**TITLE:** SELECTION OF STARTER AND PROBIOTIC CULTURES FOR APPLICATION IN A BLACKBERRY PROBIOTIC FERMENTED MILK SUPPLEMENTED WITH BEER'S INDUSTRY BY-PRODUCT

**AUTHORS:** BATTISTINI, C.; BEDANI, R.; SAAD, S. M. I.

**INSTITUTION:** FACULDADE DE CIÊNCIAS FARMACÊUTICAS, UNIVERSIDADE DE SÃO PAULO (AV. PROF. LINEU PRESTES, 580, CEP 05508-000, SÃO PAULO - SP, BRAZIL). FOOD RESEARCH CENTER, UNIVERSITY OF SÃO PAULO, SÃO PAULO, BRAZIL.

**ABSTRACT:**

Probiotic microorganisms and fibers can beneficially modulate the intestinal microbiota, improving the host's health and consequently reducing the risk for the development of diseases. In the beer production, is generated the brewer's spent grain, which has around 70% of fibers. The addition of brewer's spent grain flour (BSG) in probiotic fermented milk (FM) might enhance the beneficial properties to health of this type of functional food. Thus, this study evaluated the fermentability of BSG by starter and probiotic cultures and selected a suitable strain combination for the application in blackberry probiotic FM supplemented with BSG. Probiotic cultures *Bifidobacterium (B.) animalis* (BB-12), *B. longum* (BB-46 and BB-02), *Lactobacillus (L.) acidophilus* (LA-5), *L. rhamnosus* (GR-1 and LGG), *L. fermentum* (PCC), *L. reuteri* (RC-14), *L. paracasei* (431 and F19), and the starter cultures *Streptococcus thermophilus* (TH-4 and STM-6) were evaluated regarding their ability to ferment the BSG. The fermentability assay consisted of supplementing UHT skimmed milk (MILK) with 1 g·100 $mL^{-1}$ of BSG, followed by inoculation with each strain individually. The counts of each microorganism were determined on selective agar before (0 h) and after 24 h after incubation at 37 °C. The two cultures that showed the highest growth in the presence of BSG were selected for FM production. Four FM formulations with blackberry pulp were assessed: FM1 (control); FM2 (with BSG); FM3 (with probiotic culture); FM4 (with probiotic culture and BSG); the starter culture was added in all formulations. The products were stored at 4 °C for 28 days and the viability of the microorganisms was determined weekly, using selective agar. Regarding fermentability assay, the strains that significantly increased their populations after 24 h of fermentation with BSG were BB-46, PCC, RC-14, F19, and LGG ($p<0.05$). However, TH-4, STM-6, and F19 showed the highest population after 24 h, with counts around 9.1 CFU·$mL^{-1}$. Thus, the co-culture chosen for application in blackberry probiotic FM was composed by F19 and TH-4. In the FM formulations, both TH-4 and F19 remained stable, respectively, above 8.0 and above 8.5 CFU·$mL^{-1}$ over 28 days, and the addition of BSG did not influence their counts. Therefore, all the FM formulations tested have potential as probiotic fermented milks, since the probiotic populations were always above $10^{10}$ CFU in a daily portion of 100 mL of the FM.

**Keywords:** blackberry, brewer's spent grain, fiber, functional food, probiotic

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