Food Bioscience 19 (2017) 121–127

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Investigation of potential prebiotic activity of rye sprout extract

Negin Noori[a], Hassan Hamedi[b,*], Mina Kargozari[c], Peyman Mahasti Shotorbani[d]

[a] Department of Food Hygiene, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[b] Department of Food Safety and Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran
[c] Department of Food Science, Tehran-North Branch, Islamic Azad University, Tehran, Iran
[d] Department of Food Quality Control and Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran

ARTICLE INFO

Keywords:
Rye sprout extract
Prebiotic
Lactobacillus acidophilus
Bifidobacterium animalis
Antimicrobial activities
Synbiotic yogurt

ABSTRACT

Rye (*Secale cereale*) is one of a number of cereal species that grow wild in the northwest of Iran, Turkey, and Central Asian countries. It is revealed that sprouts possess higher vitamins, antioxidant, flavonoids and fiber than grains. For evaluation of the potential prebiotic activity of rye sprout extract (RSE), the grains were soaked in water, germinated and dried by a freeze-dryer. The in-vitro viability and relative growth ratio (RGR) of probiotic bacteria, as well as their antimicrobial activities and their retention in synbiotic yogurt during 56 days cold storage in presence of RSE, were investigated. The sensory properties (odor, color, texture, taste and overall acceptance) of symbiotic yogurt with different amounts of RSE in comparison with control group were evaluated. The results showed that adding various concentrations of RSE increased the viability of both *Lactobacillus acidophilus* and *Bifidobacterium animalis* ($p < 0.05$), although this increase in *B. animalis* was better than L. *acidophilus*. Comparison of RGR of treated samples indicated that adding RSE even 0.25% can increase the growth and survival of probiotic in comparison with control. The results also showed that adding of RSE promoted the antimicrobial activities of probiotics. In synbiotic yogurt, probiotic populations remained higher than the minimum recommended therapeutic dose for an extended period of cold storage. There was no significant difference between sensory parameters of control specimens and synbiotic yogurt. According to our results, the synbiotic effect of RSE and probiotics highlights the potential application of RSE as a prebiotic in dairy and other functional products.

1. Introduction

The term “functional food” refers to a normal type of food with an additional ingredient that provides a health benefit beyond satisfying traditional nutrient requirements (Gonzalez, Adhikari, & Sancho-Madriz, 2011). Nowadays, functional foods are an exciting trend in food industries and nutrition field (Bigliardi & Galati, 2013). Probiotics and prebiotics are used as functional components in dairy products in order to obtain maximal health benefits. The term probiotic is defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host (Hill et al., 2014) and prebiotic is specified as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of the gastrointestinal microflora, and thus improves the host health (Gibson, 2004; Roberfroid, 2007). Probiotic bacteria cannot thrive well in digestive tract without prebiotics (Cruz et al., 2010). The combination of probiotics with prebiotics has a synergistic effect on the host health (Krumbeck, Maldonado-Gomez, Ramer-Tait, & Hutkins, 2016). It has been suggested that adding non-digestible food ingredients known as prebiotics to certain foods may increase the viability of bacteria passing through the gastrointestinal tract and thus exert a beneficial effect on human health (Iyer & Kailasapathy, 2005; Khalf, Dabour, & Fliss, 2010; Mookiah, Sieo, Ramasamy, Abdullah, & Ho, 2014). Prebiotic activities of some cereal ingredients such as inulin, fructooligosaccharides (FOS) and galactooligosaccharides (GOS) have been confirmed (Bhatia et al., 2015; Fernandes, do Rosario, Mocellin, Kuntz, & Trindade, 2016).

Rye (*Secale cereale*) is one of a number of cereal species that grow wild in the northwest of Iran, Turkey and Central Asian countries (Zeder, 2008). Rye is widely used to make crisp bread, and it has high amount of gliadin and low content of glutenin. It also contains a higher proportion of soluble fiber, which may be useful in controlling energy intake and reducing the risk for development of obesity (Astrup & Brand-Miller, 2012; Pietzak, 2012). Rye plant has a rather high content of natural antioxidants compared to wheat and other cereals. It is revealed that rye sprout possesses higher vitamins, antioxidant, flavonoids and fiber than its grain (Goncharenko & Timoshchenko, 2014).

Additionally, yogurt is the most popular dairy fermented product and traditional food for a lot of consumers in the world due to its

* Corresponding author.
E-mail address: hamedih@ut.ac.ir (H. Hamedi).

http://dx.doi.org/10.1016/j.fbio.2017.07.001
Received 29 March 2017; Received in revised form 2 July 2017; Accepted 3 July 2017
Available online 04 July 2017

therapeutic, nutritional, and sensory properties (Yildiz, 2016).

According to mentioned issues and nutritional value of rye as well as the possibility of use of RSE as prebiotic in fermented products and food supplementation, the main goal of the present study was to investigate the potential prebiotic activity of rye sprout extract including the in-vitro viability and relative growth ratio (RGR) of probiotic bacteria, as well as their antimicrobial activities, sensory characteristics and their retention in synbiotic yogurt during cold storage at 4 °C.

2. Materials and methods

2.1. Microorganisms and chemicals

Yogurt starter culture containing *Lactobacillus delbreukii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophiles* were purchased from Chr. Hansen Company (Horsholm, Denmark). Lyophilized strains of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium animalis* subsp. *lactis*, strain BB-12 were obtained from the microbial collection of Department of Food Hygiene, University of Tehran, Iran.

All chemicals used were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Preparation of water extract of rye sprout

Rye (*Secale cereale*) was supplied from Jolfa City (northwest of Iran) and identified by Iranian Institute of Medical plants. Grains of rye were soaked in water for 72 h and after six days the sprouts will have grown to 5–8 cm. Fresh rye sprouts were dried by a freeze-dryer (Operon Co, FDO-8606, Gyeonggi-do, South Korea). The dried germinated grains were powdered using a grinder soaked in distilled water, shaken for 48 h and then filtered. This procedure was repeated again by distilled water and the pooled filtrate was freeze-dried again for 48 h. The dried extract was stored at 4 °C until use.

2.3. In vitro prebiotic activity of rye sprout extract

The necessary inoculum of each probiotic was prepared by inoculating from the lyophilized culture in de man-rogosa-sharpe (MRS) broth which was incubated for 18 h at 37 °C. Second subcultures were prepared in the same condition. The bacterial suspension was adjusted to optical density of 0.1 at 600 nm, using a Spectronic 20 spectrophotometer (Milton Roy Company, Rochester, NY) and enumerated by duplicate plating from 10-fold serial dilutions on MRS agar and counting the colonies after 24 h incubation at 37 °C. Working cultures were adjusted to the required concentration of 10^6 CFU ml^{-1}. To evaluate the prebiotic activity of rye sprout extract (RSE), the media with different concentrations of RSE (0%, 0.25%, 0.5%, 0.75%, and 1%, w/v) were prepared and inoculated with approximately 10^6 CFU ml^{-1} of activated L. *acidophilus* LA-5 and *B. animalis* BB12. The modified media that contain glucose was used for incubation of probiotic inoculum at 37 °C for 48 h. The enumeration of LA-5 and BB12 was carried out on MRS and MRS-C (0.05% L-cysteine, w/w) agar (Merck, Darmstadt, Germany) at different incubation times (0, 12, 24 and 48 h) aerobically and anaerobically, respectively.

2.4. Relative growth ratio (RGR)

For assessment of the in-vitro prebiotic activity of RSE samples, the relative growth ratio of the probiotic LA-5 and BB-12 were calculated using the expression:

$$RGR = \left(\frac{P_t - P_0}{G_t - G_0}\right)_p$$

where *p* subscript indicates probiotic bacteria (LA-5 and BB-12), *P* represent the growth rates of probiotics with different concentration of RSE (0.25%, 0.5%, 0.75%, and 1%, w/v) in comparison with *G* as a basal carbon source of the medium (glucose; 0.5% w/v), at different times of incubation (t = 2, 4, 8, 12 and 24 h) compared to zero time ($t = 0$), as indicated by the corresponding subscript. An RGR value greater than unity indicates that the tested RSE exerts a growth stimulant effect on LA-5 and BB-12, in comparison to *G* as a simple carbon source (Rubel, Pérez, Genovese, & Manrique, 2014).

2.5. Antibacterial activities of probiotics incorporated with RSE

Antibacterial activities of the LA-5 and BB-12 culture (ca.10^8 CFU ml^{-1}) grown in mMRS (modified MRS supplemented with 0.5% glucose) broth media containing different concentration of RSE (0.5% and 1%, w/v) was determined and compared with that grown in mMRS without RSE (James, 2014). The test organisms used were *E. coli* O157: H7, *Salmonella* Typhimurium phagetype II, *Listeria monocytogenes* ATCC 19118 and *Staphylococcus aureus* ATCC 6538.

2.6. Production of synbiotic yogurt

The pre-culture of probiotics (LA-5 and BB-12) were prepared by dissolving 100 mg of freeze-dried culture in 50-ml of sterilized skim milk (121 °C for 20 min). After blending and activation at 42 °C for 30 min, 1 ml of the pre-culture was inoculated into 250 ml of skim milk. Enumerations of these pre-cultures ranged from 7.1 to 7.4 Log CFU ml^{-1}. The yogurt starter cultures (L. *bulgaricus* and *S. thermophilus*) were prepared according to the recommendations received from Chr. Hansen procedure. To prepare the synbiotic yogurt, the pre-culture of probiotics and yogurt starter cultures were used to inoculate 3 L of reconstituted skim milk that had been heat-treated at 95 °C for 5 min, and finally RSE in different concentrations (0%, 0.25%, 0.5%, 0.75% and 1%, w/v) was added and cooled to fermentation temperature (42 °C). The incubation was then carried out and when pH reached 4.5 at the end of the fermentation period, the samples were cooled and kept at 4 °C until the probiotic bacteria were counted.

2.7. Evaluation of prebiotic activity of rye sprout extract in yogurt

Enumeration of L. *acidophilus* was performed by the standard enumeration techniques using ten-fold serial diluting prepared in 0.1% v/v buffered peptone water at pH 7.0. MRS-Maltose agar (MRS enriched with 0.2% Tween 80 and supplemented with 1% maltose, 0.05% cysteine, and 1.5% agar) was specifically used for the differential enumeration of L. *acidophilus* (Tabasco, Paarup, Janer, Peláez, & Requena, 2007). The enumeration of *B. animalis* was carried out by the method of Roy (2001) using a selective MRS medium supplemented with neomycin, paromomycin, nalidixic acid and lithium chloride. Duplicate plates were incubated anaerobically using a GasPak system (Merck, Darmstadt, Germany) at 37 °C for 72 h.

2.8. Sensory assessment

A panel of 10 trained panelists was selected among the staff of the University of Tehran on the basis of their experience in the sensory analysis. In each analysis, different amounts of RSE (0.25%, 0.5%, 0.75% and 1%, w/v) were added to the yogurts compared with the control group. Next, the samples were assessed using an acceptability analysis (color, odor, flavor, texture and overall acceptability) on a 9-point hedonic scale that included 9, like extremely; 8, like very much; 7, like moderately; 6, like slightly; 5, neither like nor dislike; 4, dislike slightly; 3, dislike moderately; 2, dislike very much; 1, dislike extremely (Hamedi, Razavi-Rohani, & Gandomi, 2014)

2.9. Statistical analysis

The experimental data of bacterial counts and sensory assessment