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Short communication

Probiotic/synbiotic enriched ayran as functional food product – quality and therapeutic benefits

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Introduction

The demand for functional foods that promote health beyond basic nutrition increases along with the increased consumer awareness for the influence of diet on their health. In respect to probiotic foods both the viability of the probiotic within foods and bioavailability within the host has to be studied (Figueroa-González et al., 2011), while at least maintaining the quality of conventional product used as a carrier. Microencapsulation may offer good protection of probiotic viability loss during manufacture, product storage and after ingestion of probiotics, especially during exposure to unfavorable GI conditions (Sánchez et al., 2012).

The aim of the study was to obtain a functional product ayran with therapeutic level of viable and metabolic active cells of L. casei 01 (a minimum of 107 log CFU per mL ayran). Quality of the prepared probiotic and synbiotic samples was examined as well as their therapeutic benefits in animal model with chemically induced colitis.

Materials and methods

Ayran samples were prepared by fortifying commercially available product (Ayran, Zdravje, Macedonia) with non-encapsulated probiotic L. casei 01 (Chr. Hansen, Denmark) and/or prebiotic oligofructose-enriched inulin (Synergy 1, Orafti-Rue L. Maréchal, Belgium) and encapsulated synbiotic. Synbiotic microparticles were produced when an overnight activated culture in MRS broth (37 °C, 24 h) with a cell load ca. 11-12 log CFU/mL in a mixture with 4% w/w alginate (Protanal LF 10/60 LS, fG 35-45%, FMC BioPolymer, IMCD, UK) and prebiotic (1.5% w/w) was infused to the spray-dryer (Büchi Mini Spray Dryer B-290, SW). Spray-drying was performed at inlet and outlet temperature of 120 °C and 58±3 °C, respectively, flow rate 6 ml/min, nozzle diameter 0.7 mm, aspirator pressure 90% and atomizer pressure 600 Nlh-1. Polyelectrolyte complexation/cross-linking of the dried powders was done under continuous stirring for at least 3 h with 0.5% w/w chitosan (Chitine, France) and 5% w/w CaCl2 (Merck, Germany) dissolved in 1% v/v acetic acid. The hardened microparticles were freeze-dried (-50 °C, 0.070 mbar, 24 h) (FreeZone Freeze Dry System, Labconco, USA) (Petreska Ivanovska et al., 2014). Analyses of the quality of ayran samples include determination of protein, fat and carbohydrate content of the ayran by Bradford, Gerber and phenol-sulphuric acid methods, respectively, while the total solids were determined using gravimetric method. Titration acidity (lactic acid,%) and pH measurements were also evaluated. AAS and HPLC was applied to quantify minerals and organic acid production, respectively. Then, to female Wistar rats (n=6, 180-250 g, 10-14 weeks old), functional ayran samples (8.5-8.9 log CFU/mL of the food product) were administered orally, once and twice daily. Plain ayran and drinking water were given to positive controls. Two weeks after starting the experiment, the rats were fasted overnight and those from the positive controls and treated groups were rendered colitic. Colitis was induced intrarectally at 8 cm proximal to the anus using TNBS (trinitrobenzenesulfonic acid) dissolved in 50% ethanol at a dose 10 and 30 mg/kg. After 6 days of continued treatment the

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rats were sacrificed. Negative controls treated with 0.9% NaCl were also included in the protocol. The anti-inflammatory effects of functional ayran samples were evaluated in respect to the clinical activity/total damage score (quantified by loss on weight, consistency of feces and rectal bleeding), macroscopic and histopathological changes, colon weight/colon length and colon weight/body weight ratios, and myeloperoxidase (MPO) activity.

Results and discussion

At the day of preparation, probiotic counts of 9.43, 9.7 and 9.58 log CFU/mL were determined in ayran enriched with non-encapsulated probiotic and synbiotic, and encapsulated synbiotic, respectively. Viability loss during the shelf-life of the product was significant with viable level above the therapeutic minimum in ayran containing encapsulated synbiotic of 8.22 log CFU/mL. Total solids in the ayran samples fortified with probiotic, synbiotic and synbiotic microparticles were moderately increased due to added dry matters with no negative effect on the texture or sensory properties of the product. Significant differences of acidity and pH values were not detected between the samples, which showed no excess of acidity when probiotic was added. The content of proteins and fats in ayran samples was in accordance with the required levels (2.8 and 1 g per 100 mL of product, respectively) during the shelf-life of 15 days at 4 °C. Analyses of carbohydrates have shown increased content in samples containing non-encapsulated and encapsulated synbiotic due to the carbohydrate nature of the prebiotic, with the latter to be more significant. No significant difference for sodium content in prepared samples were observed compared to the plain ayran, while potassium and calcium content differed significantly among the samples with decreasing trend probably due to the ability of probiotic bacteria to use these minerals as nutrient sources. Exception was observed with the increased calcium content in the sample containing synbiotic microparticles which were prepared using calcium as a cross-linking agent. Increased metabolic activity of the probiotic was observed in ayran containing encapsulated synbiotic due to the higher production of lactic and acetic acid up to 81.83 mmol/L and 79.93 mmol/L, respectively. Low quantity of propionic acid (12.15-15.03 mmol/L) was determined in functional ayran samples only, but no butyric acid was detected.

In rats with induced colitis using 10 mg/kg TNBS, most significant reduction of parameters of inflammation was found in group treated by ayran containing encapsulated synbiotic. Microscopic assessment have shown ulcerations on mucosa and sub-mucosa, accompanied by extensive inflammatory infiltrate and congested blood vessels, in groups receiving plain ayran or drinking water. In the groups treated with ayran containing non-encapsulated probiotic/synbiotic visible segments of ulcerations and subepithelial polymorph nuclear infiltration were found, while higher integrity of mucosal architecture of colon tissue was seen in group treated with ayran enriched by encapsulated synbiotic. Hence, when colitis was induced using 30 mg/kg TNBS, sample containing encapsulated synbiotic was administered. The lowest value of MPO activity was observed when ayran with microparticulated synbiotic was given twice daily, but parameters of inflammation were not significantly different between groups administered once and twice daily. Dilated blood vessels in submucosal layer as well dilated intestinal glands were observed in the rats treated by ayran with microencapsulated synbiotic, regardless of the frequency of administration. These findings indicate that a single administration of ayran enriched by microencapsulated formulation successfully protect the viability of the probiotic through the upper GIT and ensure prolonged residence time of viable cells in the lower intestinum with no necessity to increase the frequency of usage.

Conclusion

Functional samples with maintained quality of the product ayran within its shelf-life were developed, while the ayran containing synbiotic microparticles has the advantage of increased probiotic viability and better profile of the bacterial metabolism end products known to maintain morphologic and functional integrity of colonic epithelium. Ayran enriched by microencapsulated synbiotic administered once a day provided efficient anti-inflammatory activity due to adhesive properties of the microparticles and their favorable interaction with the ayran as medium, thus showing potential to be used as adjuvant therapy in IBD.

References

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