Millet grains as a support for immobilization of probiotic bacteria in fermented sausage

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Introduction: Immobilization of probiotics in food matrices is an interesting method to maintain viability and functionality of these bacteria during processing and oro-gastrointestinal digestion (OGI). This study assessed the effect of addition of immobilized L. plantarum in millet grains during the production of fermented sausage and OGI.

Materials and methods: Millet grains were sterilized at 130°C for 15min. L. plantarum 343 (LapiAgro PUCPR, Brazil) was grown at 37°C for 72h in MRS broth. Cell immobilization was carried out as described by Bosnea et al. (2009), at 37°C for 72h. Four batches of fermented sausages were prepared: FC (free cell), inoculated with free L. plantarum, NI4% (4% millet + free L. plantarum), I2% (2% immobilized L. plantarum in millet), I4% (4% immobilized L. plantarum in millet). Each batch contained 60% ground pork meat, 20% ground beef, 12%, pork fat, 1.8% salt, 1% sodium isoascorbate, 0.5% glucose, 0.5% sucrose, 0.3% garlic, 0.2% white pepper, 0.02% nutmeg, 0.005% sodium nitrate and 0.015% sodium nitrite. After mixing, commercial starter culture (Staphylococcus xylosus) and the free or immobilized probiotic were added to the batter. The mixture was stuffed into cellulose casing and ripened during 21d under controlled temperature (25 to 18°C) and relative humidity (91 to 80%) conditions. Samples were collected at 0, 2, 7,14 and 21 d. The initial count of L. plantarum was ~109 CFU/g in all treatments.

Lipid oxidation was measured by TBARS (Vyncke (1970) and pH using a digital pHmeter with a penetration probe.

Viability of L. plantarum in sausages was evatuated during OGI according to Liserre et al. (2007) and Gilz-Izquierdo et al. (2003). At the oral stage, fermented sausages (5 g) were homogenized for 2 min in a vortex with 10 mL of water and 3 mL of artificial saliva (α-amylase; pH 6.8); the conditions of the gastric stage were 120 min transit; pepsin, pH 2.0, following small intestine (120 min transit; pancreatin + bile salts, pH 5,6); and large intestine stages (120 min transit; pH 7.0). Samples were collected at the end of each stage(2, 120, 240 and 360 min).

Count of L. plantarum during ripening and OGI was performed on MRS agar incubated at 37°C for 48h.

Results: The addition of millet with immobilized or free cells of L. plantarum did not affect the pH nor the lipid oxidation of fermented sausages. L. plantarum viability increased during ripening and there was no difference among treatments ($P>0.05$), however at 21d, sausages containing immobilized probiotic (I2% and I4%) showed higher count ($P<0.05$). The count of L. plantarum in I2% and I4% at the end of ripening was 10.18 and 10.30 log CFU/g, respectively. During OGI, L. plantarum count decreased on the gastric stage, but increased on the intestinal stages in all treatments. Immobilized L. plantarum cells (I2% and I4%) showed higher viability during OGI than L. plantarum free cells confirming the ability of millet to protect bacterial cells during gastrointestinal transit.

Conclusions: Millet is an efficient food matrix for the immobilization of L. plantarum for addition as probiotic in fermented sausage.

Acknowledgements and Financial support statement: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior–Brasil (CAPES)-Finance Code 001.

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