactic acid bacteria and their applications in meat and meat products. Meat Science 120: 118-132.
4. Barbieri, F., Tabanelli, G., Montanari, C., Dall'Osso, N., Šimat, V., Smole Možina, S., Baños, A., Özogul, F., Bassi, D., Fontana, C., & Gardini, F. (2021). Mediterranean spontaneously fermented sausages: spotlight on microbiological and quality features to exploit their bacterial biodiversity. Foods 10: 2691.
5. Franciosa, I., Ferrocino, I., Giordano, M., Mounier, J., Rantsiou, K., & Cocolin, L. (2021). Specific metagenomic asset drives the spontaneous fermentation of Italian sausages. Food Research International 144: 110379.