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Development of Functional Apple Juice Naturally Enriched with Folates through Fermentation by Probiotic Lactobacilli

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The fermentation of a plant matrix by probiotic microorganisms is a challenge due to initial acidic environment and frequent presence of natural antimicrobials. However, undesirable compounds can be consumed and beneficial elements can be produced through fermentation of a plant food matrix by lactic acid bacteria. We evaluate here the possibility to ferment microfiltrated apple juice (MAJ) by commercial probiotic Lactobacilli strains and explore the modifications of sugars and organic acid profiles and color of the MAJ, as well as the production of folates.

MAJ (var Golden) was fermented by single cultures of *Lactobacillus acidophilus* L10, *L. casei* L26, *L. paracasei* L33, *L. plantarum* 299v and *L. rhamnosus* LGG at 40°C for 48h. Bacteria were inoculated at 7 log CFU/ml into the fruit juice and their growth during fermentation (4, 8, 24, 32, 48h) and viability during shelf-life at 5°C for 28 days were evaluated through the pour plate method on MRS agar medium. CIELab* parameters lightness (L*), redness (a*), and yellowness (b*) were determined using a CR-400 Minolta chromameter and color difference ($\Delta E = ((L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2)^{0.5}$) was calculated. Sugars and organic acids were quantified by colorimetric enzymatic methods adapted to microplate. Folate polyglutamates were deconjugated using chicken pancreatic enzymes prior to HPLC with fluorescence detection (excitation 295 nm/ emission 356 nm). Mono- and diglutamyl folates were quantified against standard curves of 5CH₃-tetrahydrofolate, 10 formyl-tetrahydrofolate and 5 formyl-tetrahydrofolate. Measurements were made in triplicate.

The pH of MAJ decreased from 3.75 to 3.45 on average during fermentation. The strains reached 8.5 -10 log CFU/ml in 48h of fermentation. Strains L10, L26 and LGG maintained counts over 8 log CFU/ml and L299v and L33, over 6 log CFU/ml during 28 days of shelf-life. The consumption/ production of glucose followed different patterns amongst the strains. LGG reduced significantly glucose concentration (from 23 to 20 g/l). All the strains reduced significantly concentrations of saccharose (from 8 to 2 g/l) and fructose (from 213 to 100 g/l). Production of L-lactic acid was highest in juice fermented by LGG. The concentration of formic acid was constant in L33 and L299v. L-malic acid was reduced from 183 to 3 mg/l by all strains. L10, L26 and L299v produced succinic acid. The juice lost color as parameters a* and b*, but not L*, were significantly reduced during fermentation, the highest ΔE being obtained in the juice fermented by LGG. Both L299v and LGG were able to produce folates (10 and 13 µg/l of folic acid equivalents, respectively).

The probiotic strains of Lactobacilli tested can ferment microfiltrated apple juice and maintain cell counts above the minimum limit required for a probiotic product during 4 weeks of shelf-life. Fermentation reduced calories from sugars by almost 50%, notably through fructose reduction, which can be interesting for fructose-intolerant individuals. Production of folates by *L. plantarum* L299v and *L. rhamnosus* LGG in apple juice is reported for the first time, opening new possibilities for the development of naturally folate-enriched fruit juices.