Effect of exogenous nitrogen supplementation on the fermentation of commercial apple juice by *Bifidobacterium* strains

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INTRODUCTION

Most probiotics are dairy-based, but fruit juice offers an alternative for those with milk allergies or lactose intolerance. However, generally, fruit juices lack short peptides and free amino acids needed for probiotic metabolism. This study examined the effect of two nitrogen sources (soy peptone and yeast extract) on fermentation efficiency and the quality of the beverage.

MATERIALS AND METHODS

50 mL commercial unfiltered apple juice

Supplementation

Soy peptone (1% or 2%), or Yeast extract (1% or 2%).

Inoculation

5% inocolum of *Bifidobacterium lactis* BB12 and *Bifidobacterium longum* DSM 16603 separately was added to ferment the apple juice.

Fermentation

32h at 37°C, were carried out in triplicates and samples taken at 0h, 8h, 16h, 24h and 32h.

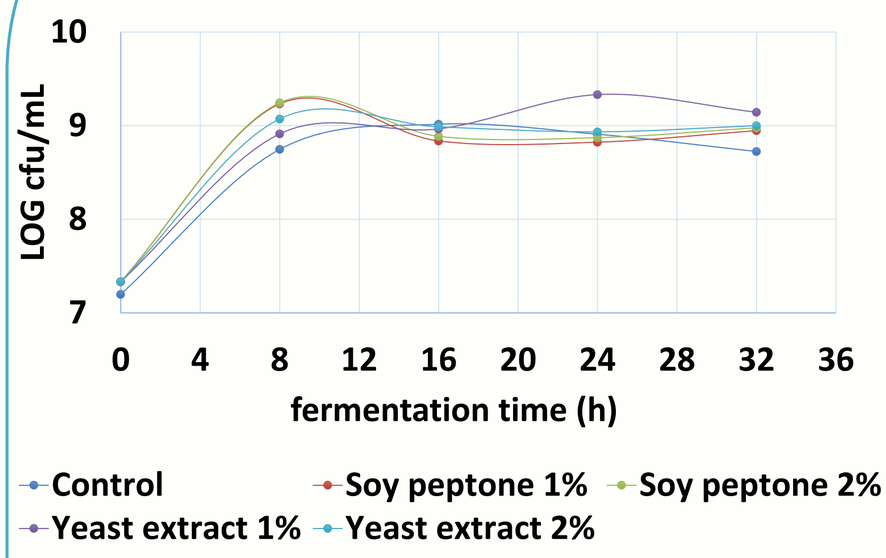
Analytical tests

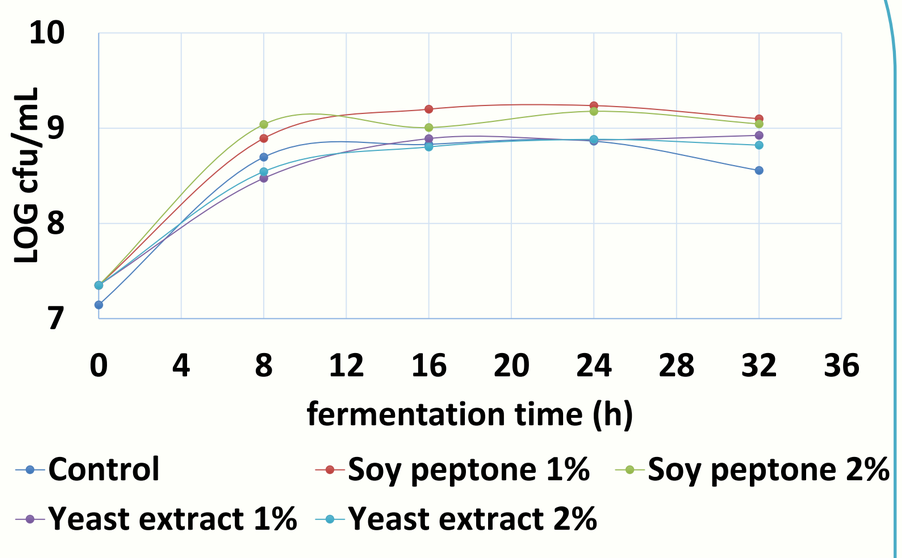
- Growth kinetics
- Sugar consumption and Organic acids production (HPLC).
- Antioxidant capacity and total phenolic content (FRAP and Folin's phenol reagent method)

Results are presented in means ± standard deviation

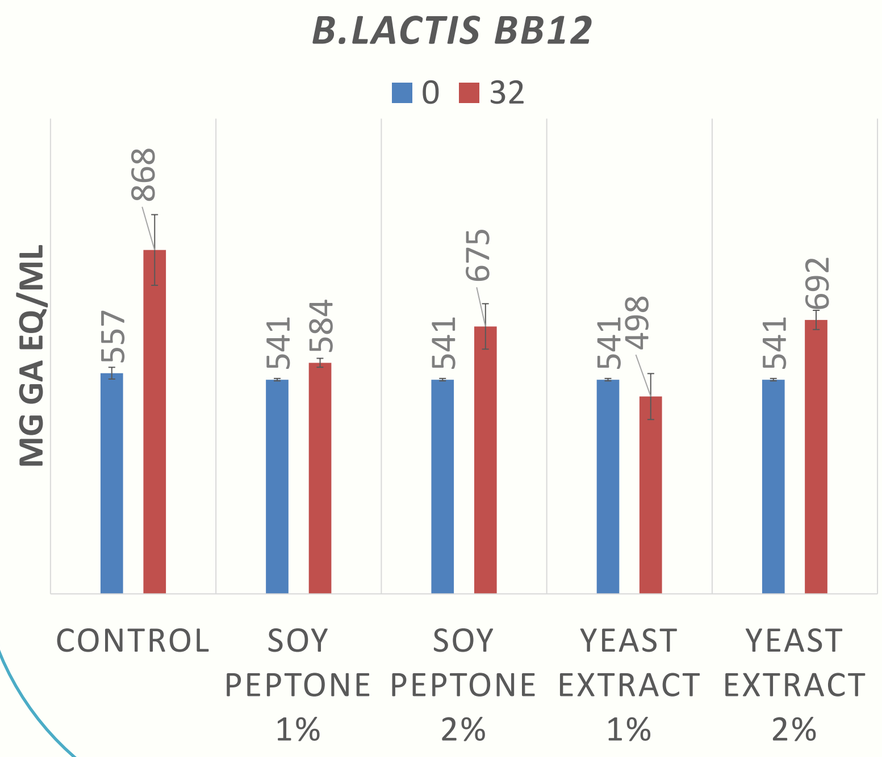
RESULTS AND DISCUSSION

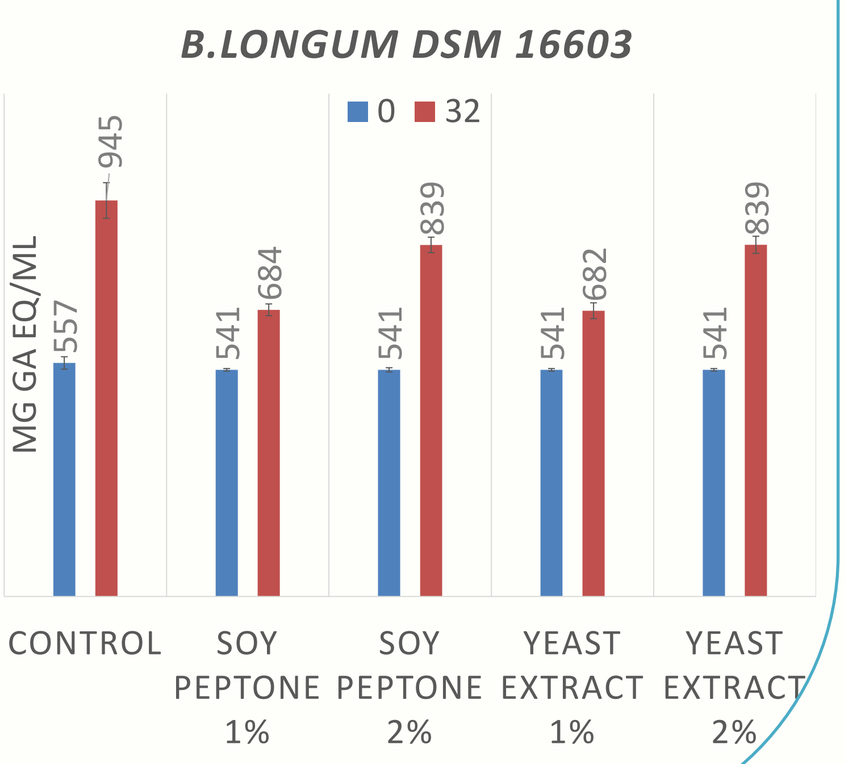
GROWTH OF *BIFIDOBACTERIUM* IN APPLE JUICE





CHANGE IN TOTAL PHENOLIC CONTENT OF FERMENTED APPLE JUICE





Supplementation maintained bacterial growth at ~9 log CFU/mL over 32h, increasing the sugar and organic acids bacterial metabolism. Before the fermentation, fresh apple juice did not contain any acetic acid and a small amount of lactic acid **(0.15g/100ml),** the fermentation increased the organic acids content under all conditions. Acidogenesis was lower when apple juice was not supplemented with exogenous nitrogen sources for Bifidobacteria strains. When nitrogen was added, *B.lactis BB12* produced lactic acid in the range **of 1.7 to 2.4 g/100**ml and **0.15 to 0.24g/100 ml** of acetic acid. Soy peptone source at 2% supplementation level showed the highest organic acids produced for both strains. However lactic acid production was superior in case of *B.longum* DSM 16603 strain under all conditions including non-supplemented juice with **1.54 g/100ml** in contrast, antioxidant and phenolic compounds were higher when apple juice was not supplemented with exogenous nitrogen.

The highest total phenolic content and antioxidant capacity were observed when the beverage was fermented with in *B. longum* DSM 16603 without nitrogen addition (**945 mg GAE/mL, 10.29 mM $FeSO_4$ EQ/mL, respectively**). These findings aligned with previous scientific studies demonstrate that nutrient supplementation including nitrogen can have a possible positive effect on organic acid production of probiotic bacteria. (Nancib et *al.*2005,. Liu *et al.*,2010., Precup *et al.* 2022., Mantzourani *et al.*2025).

CONCLUSION

Apple juice serves as a suitable matrix for the fermentation of *Bifidobacterium* strains supporting high cell viability without nutrient supplementation. Exogenous nitrogen addition, especially with soy peptone, ameliorated bacterial growth kinetics, sugar and organic acids metabolism, while highlighting strain-specific metabolic adaptations. *B. longum* DSM 16603 showed better performance in lactic acid production and yield. Curiously, the highest increases in antioxidant activity and total phenolic content were noticed in control samples, indicating that exogenous nitrogen may sometimes inhibit or shift metabolic pathways from antioxidant compound production.

Acknowledgement

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