

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

Ana Paula do ESPIRITO SANTO, Frédéric CARLIN, Caroline GARCIA, Catherine M.C. RENARD

INRA-PACA, Université d'Avignon
UMR408 "Sécurité et Qualité des Produits d'Origine Végétale". F-84000 Avignon, France.

E-mail: ana-paula.do-espirito-santo@paca.inra.fr

INTRODUCTION

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.

MATERIAL AND METHODS

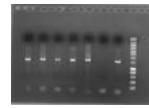


Lactobacillus acidophilus Lahti L10 (Institut Rosell – Lallemand, France)
L. casei Lahti L26 (Institut Rosell – Lallemand, France)
L. paracasei L33 (Christian Hansen, Denmark)
L. plantarum 299v (DSMZ 9843, Probi AB, Sweden)
L. rhamnosus LGG (ATCC 53103, Valio, Finland)

The identity of the bacteria were confirmed through the amplification and sequencing of the region 16S rDNA.



Microfiltrated juice made of apples var Golden



Apple juices were fermented by single bacteria strains inoculated at 7 log CFU/ml 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.

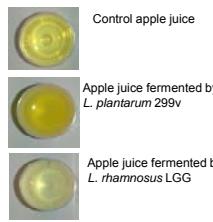
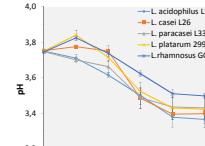
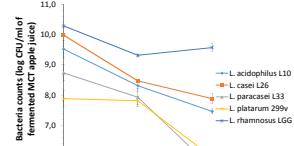
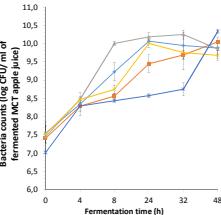
Sugars and organic acids were quantified by colorimetric enzymatic methods (r-Biopharm, Roche, Germany) adapted to microplate.

CIELab* parameters lightness (L*), redness (a*), and yellowness (b*) were determined using a CR-400 Minolta chromameter and color difference was calculated::
 $\Delta E = ((L^*-L^{*0})^2 + (a^*-a^{*0})^2 + (b^*-b^{*0})^2)^{0.5}$.



Folate polyglutamates were deconjugated using chicken pancreatic enzymes prior to HPLC with fluorescence detection (excitation 295 nm/ emission 356 nm). It was added 10µM of pABA to the apple juices and juices without this folate precursor were kept as control. Measurements were made in triplicate.

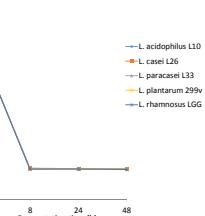
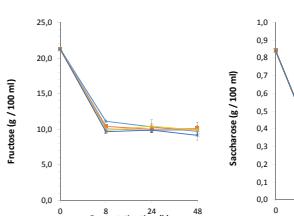
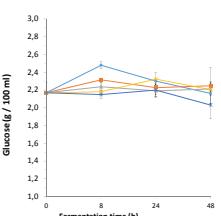
RESULTS



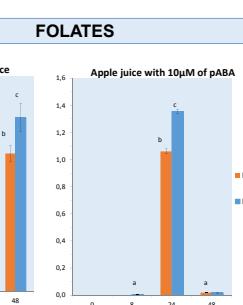
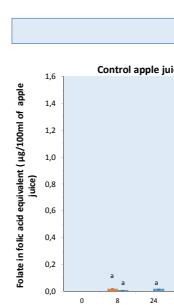
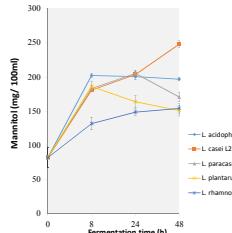
Colorimetric parameters during fermentation at 40°C of MCT apple juice by different probiotic bacteria strains, n = 3.

| Microorganism | Fermentation time (h) | L | a | b | ΔE |
|---------------------------|-----------------------|------------------|-----------------|-----------------|-----------------|
| Control | 0 | 64.26 ± 0.06 a | -5.05 ± 0.17 h | 24.54 ± 0.04 k | 1.04 ± 0.07 a |
| <i>L. acidophilus</i> L10 | 8 | 65.30 ± 0.82 abc | -3.24 ± 0.13 g | 19.07 ± 0.62 ef | 5.91 ± 0.47 def |
| <i>L. acidophilus</i> L10 | 24 | 64.72 ± 0.23 ab | -2.29 ± 0.07 ef | 16.10 ± 1.15 cd | 8.90 ± 1.08 fgh |
| <i>L. acidophilus</i> L10 | 48 | 65.39 ± 0.15 bc | -2.15 ± 0.06 c | 15.08 ± 0.57 cf | 9.97 ± 0.52 ghf |
| <i>L. casei</i> L26 | 8 | 64.88 ± 1.38 abc | -3.43 ± 0.25 ef | 19.92 ± 1.40 gh | 5.10 ± 1.28 cde |
| <i>L. casei</i> L26 | 24 | 65.04 ± 0.45 bc | -2.29 ± 0.05 ef | 16.19 ± 0.23 d | 8.85 ± 0.22 g |
| <i>L. casei</i> L26 | 48 | 64.91 ± 0.63 abc | -2.42 ± 0.46 bc | 16.36 ± 0.83 fg | 8.64 ± 1.00 fg |
| <i>L. paracasei</i> L33 | 8 | 65.37 ± 1.02 abc | -3.22 ± 0.13 ef | 20.00 ± 1.71 gh | 5.11 ± 1.70 cde |
| <i>L. paracasei</i> L33 | 24 | 64.14 ± 0.17 ab | -3.43 ± 0.07 ef | 19.43 ± 0.46 fg | 5.37 ± 0.48 def |
| <i>L. paracasei</i> L33 | 48 | 64.10 ± 0.66 ab | -3.34 ± 0.15 e | 18.50 ± 0.62 fg | 6.31 ± 0.62 def |
| <i>L. plantarum</i> 299v | 8 | 65.54 ± 0.32 bc | -4.00 ± 0.15 g | 22.45 ± 1.90 h | 2.89 ± 1.47 b |
| <i>L. plantarum</i> 299v | 24 | 64.86 ± 0.93 ab | -3.71 ± 0.18 gh | 21.90 ± 1.90 gh | 3.35 ± 1.53 bc |
| <i>L. plantarum</i> 299v | 48 | 64.76 ± 0.43 ab | -2.28 ± 0.35 ef | 15.92 ± 1.70 ef | 9.08 ± 1.79 ghf |
| <i>L. rhamnosus</i> LGG | 8 | 64.56 ± 0.25 ab | -2.09 ± 0.09 ef | 14.70 ± 0.53 c | 10.29 ± 0.50 hi |
| <i>L. rhamnosus</i> LGG | 24 | 65.26 ± 0.28 bc | -1.46 ± 0.09 ab | 12.92 ± 0.25 ef | 12.21 ± 0.23 j |
| <i>L. rhamnosus</i> LGG | 48 | 65.18 ± 0.83 abc | -1.31 ± 0.14 a | 11.58 ± 0.28 ab | 13.54 ± 0.35 k |

Different letters in the same column indicate significant differences between values (p < 0.05).



POLYOL



Conclusions

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. The addition of pABA into apple juices did not increase the folates content but reduced the fermentation time for their production. 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.

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E-mail: paulaes17@hotmail.com