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Using physical approaches for the attenuation of lactic acid bacteria in an organic rice beverage

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ABSTRACT

A wild strain of *Lactobacillus plantarum*, isolated from an Italian sourdough, was inoculated in an organic rice drink; however, it caused a strong acidification. Thus, it was preliminary processed through homogenization (single or multiple passes) or sonication (US) and then inoculated in the beverage. The samples were stored at 4 °C and analyzed to assess pH, production of lactic acid, viable count and sensory scores. A US-2-step process (power, 80%) could control acidification; viability and sensory traits were never affected by sonication. This result was confirmed on two commercial probiotics (*Lactobacillus casei* LC01 and *Bifidobacterium animalis* subsp. *lactis* Bb12).

In the 2nd step samples inoculated with attenuated strains were also stored under thermal abuse conditions (25 or 37 °C for 4 or 24 h, then at 4 °C) and the results showed that US could control acidification for a short thermal abuse. Finally, US-attenuated starter cultures were inoculated in the rice drink containing β-glucans as healthy compounds; the targets did not cause any significant change of prebiotic.

1. Introduction

Nowadays consumers are more health conscious towards food quality and safety. Traditionally dairy fermented products have been considered as the best carrier for probiotics; however, allergies or cholesterol diseases may limit the use of milk-based products. Therefore, several raw materials, including cereals and cereal-based beverages, have been extensively investigated to determine if they are suitable substrates to produce novel non-dairy functional foods (Rivera-Espinoza and Gallardo-Navarro, 2010). Thus, cereals have been also investigated as fermentable substrates for the growth of probiotic microorganisms (Nyanzi and Jooste, 2012).

Cereals have also a huge potential as vehicles for functional compounds such as antioxidants, dietary fiber, minerals, prebiotics, and vitamins (Nionelli et al., 2014). Examples of commercial products are Proviva® (Skane Dairy, Sweden), an oat-based food beverage, containing *Lactobacillus plantarum* 299v (Prado et al., 2008), and Whole Grain Probiotic Liquid® (Grainfields, Australia), inoculated with lactic acid bacteria (*Lactobacillus acidophilus*, *Lactobacillus delbrueckii*) and yeasts (*Saccharomyces cerevisiae* var. *boulardii* and *S. cerevisiae*), and supplemented with vitamins, amino acids, and enzymes (Soccol et al., 2012).

Many traditional cereal foods contain microorganisms in a living form (biza, tarhana, shalgam, fura, brembali, burukutu, etc.) (Blandino et al., 2003); therefore, the use of probiotic bacteria/functional yeasts for these products or for other traditional cereal foods could be a suitable way for their valorization. Some examples of novel functional beverages are whey-based prickly pear (Baccouche et al., 2013) and grape-based beverages (Di Cagno et al., 2010), cereal-based probiotic drinks (Rathore et al., 2012), fruit-beverages (Gad et al., 2013), and some organic beverages (Awe et al., 2013).

A main drawback of probiotics in food could be their active metabolism, e.g. some strains of lactic acid bacteria continue to produce lactic acid and cause the so-called post-acidification (the decrease of pH within the storage). Therefore many times it is important to control the metabolism of probiotic and starter cultures in foods, without affecting their viability and functional properties (Bandiera et al., 2013; Ferdousi et al., 2013). This goal could be achieved by attenuation. Some authors studied homogenization as a way to attenuate/modulate starter cultures in dairy

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products (Lanciotti et al., 2004, 2006, 2007). The topic of this paper is the evaluation of homogenization and ultrasound (US) as tools for the attenuation of three strains of lactic acid bacteria (a wild isolate of *L. plantarum*, isolated from an Italian sourdough, and two commercial probiotics, *Lactobacillus casei* LC01 and *Bifidobacterium animalis* BB12), intended for an organic rice drink.

Apart from the addition of probiotics, the functionality of a food could be improved by prebiotics and by their combination with probiotic cultures. In this paper we focused on β-glucans, due to their well-known "function claims" and "claims connected with the reduction of disease" (European Commission, 2007); European Commission reported two different claims for β-glucans:

1. β-glucans contribute to the maintenance of normal blood cholesterol levels. This claim can be used only for a food containing at least 1 g of β-glucans from oats, oat bran, barley, barley bran, or from mixtures of these sources. Consumers should be informed that the health benefit relies upon a daily intake of 3 g of β-glucans.
2. The consumption of β-glucans from oats or barley as part of a meal reduce significantly the impact of the post-prandial hyperglycemia. This claim can be used only for a food containing at least 4 g of β-glucans from oats or barley for each 30 g of available carbohydrates. Consumers should be informed that the beneficial effect relies upon the consumption of glucans from oats or barley (EFSA, 2011).

The main topic of this paper is the attenuation of 3 strains of lactic acid bacteria (*L. plantarum*, *L. casei*, and *B. animalis*) inoculated in an organic rice drink, through homogenization and ultrasound by focusing on cell viability, acidification, and lactic acid. Some other additional topics were the effects of attenuation and microbial inoculation on the sensory scores of rice drink and the effects on β-glucans supplemented as prebiotic compounds to the beverage.

2. Materials and methods

2.1. Strains and inocula preparation

The following microorganisms were used throughout this study: i) *L. plantarum* 12 isolated from sourdough and belonging to the Culture Collection of the Laboratory of Predictive Microbiology (Dept. of the Science of Agriculture, Food and Environment-University of Foggia) (Corbo et al., 2014); ii) *L. casei* LC01 and *B. animalis* subsp. *lactis* BB12 purchased from Chr. Hansen (Hørsholm, Denmark).

Lactic acid bacteria were stored at −20 °C in MRS broth (Oxoid, Milan, Italy), containing 33% of sterile glycerol (J. T. Baker, Milan); before each assay the strains were grown under the optimal conditions. LAB cultures were centrifuged at 4000 rpm/4 °C for 10 min; then, the strains were washed with sterile tap water and inoculated into a commercial rice drink. The initial cell count of LAB was 9 log cfu/ml.

2.2. Rice drink

A commercial organic rice-drink gluten and dairy free and with no added sugar (water; Italian organic rice, 20%; organic sunflower seed oil cold-pressed, 1.2%; sea salt, 0.1%; pH, 6.8–6.9; soluble solids, 14.1°Bx) was purchased from a local market in Foggia.

2.3. HPH-treatment

An aliquot of rice drink (1 l) inoculated with *L. plantarum* was processed in a pressure range of 50–100 MPa for 1 time or for 2–3 consecutive times through a high-pressure homogenizer PANDA 2K (Niro Soavi s.p.a., Parma, Italy). The circuits were cleaned with sterile distilled water (60–70 °C) and cooled with cold water to obtain an exit temperature of the samples of 40 °C. Untreated aliquots of rice drink, inoculated with the targets, were used as controls.

HPH-treated rice drink was used as a mother culture to inoculate fresh samples of rice drink (300 μl in 30 ml); thereafter the samples were stored at 4 °C or 30 °C and analyzed periodically to assess the viable count, pH, and lactic acid. The analyses were repeated two times and for each time two independent batches were analyzed (n = 4); for each sample the analyses were performed with two technical replicates.

2.4. Ultrasound-treatment on L. plantarum

Rice drink inoculated with *L. plantarum* (30 ml) was processed for 4 min (pulse set to 2 s) through a VC Vibra Cell Ultrasound (US) equipment, model VC 130 (Sonics and Materials Inc., Newtown, CT, USA); the power was set to 60–100% (single treatment) or to 80% (2 or 3 consecutive treatments). Before each treatment, the ultrasonic probe was washed with sterile distilled water; immediately after processing, beverage was cooled in ice. US-treated rice drink was used to inoculate fresh samples of rice drink, as reported above; the samples were stored at 4 °C and analyzed by assessing cell count, pH, and the content of lactic acid and ethanol. The analyses were repeated two times and for each time two independent batches were analyzed (n = 4); for each sample the analyses were performed with two technical replicates.

2.5. Confirmation of US-attenuation on L. casei and B. animalis

Aliquots of rice drink (30 ml) inoculated with either *L. casei* LC01 and *B. animalis* BB12 (ca. 9 log cfu/ml) were US-treated for 2 times (power, 80%; time, 4 min; pulse, 4 s). These aliquots were used as mother cultures to inoculate fresh samples of rice drink, as reported above. The samples were stored at 4 °C for 11 days and analyzed to assess cell count, pH, lactic acid, and sensory scores. The analyses were repeated two times and for each time two independent batches were analyzed (n = 4); the analyses for each sample were performed with two technical replicates.

2.6. Effect of a thermal abuse on US-attenuation

Aliquots of rice drink inoculated with *L. casei* (9 log cfu/ml) were US-treated for 2 times (power, 80%; time, 4 min; pulse, 4 s) and used to inoculate fresh samples of rice drink, as reported above. Thereafter, the samples were stored as follows:

1. 4 °C
2. 4 h at 25 °C and then at 4 °C
3. 4 h at 37 °C and then at 4 °C
4. 24 h at 25 °C and then at 4 °C
5. 24 h at 37 °C and then at 4 °C.

The samples were periodically analyzed (pH, viable count, lactic acid); the assays were performed with n = 4, as reported above.

2.7. β-Glucan supplemented rice drink inoculated with attenuated probiotics

US-attenuated *L. casei* and *L. plantarum* (2 cycles-power, 80%; time, 4 min; pulse 4 s) were inoculated in rice drink supplemented with 1% of β-D-glucans from barley (Sigma–Aldrich, Milan, Italy; product code G6513-5G, CAS 9041-22-9); the following samples