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ORIGINAL ARTICLE

Development of a functional synbiotic beverage fortified with different cereal sprouts and prebiotics

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Abstract The study was devoted to developing a novel synbiotic beverage based on with millet, rye and alfalfa sprouts with a mixed culture of *Lactobacillus casei* and *Lactobacillus plantarum*. In this regard, the influences of incorporated prebiotics inulin and oligofructose on probiotics viability during the refrigerated storage (4 ± 1 °C, 28 days) as well as under the simulated gastric condition were investigated. The characteristics such as microbial viability, physicochemical properties (viscosity, pH, titrable acidity and radical scavenging activity) and sensorial evaluation were assessed. The synbiotic beverage produced contained 10^8 CFU ml^{-1} for *L. casei*, with a good survival throughout the storage period (10^8 CFU ml^{-1}) and *L. plantarum* at sufficient levels (10^6 CFU ml^{-1}) after about 21 days. Inulin and oligofructose promoted the growth of the strains and their viability under cold storage while conferring higher sensory scores. In this context, the beverages demonstrated acceptable sensory attributes. The viability (bacterial survival) of over 55% for all the strains was achieved under simulated gastric condition. Therefore, the introduced fermented beverage was a good food matrix from the viability of probiotics as well as under the gastric condition and sensory characteristics

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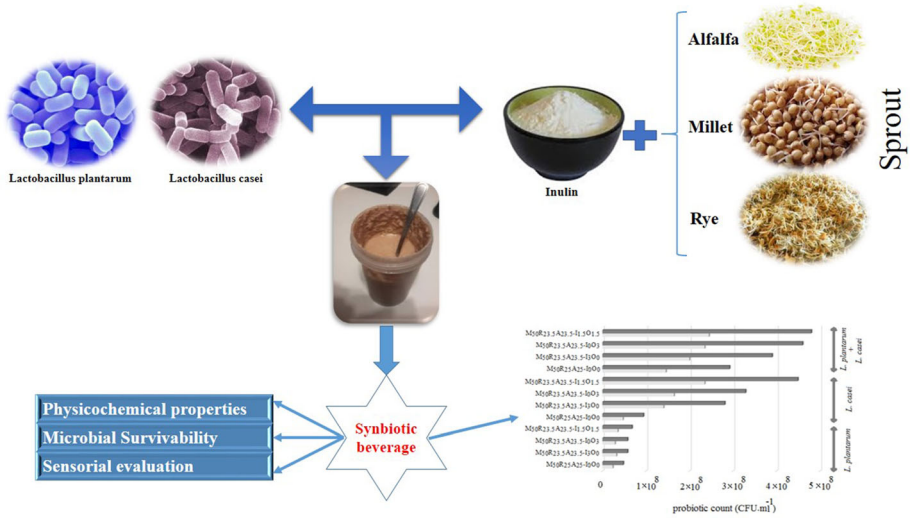
Graphic abstract



Keywords Millet · Prebiotic · Probiotic · Rye · Sprouts · Symbiotic · Healthy food

Introduction

Recently, functional foods are occupying an important place among both academic and industrial trends as they improve the quality of dietary intake by providing the required nutritional components for the human organism (Jafari et al. 2017). On the other hand, beverages are one of the most accessible food which could provide nutrients such as vitamins, mineral substances, antioxidants, organic acids and other active biological substances for the body (Mohammadi and Mortazavian 2011). In this regard, there are huge demands for the development of new non-dairy based probiotic beverages to the functional food market by using controlled fermentation by probiotics and incorporation of prebiotics.

Probiotics are live microorganisms which, upon consumption in sufficient quantities, provide health benefits higher than the intake of the inherent nutrition to the host, by improving the microbial balance of the intestine (Jafari et al. 2017). Lactic acid bacteria (LAB) mainly lactobacilli and bifidobacteria as the most widely used strains of probiotic can survive in the intestine system. LAB improve the host immune system and show anti-cancer and anti-tumor activity. Also, they play an important roles in colonization resistance in the intestinal, respiratory, and urogenital tracts, cholesterol metabolism, lactose metabolism, absorption of calcium, and synthesis of vitamins (Fuller 2012).

The term "prebiotic" is defined as non-digestible food that selectively stimulates the growth and/or activity of one or a few bacteria in the intestine and further improvements in the health of the host (Gibson and Roberfroid 1995). Among the prebiotics, inulin and oligofructose have shown convincing evidence of health-promoting effects (Afshari et al. 2015; Sarteshnizi et al. 2017). Moreover, by combining probiotics and prebiotics (synbiotic), synergistic benefits may be observed (Fernandez and Marette 2017).

Sprouted cereals have higher enzymatic activity, total protein, sugars, crude fat and fiber, vitamins and minerals (Lorenz and D'Appolonia 2009), total phenolic, flavonol, tannin, phytic acid, tocopherol and antioxidant activity and lower total dry matter and starch compared to unsprouted cereals (Kaur et al. 2017).

Sprouts are rich in digestible energy, bioavailable vitamins, minerals, amino acids, proteins, and phytochemicals, are necessary for a germinating of the plants. However, the nutritional content of sprouted seeds can be varied due to differences in species, they contain remarkable amounts protein, fiber, vitamin C, B vitamins, and small amounts of calcium, iron, magnesium, phosphorus, potassium, and zinc (El-Adawy 2002). Alfalfa also called lucerne and called *Medicago sativa* is a perennial flowering plant in the legume family Fabaceae, has been used traditionally as a liver protectant, antioxidant agent and also for the treatment of bleeding and digestive problems (Cornara et al. 2016). Their sprouts contain high amounts of vitamins A, and C (Hong et al. 2010).

Probiotic fermentation of bran such as rye not only aid to the proliferation of targeted microorganism in higher values but also improve the healthy composition. In this regard, the improvements in bioactivity as well as technical characteristics of rye bran was investigated by (Lamsal and Faubion 2009). Moreover, according to Morah 2017, the incorporation of rye sprouting improved the nutritional

Table 1 The formulation characteristics of treatments

Probiotic	Treatment symbol	Millet sprout (% m/m)	Rye sprout (% m/m)	Alfalfa sprout (% m/m)	Inulin (% m/m)	Oligofructose (% m/m)
L. plantarum	$M_{50}R_{25}A_{25}$–I_0O_0	50	25	25	0	0
	$M_{50}R_{23.5}A_{23.5}$–I_3O_0	50	23.5	23.5	3	0
	$M_{50}R_{23.5}A_{23.5}$–I_0O_3	50	23.5	23.5	0	3
	$M_{50}R_{23.5}A_{23.5}$–$I_{1.5}O_{1.5}$	50	23.5	23.5	1.5	1.5
L. casei	$M_{50}R_{25}A_{25}$–I_0O_0	50	25	25	0	0
	$M_{50}R_{23.5}A_{23.5}$–I_3O_0	50	23.5	23.5	3	0
	$M_{50}R_{23.5}A_{23.5}$–I_0O_3	50	23.5	23.5	0	3
	$M_{50}R_{23.5}A_{23.5}$–$I_{1.5}O_{1.5}$	50	23.5	23.5	1.5	1.5
L. plantarum + *L. casei*	$M_{50}R_{25}A_{25}$–I_0O_0	50	25	25	0	0
	$M_{50}R_{23.5}A_{23.5}$–I_3O_0	50	23.5	23.5	3	0
	$M_{50}R_{23.5}A_{23.5}$–I_0O_3	50	23.5	23.5	0	3
	$M_{50}R_{23.5}A_{23.5}$–$I_{1.5}O_{1.5}$	50	23.5	23.5	1.5	1.5
Without probiotic	$M_{50}R_{25}A_{25}$–I_0O_0	50	25	25	0	0
	$M_{50}R_{23.5}A_{23.5}$–I_3O_0	50	23.5	23.5	3	0
	$M_{50}R_{23.5}A_{23.5}$–I_0O_3	50	23.5	23.5	0	3
	$M_{50}R_{23.5}A_{23.5}$–$I_{1.5}O_{1.5}$	50	23.5	23.5	1.5	1.5

value (i.e., folate content by 1.7–3.8 fold) of millet and makes it safer for human consumption. According to researchers sprouting increases its (Morah 2017).

Therefore, the current investigation was aimed to produce a novel cereal sprout-based synbiotic beverage fermented by a mixed culture of *Lactobacillus casei* and *Lactobacillus plantarum*, with appropriate quality attributes.

Materials and methods

Chemical reagents and microbial strains

The inulin, oligofructose, MRS agar culture medium, MacConkey agar, potato dextrose agar sodium chloride and hydrochloric acid were supplied by Merck (Darmstadt, Germany). *L. plantarum* and *L. caesi* were obtained from the Iranian Research Organization for Science and Technology, Iran with an initial viable cell count of 10^8 CFU ml^{-1}

Preparation of cereal sprouts

Two hundred g of each cereal, including alfalfa, millet, and rye (provided from the local market, Tehran, Iran) were soaked in 1000 mg of sodium hypochlorite (Merck, Germany) for 30 min. The seeds were then washed with distilled water to reach the neutral pH. The seeds were placed in germination machine at 20 °C with a relative humidity of 99% for 5 days (Fernandez-Orozco et al. 2006).

Preparation of synbiotic beverage

The sprouts were shredded and compressed before the green leaf emerged and after pasteurization were kept in a cool place until incorporation in the proposed formulations. The various proportions of obtained sprouts' juices were

inoculated with *L. plantarum* and *L. caesi* and supplemented with inulin or oligofructose according to Table 1. The mixture of suspension was incubated at 30 °C until reaching pH 4.8. The fermented beverages were kept at 4 ± 1 °C and used for analysis per 7-day intervals till day 28.

Microbiological analysis

Viable counts of probiotics in beverages were measured using serial dilution technique and MRS agar culture medium (Tharmaraj and Shah 2003). Plates were incubated in anaerobiosis (37 °C, 72 h) using AnaeroPack system. Coliform and total yeast-mold counts were checked using MacConkey agar (37 °C, 48 h) and potato dextrose agar (25 °C, 72 h), respectively.

Determination of pH and titrable acidity

The pH was determined using a pH-meter (Model 691; Metrohm, Herisau, Switzerland) equipped to a food penetration probe (AOAC 2010). Also, the titrable acidity was determined by the titration method (10 g beverage with 90 ml distilled water) using a 0.1 N NaOH (Merck, Germany) solution (Coda et al. 2011).

Sensory evaluation

Sensory evaluation was performed using a descriptive test for overall quality (flavor, odor, color, and overall acceptability) of samples using a panel of 12 trained panelists using a hedonic scale (5 and 1 points showing like extremely and dislike extremely) (Stone 2012).

Probiotic survival under the simulated gastrointestinal condition In order to simulate the gastrointestinal condition, 3.2 g of pepsin (Sigma-Aldrich, USA) was mixed with 0.5% sodium chloride solution and 0.1 M hydrochloric acid to reach the pH 1.5 ± 0.02. The acidic solution was sterilized for 15 min at 121 °C (Jain et al. 2007).

To assess the survival of probiotics under acidic conditions, 0.5 g of each sample was incubated in a sterile flask containing 4.5 ml of acidic solution at 37 °C for 2 h; then centrifugation was performed at 11,952 g for 10 min. 0.5 ml of the supernatant solution after serial dilution was used for surface cultivation on MRS agar medium (Semyonov et al. 2012).

Statistical analysis

All experiments were performed in triplicate were carried out and the results presented as a mean of the three values

Table 2 pH and titrable acidity in treatments during refrigerated storage

Probiotic	Treatment symbol	pH					Titrable acidity (%)				
		0	7	14	21	28	0	7	14	21	28*
L. plantarum	$M_{50}R_{25}A_{25}–I_0O_0$**	4.40[a]***	3.62[a]	3.32 [c]	3.20[c]	3.92[c]	0.46 [d]	0.43[d]	0.61[f]	0.66[f]	0.66[f]
	$M_{50}R_{23.5}A_{23.5}–I_3O_0$	4.12[a]	3.34[a]	3.34[c]	3.32[c]	3.31[c]	0.36[d]	0.45[d]	0.62[f]	0.63[f]	0.63[f]
	$M_{50}R_{23.5}A_{23.5}–I_0O_3$	4.07[a]	3.47[a]	3.32[c]	3.30[c]	3.40[c]	0.32[d]	0.44[d]	0.66[f]	0.62[f]	0.63[f]
	$M_{50}R_{23.5}A_{23.5}–I_{1.5}O_{1.5}$	4.06[a]	3.57[a]	3.33[c]	3.33[c]	3.21[c]	0.36[d]	0.45[d]	0.61[f]	0.63[f]	0.63[f]
L. casei	$M_{50}R_{25}A_{25}–I_0O_0$	4.09[a]	4.79[a]	4.72[a]	4.62[b]	3.23[c]	0.36[d]	0.45[d]	0.63[f]	0.64[f]	0.63[f]
	$M_{50}R_{23.5}A_{23.5}–I_3O_0$	4.13[a]	4.96[a]	4.59[a]	4.33[b]	3.09[c]	0.36[d]	0.43[d]	0.64[f]	0.66[f]	0.66[f]
	$M_{50}R_{23.5}A_{23.5}–I_0O_3$	4.08[a]	4.59[a]	4.50[a]	4.21[b]	3.18[c]	0.36[d]	0.44[d]	0.65[f]	0.63[f]	0.63[f]
	$M_{50}R_{23.5}A_{23.5}–I_{1.5}O_{1.5}$	4.00[a]	3.86[a]	3.96[a]	3.68[b]	3.11[c]	0.39[d]	0.48[d]	0.68[f]	0.69[f]	0.69[f]
L. plantarum + *L. casei*	$M_{50}R_{25}A_{25}–I_0O_0$	4.13[a]	4.77[a]	4.74[b]	4.65[b]	4.47[b]	0.36[d]	0.42[d]	0.64[f]	0.61[f]	0.61[f]
	$M_{50}R_{23.5}A_{23.5}–I_3O_0$	4.94[a]	3.57[a]	3.59[b]	3.33[b]	4.81[b]	0.36[d]	0.44[d]	0.63[f]	0.64[f]	0.64[f]
	$M_{50}R_{23.5}A_{23.5}–I_0O_3$	4.22[a]	4.12[a]	4.27[b]	4.02[b]	4.98[b]	0.36[d]	0.45[d]	0.67[f]	0.62[f]	0.62[f]
	$M_{50}R_{23.5}A_{23.5}–I_{1.5}O_{1.5}$	4.19[a]	4.46[a]	3.56[b]	3.42[b]	4.18[b]	0.36[d]	0.40[d]	0.62[f]	0.61[f]	0.61[f]
Without probiotic	$M_{50}R_{25}A_{25}–I_0O_0$	6.91[b]	6.62[b]	5.66[b]	5.56[b]	5.15 [ab]	0.06 [e]	0.08[e]	0.09[e]	0.09[e]	0.09[e]
	$M_{50}R_{23.5}A_{23.5}–I_3O_0$	6.07[b]	5.51[b]	5.54[b]	5.36[b]	5.25[ab]	0.06[e]	0.07[e]	0.07[e]	0.08[e]	0.08[e]
	$M_{50}R_{23.5}A_{23.5}–I_0O_3$	5.94[b]	5.87[b]	5.77[b]	5.62[b]	5.29[ab]	0.06[e]	0.05[e]	0.06[e]	0.06[e]	0.06[e]
	$M_{50}R_{23.5}A_{23.5}–I_{1.5}O_{1.5}$	6.22[b]	6.05[b]	5.69[b]	5.58[b]	5.32[ab]	0.06[e]	0.05[e]	0.06[e]	0.06[e]	0.06[e]

*(4 °C, 28 day)

**Letters 'M, R, A, I and O' represents millet, rye, alfalfa, inulin and oligofructose, respectively

***Means in a column shown with different English letters are significantly different ($p < 0.5$)

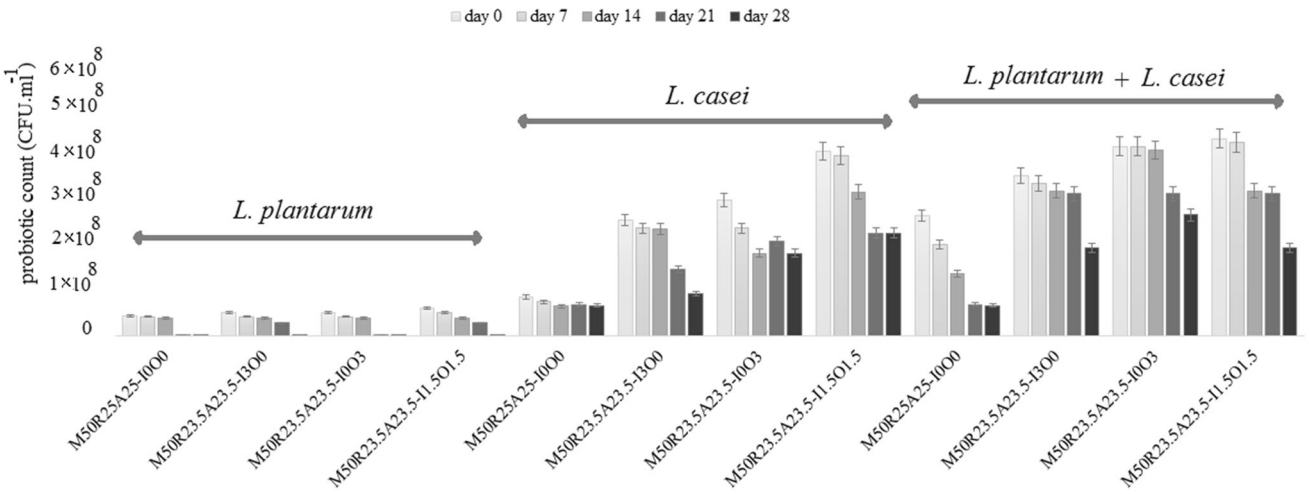


Fig. 1 Viability of probiotic bacteria in beverages during storage (letters 'M, R, A, I and O' represents millet, rye, alfalfa, inulin and oligofructose, respectively)

with the standard deviation. Analysis of variance procedure followed by Duncan's test using SPSS 13 (SPSS Inc., Chicago, IL, USA) software was applied to determine the significant differences ($P < 0.05$) among treatment means.

Results and discussion

pH and titrable acidity

Fermentation in samples although became limited but slowly continued by storing them under refrigerated conditions (4 ± 1 °C). Nighswonger et al. (1996) revealed that there was a slight fermentative activity by the probiotic even at 4 °C (Nighswonger et al. 1996). Changes in pH and titrable acidity in different treatments during the refrigerated period were demonstrated in Table 2. By increasing in the storage time up to 28 days, there is a significant decrease in pH among all the treatments except those not inoculated with probiotics. The highest reduction in pH and acidity augmentation was noted in samples inoculated with *L. casei* combined with inulin and oligofructose. The results showed that this combination increased the sensory acceptability of this functional synbiotic beverage. In general, fillers have a positive effect on reducing the unpleasant sensation of probiotic fermented beverages.

Similar findings were reported by Garro et al. (1998), who observed a more pronounced drop in pH and an increase in acidity in a synbiotic beverage fermented with *L. casei* (Garro et al. 1998). On the other hand, inulin and oligofructose stimulated the metabolic activity of probiotics which led to further increments in the acidity and decrement in pH (Cardarelli et al. 2008). The production of acidic metabolites by carbohydrates fermentation by the bacteria led to a decrease in pH and an increase in acidity.

In fermented beverages, the pH plays an important role in the microbiological durability of the product against foodborne pathogens. Generally, pH < 3.8 makes a rough condition for pathogens (Jayamanne and Adams 2009).

The viability of probiotics during storage

In Fig. 1, the survivability of probiotics during storage for 28 days in a refrigerated condition was demonstrated. *L. casei* survived well throughout the storage with the corresponded values for population ranged from 7.39×10^7 (d28) to 4.49×10^8 CFU ml^{-1} (d0). However, the population of *L. plantarum* was reported as 6.77×10^7 (d0) CFU ml^{-1} before storage and decreased during the storage to 2.32×10^6 (d28). The reduction of viability of *L. casei* and *L. plantarum* during refrigerated storage were in good agreement by the findings of Jayamanne and Adams (2009) reporting the reduction in viable cells of *Bifidobacterium lactis* in products over cold storage (Jayamanne and Adams 2009). The loss of viability of probiotic organisms can be associated with decreasing in the pH of the medium and accumulation of organic acid as a result of growth and fermentation of bacteria (Mohammadi et al. 2017; Ebrahimi et al. 2018). Addition of inulin and oligofructose had significant similar ($P > 0.05$) effect on *L. casei* and *L. plantarum* viability in synbiotic beverages. The stimulating effect of probiotics in the product was in accordance with previous studies (Khanniri et al. 2018; Heydari et al. 2018).

The prebiotic inulin and oligofructose stimulated the growth and viability of *L. casei* and *L. plantarum*, in the product, therefore, can promote intestinal health (De Souza

Table 3 Sensory assessment of treatments during the storage

Probiotic	Treatment symbol	Taste				Odor				Color				Overall acceptability			
		0	7	14	21	0	7	14	21	0	7	14	21	0	7	14	21*
L. plantarum	$M_{50}R_{25}A_{25}$–I_0O_0**	2.01^{Aa}***	3.12^{Bb}	2.00^{Aa}	3.20^{Bb}	2.98^{Aa}	2.99^{Aa}	3.00^{Bb}	3.01^{Bb}	3.53^{Bb}	3.97^{Bb}	3.66^{Bb}	3.65^{Bb}	4.56^{Cc}	4.58^{Cc}	4.25^{Cc}	4.32^{Cc}
	$M_{50}R_{23.5}A_{23.5}$–I_3O_0	2.65^{Aa}	2.41^{Aa}	2.25^{Aa}	4.62^{Cc}	3.42^{Bb}	3.33^{Bb}	3.25^{Bb}	3.00^{Bb}	3.50^{Bb}	3.60^{Bb}	3.54^{Bb}	3.69^{Bb}	4.50^{Cc}	5.00^{Dd}	4.00^{Cc}	4.00^{Cc}
	$M_{50}R_{23.5}A_{23.5}$–I_0O_3	2.00^{Aa}	2.74^{Aa}	2.32^{Aa}	4.36^{Cc}	3.30^{Bb}	3.21^{Bb}	3.35^{Bb}	3.36^{Bb}	3.05^{Bb}	3.50^{Bb}	3.05^{Bb}	3.98^{Bb}	3.50^{Bb}	5.00^{Dd}	4.00^{Cc}	4.00^{Cc}
	$M_{50}R_{23.5}A_{23.5}$–$I_{1.5}O_{1.5}$	2.52^{Aa}	3.45^{Bb}	3.36^{Bb}	5.00^{Dd}	3.37^{Bb}	3.32^{Bb}	3.23^{Bb}	3.00^{Bb}	3.62^{Bb}	3.53^{Bb}	3.28^{Bb}	3.67^{Bb}	5.00^{Dd}	5.23^{Dd}	4.33^{Cc}	4.20^{Cc}
L. casei	$M_{50}R_{25}A_{25}$–I_0O_0	2.05^{Aa}	2.00^{Aa}	2.02^{Aa}	4.01^{Cc}	2.00^{Aa}	2.12^{Aa}	2.03^{Aa}	2.30^{Aa}	3.85^{Bb}	3.98^{Bb}	3.31^{Bb}	3.68^{Bb}	3.32^{Bb}	4.25^{Cc}	3.31^{Bb}	4.30^{Cc}
	$M_{50}R_{23.5}A_{23.5}$–I_3O_0	2.12^{Aa}	2.15^{Aa}	2.33^{Aa}	4.00^{Cc}	3.11^{Bb}	3.31^{Bb}	3.12^{Bb}	3.22^{Bb}	3.54^{Bb}	3.58^{Bb}	3.50^{Bb}	3.51^{Bb}	4.30^{Cc}	5.32^{Dd}	4.00^{Cc}	4.31^{Cc}
	$M_{50}R_{23.5}A_{23.5}$–I_0O_3	2.42^{Aa}	2.12^{Aa}	2.36^{Aa}	4.30^{Cc}	3.64^{Bb}	3.35^{Bb}	3.32^{Bb}	3.34^{Bb}	3.44^{Bb}	3.91^{Bb}	3.95^{Bb}	3.99^{Bb}	4.28^{Cc}	4.00^{Cc}	4.08^{Cc}	5.00^{Dd}
	$M_{50}R_{23.5}A_{23.5}$–$I_{1.5}O_{1.5}$	2.86^{Aa}	3.39^{Bb}	3.48^{Bb}	5.00^{Dd}	3.76^{Bb}	3.62^{Bb}	3.64^{Bb}	3.48^{Bb}	3.52^{Bb}	3.56^{Bb}	3.77^{Bb}	3.58^{Bb}	4.42^{Cc}	4.00^{Cc}	4.55^{Cc}	4.42^{Cc}
L. plantarum + *L. casei*	$M_{50}R_{25}A_{25}$–I_0O_0	2.02^{Aa}	2.00^{Aa}	2.30^{Aa}	3.22^{Bb}	2.30^{Aa}	2.41^{Aa}	2.12^{Aa}	2.33^{Aa}	3.50^{Bb}	3.59^{Bb}	3.68^{Bb}	3.32^{Bb}	4.68^{Cc}	4.54^{Cc}	4.51^{Cc}	4.52^{Cc}
	$M_{50}R_{23.5}A_{23.5}$–I_3O_0	2.21^{Aa}	2.03^{Aa}	2.25^{Aa}	4.30^{Cc}	3.37^{Bb}	3.31^{Bb}	3.21^{Bb}	3.00^{Bb}	3.53^{Bb}	3.95^{Bb}	3.58^{Bb}	3.94^{Bb}	4.30^{Cc}	4.21^{Cc}	4.20^{Cc}	4.22^{Cc}
	$M_{50}R_{23.5}A_{23.5}$–I_0O_3	2.35^{Aa}	2.36^{Aa}	2.21^{Aa}	4.23^{Cc}	3.69^{Bb}	3.65^{Bb}	3.41^{Bb}	3.47^{Bb}	3.58^{Bb}	3.69^{Bb}	3.98^{Bb}	3.50^{Bb}	4.36^{Cc}	4.00^{Cc}	4.12^{Cc}	4.84^{Cc}
	$M_{50}R_{23.5}A_{23.5}$–$I_{1.5}O_{1.5}$	3.62^{Bb}	3.67^{Bb}	3.35^{Bb}	5.00^{Dd}	3.56^{Bb}	3.48^{Bb}	3.14^{Bb}	3.27^{Bb}	3.65^{Bb}	3.53^{Bb}	3.35^{Bb}	3.58^{Bb}	4.68^{Cc}	4.89^{Cc}	4.64^{Cc}	4.57^{Cc}
Without probiotic	$M_{50}R_{25}A_{25}$–I_0O_0	2.00^{Aa}	2.01^{Aa}	–	–	3.00^{Bb}	3.21^{Bb}	3.00^{Bb}	3.12^{Bb}	3.98^{Bb}	3.55^{Bb}	3.85^{Bb}	3.69^{Bb}	–	–	–	–
	$M_{50}R_{23.5}A_{23.5}$–I_3O_0	2.21^{Aa}	2.28^{Aa}	–	–	3.54^{Bb}	3.90^{Bb}	3.34^{Bb}	3.65^{Bb}	4.02^{Cc}	3.94^{Bb}	3.71^{Bb}	3.98^{Bb}	–	–	–	–
	$M_{50}R_{23.5}A_{23.5}$–I_0O_3	2.50^{Aa}	2.34^{Aa}	–	–	3.51^{Bb}	3.90^{Bb}	3.33^{Bb}	3.98^{Bb}	3.85^{Bb}	3.50^{Bb}	3.59^{Bb}	3.65^{Bb}	–	–	–	–
	$M_{50}R_{23.5}A_{23.5}$–$I_{1.5}O_{1.5}$	2.52^{Aa}	2.27^{Aa}	–	–	3.21^{Bb}	3.67^{Bb}	3.36^{Bb}	3.85^{Bb}	3.65^{Bb}	3.99^{Bb}	3.55^{Bb}	3.38^{Bb}	–	–	–	–

*(4 °C, 21 day)

**Letters 'M, R, A, I and O' represents millet, rye, alfalfa, inulin and oligofructose, respectively

***Means in a column or row shown respectively with different small or capital English letters, are significantly ($p < 0.05$) different

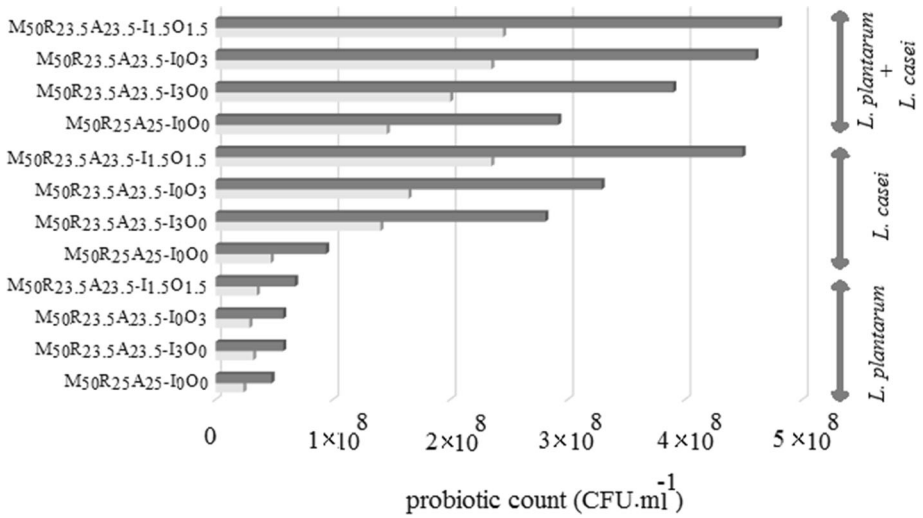


Fig. 2 Probiotic cell survival under simulated gastric condition (letters 'M, R, A, I and O' represents millet, rye, alfalfa, inulin and oligofructose, respectively)

Oliveira et al. 2011). Treatments containing the mixture of the strains showed significantly higher viable cells for each strain compared to those with single strains which can be associated with the symbiotic effect of probiotic bacteria and the production of required compounds and vitamins B in the environment in the presence of each other (Hong et al. 2010). Investigated the mixture of probiotics in formulating a probiotic yogurt and found the synergistic effects of the mixed strains on their growth and viability. Noori et al. (2017) declared that rye sprout extract could enhance the growth and survival of probiotic bacteria and showed prebiotic activity, while sprout extracts used in this study did not significantly affect the growth of probiotic bacteria (Noori et al. 2017). Generally speaking, the designed matrix in this study met the requirement of being a probiotic food from a viability standpoint.

Sensory evaluation

The sensory evaluation scores for the 16 treatments during 21 days of storage were presented in Table 3. The samples with no added inoculation showed evidence of mold and yeast contamination. Hence, they were not presented for the panelists. Panelists did not declare a significant sensorial change in beverages during 21 days of storage. However, the inulin and oligofructose besides inoculation with *L. casei* and *L. plantarum* significantly increased the acceptability of beverage as higher proportions of inulin and oligofructose in formulations resulted in the higher acceptance. The positive effect of fillers in recovering the grittiness sense of the fermented beverage with probiotics was previously documented (Rouhi et al. 2015). Color is one of the main factors affecting beverage early acceptance or rejection for consumers, which influences the purchase and the general consumption of products (Tárrega and Costell 2007). According to results, no significant differences ($P < 0.05$) in the accepted values of formulations were observed, and the light color of the beverages was satisfactory in the point of panelists. The odor did not please the panelist, probably because of the acidic smell of formulations.

According to the sensorial analysis, although slight acidification of the beverage was detected by panelists, the beverage was acceptable for 21 days at 4 °C. All the beverages introduced in this study were in their natural form, without any additives or flavorings. The use of flavorings would considerably improve the sensorial characteristics of beverages.

Probiotic survival under the simulated gastric condition

The resistance between the strains was noted as significant, while *L. plantarum* showed better resistance (Fig. 2) while based on the previous investigations, the highest resistance to simulated gastric condition was nominated for *L. plantarum* strains (Khalil et al. 2007; Pan et al. 2009; Kirtzalidou et al. 2011). It should be noted that the combination of strains had no significant effect on increasing the chance of survival of bacteria. The presence of oligofructose or inulin preserved the two strains in the simulated gastric condition. In this regard, Nazzaro et al. also declared inulin protected *L. acidophilus* against gastrointestinal juices (Nazzaro et al. 2012).

Probiotic cell survival in the digestive tract is the most important factor for selecting probiotic strains and for their efficiency (FAO/WHO 2006). However, various mechanisms are involved in surviving the bacteria under the gastric environment. The two bacteria evaluated in this study had suffered significantly under gastric conditions, although the number of viable cells was still more than 55%. High bacterial survival under simulated gastric

condition increases the possibility of the viability of these cultures in the digestive tract.

Conclusion

For the first time, a synbiotic cereal sprout-based beverage was developed to evaluate the combined benefits of the probiotics *L. casei* and *L. plantarum*, and the prebiotic property of inulin and oligofructose besides nutritional profits of millet, rye and alfalfa sprouts. *L. casei* survived well ($>10^7$ CFU ml^{-1}) throughout the refrigerated storage (4 °C, 28 days), while *L. plantarum* only survived at sufficient levels until day 21. Inulin and oligofructose positively affected the viability and sensory properties of beverage throughout the storage. Evaluation of bacterial survival under simulated gastric condition declared viability of over 55% of the strains. Moreover. The final product had acceptable sensory attributes. Therefore, the developed functional fermented beverage could be deemed as a standard healthy ready-to-drink product from functional, sensory and economical points of view. Further investigations might focus on finalizing and optimizing the sensory characteristics of the mentioned product using adequate flavorings and stabilizers.

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Compliance with ethical standards

Conflict of interest The authors declare no potential conflicts of interest concerning the research, authorship, and publication of this article.

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