Comparative survival of Lactobacillus rhamnosus strains in goat cheese matrix to in vitro simulated gastrointestinal conditions

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The survival of probiotic bacteria in the gastrointestinal tract (GIT) is a condition for their beneficial effects in the host. A high probiotic viability in food products at the moment of consumption, however, does not guarantee their survival until the arrival and persistence in the intestine. Nevertheless, the food matrix in which the microorganism is incorporated might exert an important role in probiotic protection. Therefore, the viability of a probiotic strain in each food matrix should be investigated after exposure to GIT conditions, complementing the study of probiotic viability in the product. The present study aimed to evaluate the resistance of a potentially probiotic *Lactobacillus rhamnosus* wild strain (Lr-EM1107) incorporated to a caprine spreadable fresh cheese matrix to in vitro simulated GIT conditions, in comparison with a commercial *L. rhamnosus* Lr-32 probiotic culture incorporated to the same type of cheese.

The cheeses were manufactured using a combined process of lactic acid fermentation and coagulant enzymes for the goat milk coagulation. Two pilot-scale trials were produced from pasteurized goat milk inoculated with *Streptococcus thermophilus*, as starter culture, and Lr-EM1107 or Lr-32, and with the addition of small amounts of a commercial coagulant (1/10 of manufacturer recommended dose). Milk fermentation was conducted at 37 °C for around 7 hours, until coagulation was achieved. Next, syneresis and the beginning of, followed by whey drainage in sterile cotton cheesecloth at 4 °C for 16 hours. The resulting curd was stirred and salted, packed in polypropylene pots and stored at 4 °C for up to 28 days. The protein, fat and dry matter content of cheeses samples were determined, and pH values were monitored fortnightly.

Cheese samples were collected at 14 days of storage for the evaluation of *L. rhamnosus* survival to gastric and enteric simulated conditions. Samples were decimally diluted in a sterile 0.85% (w/v) NaCl solution and, for the gastric phase simulation, the pH was set at 2.5 with 1 M HCl solution, and pepsin and lipase solutions were added to reach final concentrations of 3.0 g/l and 0.9 g/l, respectively. The flasks were incubated at 37 °C for 2 hours under agitation (150 rpm). Subsequently, enteric conditions were simulated in two phases. In the enteric phase 1, the pH was increased up to 5.0 with a sterile alkaline solution, with the addition of bovine bile and pancreatin to reach a concentration of 10 g/l and of 1 g/l, respectively. After two hours of incubation at the same conditions, the pH was adjusted to 7.0, and bile and pancreatin concentrations were adjusted, respectively, to 10 g/l and 1 g/l for the second enteric phase, followed by an additional two hours of incubation. For *L. rhamnosus* counts, samples were collected at the assay baseline (0 h) and after 2, 4, and 6 hours, serially diluted in peptone water solution and 1 mL of each dilution were pour plated on acidified MRS agar, followed by anaerobic incubation at 37 ºC for 48 hours. A survival ratio (SR%) was calculated based on the initial and final populations to estimate the relative resistance of each *L. rhamnosus* strain to the simulated GIT conditions.

The protein, fat, and dry matter content of cheeses with Lr-EM1107 were 10.0, 10.6 and 27.2 g/100g, respectively, and 10.6, 12.7, and 27.4 g/100g for cheeses with Lr-32, respectively. The cheese pH values decreased from around 4.28 to 4.01 during the storage period of 28 days for both cheese trials ($P > 0.05$). At the beginning of the survival assay, the populations of *Lr*-EM1107 and Lr-32 in the goat fresh cheeses were 8.9±0.2 and 7.9±0.1 log CFU/g, respectively. The populations of *Lr*-EM1107 declined around 4.3 log CFU/g after the gastric phase, in average, whereas a decrease of 4.2 log CFU/g was registered for Lr-32 in the same phase. Populations of Lr-EM1107 had a further reduction of 1.5 log CFU/g after the enteric phase 1, followed by a mean increase of 2.3 log CFU/g during the second enteric phase. The same trend was observed for Lr-32; populations counts decreased 1.9 log CFU/g after the first enteric phase, and a recovery of 1.34 log CFU/g was registered after the subsequent period. At the end of the assay, the respective final populations of Lr-EM1107 and Lr-32 were 5.3 and 3.1 log CFU/g. Considering the whole assay, the SR% was 59.6±6.5% for Lr-EM1107 and 38.8±2.2% for Lr-32.

In general, *lactobacilli* is considered resistant to acidic environments, although the survival rates to GIT conditions varies among the *Lactobacillus* species, and some variation at the strain level has been reported. According to these results, the wild *L. rhamnosus* EM1107 achieved a higher comparative survival in the goat cheese matrix, maintaining high populations after the exposure to *in vitro* simulated GIT conditions. The spreadable goat cheese studied showed to be an adequate vehicle for the probiotic candidate strain *L. rhamnosus* EM1107.

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