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Enhanced probiotic viability and aromatic profile of yogurts produced using wheat bran (*Triticum aestivum*) as cell immobilization carrier

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ABSTRACT

The effect of wheat bran (*Triticum aestivum*) as a cell immobilization carrier for probiotic yogurt production on cell viability, composition of volatile compounds and sensory characteristics, was studied. Wheat bran was delignified and separately used as a carrier for the immobilization of *Lactobacillus casei* ATCC393 and *Lactobacillus bulgaricus* DSM20081. Both biocatalysts were freeze-dried without the addition of cryoprotectants and were used for yogurt fermentation at 40 °C. Their operational stability was evaluated during successive yogurt fermentation batches until they were inactivated. The yogurts fermented using the immobilized biocatalysts were compared with those fermented with free *Lactobacillus* cells and with conventional yogurt culture (*Streptococcus thermophilus* and *L. bulgaricus*). The novel yogurts showed significantly higher cell viabilities during storage at 4 °C. In addition, the immobilized biocatalysts showed higher survival rates during exposure to simulated gastric juice (pH 3.0). The immobilized biocatalysts significantly affected the production of volatile compounds, indicating, in combination with the sensory evaluations, potential for good-quality probiotic yogurt production by freeze-dried ready-to-use immobilized starters.

1. Introduction

Currently, there is an increase in interest for developing functional food products containing probiotic microorganisms combined with prebiotic ingredients. Such combinations demonstrate a great potential in promoting human health and can easily be provided to the consumer by incorporation into dairy products such as yogurt [1–3]. Yogurt is a fermented dairy product consumed worldwide. It has a high nutritional value with well-established health benefits, especially when reinforced with prebiotic ingredients and probiotic bacteria [1–4]. Traditionally, yoghurt is produced from milk by the synergistic action of lactic acid bacteria (LAB) such as *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* [5]. The addition of beneficial probiotic bacteria (bifidobacteria and lactobacilli) to yogurt presents a challenge, basically because of their interaction with other microbial species present in yogurt and sensitivity to yogurt constituents, processing, and storage conditions (pH, temperature, lactic acid concentration, oxygen, micronutrients, etc.), which might lead to important losses in cell viability [4]. To deliver the health benefits to the consumer, it is utmost important to maintain viable probiotic cells in a product until consumption, at the minimum level usually ranging from 10^6 to 10^9 cfu mL^{-1} [5], to survive the acidic conditions of the upper gastrointestinal (GI) tract and proliferate in the intestine.

To enhance probiotic cell viability, several methods have been proposed, such as use of an appropriate administration matrix and microencapsulation techniques [4,6,7], cell immobilization [8–10], addition of prebiotics [9], and use of mixed starters [11]. Furthermore, there is an increasing trend for application of synbiotics (the combined use of probiotics and prebiotics) in functional food products. Prebiotics can increase the survival rates and stability of probiotics during processing and storage, especially when the cells are used in an immobilized state (with the prebiotic as the immobilization carrier) [8,12].

Cereal dietary fibers can exert several beneficial physiological effects because of their content of specific non-digestible carbohydrates and can act as prebiotics promoting the growth and survival of probiotic species [1–3,8,9,12]. Wheat bran, an edible material containing dietary fiber, protein, inorganic elements, fat, and antioxidant components [8,12,13], was used in the present study as a prebiotic immobilization carrier for probiotic yogurt cultures. Specifically, the objective of the present study was to develop and

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evaluate freeze-dried, ready-to-use biocatalysts by immobilization of *Lactobacillus casei* ATCC 393 and *Lactobacillus delbrueckii subsp. bulgaricus* on wheat bran for probiotic yogurt production.

2. Materials and methods

2.1. Starter cultures and immobilized biocatalysts preparation

The probiotic strain *L. casei* ATCC 393 (*L. casei* henceforth) and the strain *L. delbrueckii subsp. bulgaricus* DSM 20081 (*L. bulgaricus*), isolated from Bulgarian yogurt (DSMZ, Braunschweig, Germany), were grown at 37 °C and 45 °C, respectively, for 48 h on de Man, Rogosa, and Sharpe (MRS) broth (Fluka, Buchs, Switzerland). *S. thermophilus* was isolated from commercial freeze-dried yogurt culture (FD-DVS CH-1 – Yo-Flex, Chr. Hansen, Horsholm, Denmark) and was grown at 45 °C in MRS medium for three generations before use. All cultures were harvested by centrifugation at 5000 rpm for 10 min at 25 °C (Sigma Laborzentrifugen GmbH, Germany).

Wheat bran (*Triticum aestivum* L.) was supplied by the company Kepenos Flour Mills S.A. (Patras, Greece). It consisted of approximately 20% protein, 7% ash, 5% lipids, and 50% dietary fiber of which 30% was arabinoxylan and 18% was starch; this composition was comparable with those of other bran types reported in the literature [14,15]. Wheat bran was delignified by alkali treatment [16] to increase the porosity of the biocatalyst by lignin removal. The delignified wheat bran (DWB) was dried overnight at 50 °C until complete moisture removal and was divided in equal amounts of 5 g. *L. casei* and *L. bulgaricus* cells were separately immobilized on DWB. Immobilization was performed by mixing 1 g of harvested wet cell mass with 5 g of DWB in MRS broth and incubating at 37 °C and 45 °C, respectively, for 48 h. The biocatalysts were washed twice with sterile Ringer's solution (1/4 strength) for the removal of free cells. The immobilized biocatalysts were frozen to −44 °C at a cooling rate of 5 °C min^{-1} [8]. The frozen samples were freeze-dried for 48–72 h at 5×10^{-3} mbar and −44 °C in a Freeze Drying System, Freezone 4.5 (Labconco, Kansas City, Missouri, USA). Similarly, free cell cultures of *L. casei, L. bulgaricus*, and *S. thermophilus* were frozen and freeze-dried separately. No cryoprotectant was used during freeze-drying [9].

2.2. Yogurt production

Homogenized and pasteurized cow's milk (3.5% fat, 13% total solids, pH 6.7) was used for yogurt production. For the fermentation process, the milk was preheated to 90 °C for 10 min, cooled down to 40 °C, divided into eight equal portions (1000 mL each), and placed in sterile glass beakers.

The first milk portion (C: Control) was inoculated with classical yogurt culture (*S. thermophilus and L. bulgaricus,* 1:1 proportion, 1% w/v inoculum), the second (F) was inoculated with freeze-dried free *L. casei* and *L. bulgaricus* cells (1% w/v inoculum; 1:1 proportion), and the third (IM) was inoculated with the freeze-dried immobilized biocatalyst (*L. casei* and *L. bulgaricus* cells immobilized on wheat bran; 1:1 proportion; 1% w/v inoculum) that remained in yogurt after fermentation. In the same manner, the fourth milk portion (IR_1) was inoculated with the immobilized biocatalyst (1% w/v inoculums; 1:1 proportion), but it was removed from yogurt at the end of fermentation (pH 4.6 ± 0.05) and was used for four more fermentation batches (IR_2, IR_3, IR_4, and IR_5). The immobilized biocatalysts, which were contained in a thin sterile perforated fabric, were carefully removed from the product at the end of fermentation and were submerged into milk (40 °C) for the next fermentation batch. After the removal of the biocatalyst, the yogurts were allowed to set for 10 min. When each fermentation process was completed, the yogurt sample was stored in the refrigerator (4 °C) for 30 days.

2.3. Microbiological analysis of yogurt

For the enumeration of viable cells, 10-g samples were collected from each yogurt at various time intervals (1, 5, 15, 21, and 30 days) during storage at 4 °C. The samples were suspended in 90 mL of sterilized Ringer's solution (1/4 strength), homogenized (Bagmixer 400, Model VW, Interscience), serially diluted (ten-fold), and subsequently plated on media selective for each strain. *L. casei* was enumerated on V-MRS agar containing 1% vancomycin (Fluka, Buchs, Switzerland) after incubation at 37 °C for 72 h [8,17]. *S. thermophilus* was enumerated on M17 medium with 1% lactose after incubation at 45 °C for 72 h. *L. bulgaricus* was enumerated on MRS agar with pH adjusted to 5.2 and incubation at 45 °C for 72 h [17]. Viable counts of total mesophilic bacteria were enumerated on plate count agar (Fluka, Buchs, Switzerland) after incubation at 30 °C for 72 h. Total enterobacteria were enumerated on violet red bile glucose agar (Fluka, Buchs, Switzerland) after incubation at 37 °C for 24 h. Staphylococci counts were performed on Baird Parker agar (Fluka, Buchs, Switzerland) after incubation at 37 °C for 48 h. Yeasts and moulds were determined by plating on potato dextrose agar (Fluka, Buchs, Switzerland) after incubation at 30 °C for 72 h. Cell counts were expressed as log cfu g^{-1} of yogurt.

2.4. Simulated gastric digestive analysis of biocatalysts and yogurts

To investigate the influence of stomach pH on the survival rate of the probiotic *L. casei* bacteria (free or immobilized on DWB), simulated gastric solution was prepared as previously described [18]. The simulated gastric solution contained 2.0 g kg^{-1} of NaCl and 0.3 g kg^{-1} of pepsin and had pH of 3.0 (adjusted by the addition of 5 mol L^{-1} HCl). Samples contained the following: 1 mL each of free freeze-dried *L. casei* cells (S1), immobilized freeze-dried *L. casei* cells (S2), yogurt (first day after completion of fermentation) with free *L. casei* (F) cells, and yogurt with immobilized *L. casei* and *L. bulgaricus* that remained in yogurt during storage (IM). The samples were placed in sterile glass tubes containing 99 mL of simulated gastric solution. The mixtures were then blended in a stomacher for 10 min and were placed in an incubator at 37 °C for 120 min with periodic shaking. After 30, 60, 90, and 120 min, the samples were removed from the incubator and were tested for viable cells counts on MRS agar (Fluka, Buchs, Switzerland), for biocatalyst samples, or MRS-V agar, for yogurt samples, after incubation at 37 °C for 72 h [17].

2.5. Physicochemical analysis

The produced yogurts were analyzed for acidity, residual lactose, and the organic acids produced (Table 1). The pH values were measured using a digital pH meter (Hanna HI99161). The titratable acidity was determined by titrating 10 g of each yogurt sample (suspended in 20 mL deionized water) with 0.1 N NaOH using phenolphthalein as indicator.

For the determination of the lactose content and organic acids, 5 g of yogurt samples were suspended in sterile deionized water to obtain a total volume of 200 mL. The mixture was filtered and centrifuged at 4125 rpm for 20 min (Shimadzu Application, No L213). For the determination of organic acids, the samples were treated with 40% trichloroacetic acid (TCA) for protein precipitation [19]. Briefly, 9 mL of the filtrate was mixed with 1 mL TCA, incubated at 4 °C for 24 h, and then centrifuged for 30 min at 4 °C. All the samples were filtered through 0.2 μm nylon filters before analysis.