SCREENING OF PROBIOTICS OF LACTIC ACID BACTERIA FOR MEAT SYSTEM: EVALUATION IN VITRO AND IN MINI DRY FERMENTED SAUSAGE

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Abstract –The aim was to select strains of probiotic lactic acid bacteria to simulate the processing of dry fermented sausages. Nine strains (*Lactobaccillus paracasei* BGP1, *Bifidobacterium animalis* subsp. *lactis* BB-12, *Lactobaccillus rhamnosus* GG, *Lactobacillus casei* SJRP38, *Lactobacillus casei* SJRP66, *Lactobacillus casei* SJRP141, *Lactobacillus casei* SJRP45, *Lactobacillus casei* SJRP146, *Lactobacillus casei* SJRP169,) were tested *in vitro*. The strains that showed highest tolerance (BGP1, GG, SJRP38 and SJRP146) were tested in the processing of dry fermented sausage. Fermentation and ripening were monitored in relation to pH, weight loss, aw and LAB count. The pH value was between 4.7-4.9 and the aw was 0.88. The weight loss was highest (35%) for SJRP38 and BGP1. The selected strains showd potential for application in fermented meat.

Key Words – Selection, LAB probiotics strains, fermented meat, model system.

I. INTRODUCTION

The inclusion of probiotic strains in the processing of fermented meat products has become more common because of the numerous benefits provided to the consumer by the intake of these microorganisms. Fermented meat products can ensuring the survival of probiotic microorganisms through the gastrointestinal tract, the fermentation and drying process of the product under different conditions [1, 2]. The use of lactic acid bacteria in the fermentation of meat sausages is justified by its rapid acidification, which assists in the development of the sensorial characteristics of the product, besides guaranteeing the microbiological safety against pathogens [3]. The purpose of this study was to screening between nine strains of probiotic lactic acid bacteria the strains that showed more tolerance under different conditions in vitro test. For further simulation to the processing of dry fermented sausages.

II. MATERIALS AND METHODS

In vitro evaluation

Nine different strains of probiotics lactic acid bacteria were tested*: Lactobaccillus paracasei* BGP1 (SACCO Brasil) *Bifidobacterium animalis subsp. lactis* BB-12® (Chr-Hansen Ind. e Com. Ltda) e *Lactobaccillus rhamnosus* GG (ATCC 53103). Wild strains were: *Lactobacillus casei* SJRP38, *Lactobacillus casei* SJRP66, *Lactobacillus casei* SJRP141, *Lactobacillus casei* SJRP145, *Lactobacillus casei* SJRP146, *Lactobacillus casei* SJRP169, identified from gene sequencing 16S rRNA as *Lactobacillus casei* and previously characterized for safety, technological and probiotic potential. All these strains were grown in MRS broth (Difco™, Leeuwarden, The Netherlands) at 37°C for 24h, centrifuged at 7.000g for 10 minutes at 4°C, washed and resuspended in saline solution (0,85%). The cells were centrifuged again at 7.000g for 10 minutes at 4°C, washed and resuspended using the modified MRS broth and were inoculated at 37°C/24h. The result was expressed in presence (+, ++, +++) and absence (-) of growth. This procedure was performed in duplicate. The modifications were pH 4.5, 5.0, 5.5; sodium chloride 2.5%, 3%, 3.5%, 4% and 0.015% of sodium nitrite, 0.015% of sodium nitrate. The strains that presented the highest (++) tolerance to the different conditions were selected for testing in the model system.

Mini dry fermented sausage evaluation

The mini dry fermented sausages were produced using the following ingredients: pork (70g/100g), pork back fat (25g/100g), sucrose (1.7g/100g), sodium chloride (NaCl) (2.5g/100g), sodium erythorbate (0.5g/100g), sodium nitrite (0.015g/100g), sodium nitrate (0.015g/100g), white pepper (0.5g/100g), garlic (0.2g/100g), nutmeg (0.2g/100g) and starter culture composed by *Staphylococcus xylosus* e *Pediococcus pentosaceus* (0.25 g/kg; SPX Floracarn, Chr Hansen). The pork meat was ground with a disk of 10 mm, and the pork back fat was cut into cubes of 1 cm^3. The raw material was mixed with NaCl, other ingredients and the following strains for each treatment: BGP1, GG, SJRP38, SJRP146. The treatments were stuffed in collagen casings (diameter of 45 mm), and they were cut into pieces of 15 cm in length. The sausages were fermented according to the following procedure (Temperature/ Relative

humidity %): 25°C/89% for four days; 23°C/89% for three days; 20°C/85% for three days; 18°C/80% for five days and 15°C/75% until day 20. The analyses performed were pH, weight loss, aw and LAB count during fermentation and ripening. The pH was measured in triplicate in each treatment using a PG 1800 digital pH meter (Gehaka, São Paulo, SP, Brazil) with a penetration probe. To calculate the weight loss, the samples were weighed in a semi analytical balance during the processing. The water activity (Aw) was measured using a NOVASINA Aw Sprint electric hygrometer (Axair Ltd., Switzerland). Total lactic acid bacteria (LAB) counts were determined by plate counting in MRS agar (OXOID, Hampshire, United Kingdom) after 48 h at 37 °C anaerobically.

III. RESULTS AND DISCUSSION

The BB12 strain did not resist well in the given conditions. The other probiotic strains showed low growth (+) in the presence of nitrite and nitrate (curing agents). This result indicates that the incorporation of these strains in the processing of dry fermented sausage can be a challenge since curing agents are indispensable ingredients in this process. According to Ammor & Mayo [3] rapid and adequate production of lactic acid, growth rate in different temperatures, salt concentrations a pH, tolerance to other microbial components and tolerance of nitrate and nitrite are some criteria that should be evaluated in the LAB selection. Of the commercial probiotic strains, the highlights (+++ for all the modifications) were BGP1 and GG. Wild strains that showed better adaptation on modified MRS were SJRP141 and SJRP146.

Mini dry fermented sausage evaluation

According to Leistner et al. [4] shelf-stable meat products are classified as those having pH below 5.0 or water activity at or below 0.95. So, these mini dry fermented sausages can be considered shelf-stable. It shows that the addition of probiotic strains did not influence the stability of the process. Figure 1 showed a rapid drop in pH in all treatments, reaching values near to 4.9 after three days of fermentation. This shows that these LAB probiotics were good producers of lactic acid, even under adverse conditions presented by the food matrix. In relation to aw, there is great variation during the ripening of the mini dry fermented sausages, although the final products had water activity values close to 0.89.

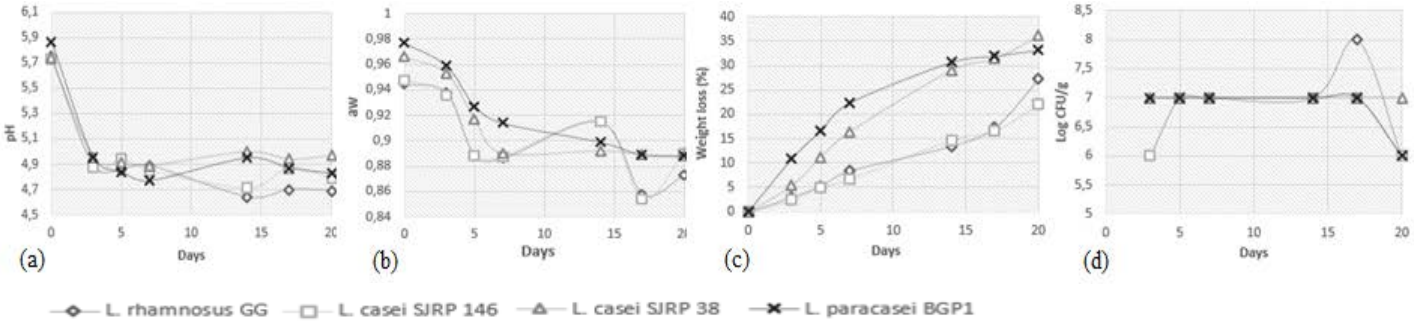

Figure 1. pH (a), water activity (aw) (b), weight loss (c) and LAB count (d) in mini dry fermented sausage with added probiotic strains, during a 20 day fermentation and ripening period.

IV. CONCLUSION

The strains *Lactobaccillus paracasei* BGP1; *Lactobaccillus rhamnosus* GG; *Lactobacillus casei* SJRP 38; *Lactobacillus casei* SJRP 146 show good tolerance to adverse conditions and with high potential for application in dry fermented sausage. These strains resulted in a meat product with appropriate technological features for a fermented meat product.

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