LWT - Food Science and Technology 71 (2016) 184–189

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Development of a potentially probiotic food through fermentation of Andean tubers

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ARTICLE INFO

Article history:
Received 19 August 2015
Received in revised form
4 March 2016
Accepted 6 March 2016
Available online 17 March 2016

Keywords:
Andean tubers
Lactobacillus brevis
Probiotic viability
In vitro gastrointestinal simulation

ABSTRACT

With the aim to obtain a non-dairy solid probiotic product, three different Andean tubers -oca (*Oxalis tuberosa)*, papalisa (*Ullucus tuberosus)* and potato (*Solanum tuberosum* spp andigena)- were assessed as fermentation substrates for the potentially probiotic *L. brevis* CJ25 strain. Fermented Churqueña potato puree, oregano and NaCl were used for the manufacture of a product called Potato Cheese due to its firm texture. This functional food contains a viable cell concentration of 8.0 log CFU/g and pH 5.1. Even after 28 days of storage at 4 °C, increases in cell counts were found and pH decreased 0.7 units, which improved food safety avoiding the growth of spoilage and pathogenic microorganisms. The *in vitro* tests indicated that *L. brevis* CJ25 exhibited high level of survival in simulated gastric juice (3 h, pH 2.5) when delivered in Potato Cheese, and also showed resistance to bile salt after 3 h of exposure. These results suggest that Potato Cheese is a promising novel functional food with high nutritional value, free of cholesterol and lactose and its development may have a favorable impact in Andean regional economies.

1. Introduction

In the Andean region of Argentina there is a wide diversity of crops, among these, potato (*Solanum tuberosum* spp *andigenum*), oca (*Oxalis tuberosa*) and papalisa (*Ullucus tuberosus*) represent an alternative to cover increasing demands in human alimentation. These tubers show an extensive variety of shapes and colors and offer a wide antioxidant profile (Condori et al., 2008). Research on local and ancient crops and their use to develop new products have a worldwide renewed interest (Coda, Cagno, Gobbetti, & Rizzello, 2014) and may represent an opportunity to enhance the regional economies.

Lactic acid bacteria (LAB) are the group most widely used in the fermented food industry since they improve nutritional, technological and sensorial characteristics and play a protective role against spoilage and pathogen microorganisms by lowering pH, competing for nutrients and producing antimicrobial compounds such as organic acids, H_2O_2, diacetyl and bacteriocins in some cases (Ramos, Thorsen, Schwan, & Jespersen, 2013; Swain, Anandharaj, Ray, & Praveen Rani, 2014). Foods containing probiotics fall within the category of functional foods, which are claimed to have a positive effect on health since these bacteria reach the intestine where they exert a number of benefic effects (Govender et al., 2014; Saad, Delattre, Urdaci, Schmitter, & Bressollier, 2013). Consequently, candidate probiotics must be able to survive through gastrointestinal (GI) simulated conditions which include *in vitro* tests to assess the tolerance to low pH and bile salts and finally *in vivo* and clinical studies to validate the functional properties (Saarela, Virkajarvi, & Alakomi, 2006). The number of viable microorganisms at the time of consumption is extremely important to provide expected health benefits. Despite the effective dose may be determined for each particular case, many authors have suggested a minimum dose between log 6.0 and log 9.0 CFU/g to assure a beneficial effect (Kim, Jang, & Yoon, 2012; Silva, Bezerra, Santos, & Correia, 2015).

Food matrix is an important factor in the development of a probiotic food. The effect of additives or spices in the bacterial growth has been studied since some authors suggested that the substrate composition could affect growth and survival during fermentation, processing and storage, and even the stability of the probiotic microorganisms throughout GI transit may be enhanced by some food components (Charalampopulos, Pandiella, & Webb, 2003; Do Espírito Santo, Perego, Converti, & Oliveira, 2011). Particularly, it was reported that some spices have positive influences in the growth of several LAB strains (Marhamatizadeh

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http://dx.doi.org/10.1016/j.lwt.2016.03.008
0023-6438/

et al., 2012; Zaika & Kissinger, 1981).

Although dairy and meat products are the most common fermented food, some particular vegetables are fermented as traditional practices in different cultures (e.g. sauerkraut, miso, soy sauce, pickled vegetables and kimchi) (Swain et al., 2014). Nowadays it is important to offer non-dairy probiotic products to cover the increasing demand of persons with lactose intolerance, high cholesterol or vegetarians (Kim et al., 2012). Fruit, vegetables and cereals are considered suitable matrices for probiotic foods because their nutrients are easily assimilated by bacteria (Martins et al., 2013). In this sense, some of them such as beetroot, apple, pear, soybean, carrot and tomato, among others, were successfully employed to design drinks as probiotic carriers since LAB were capable to produce high amounts of lactic acid and maintain desirable cell counts during shelf-life (Alegre, Viñas, Usall, Anguera, & Abadias, 2011).

The aim of this study was to determine the suitability of different Andean tubers as fermentation substrates for a potentially probiotic strain of *Lactobacillus brevis* and the development and characterization of a solid probiotic product.

2. Materials and methods

2.1. Fermentation substrates

Three varieties of Andean tubers were studied: Churqueña potato (CH) (*S. tuberosum* spp andigena), Oca morada (OC) (*O. tuberosa*) and Papalisa rosada (PL) (*U. tuberosus*). Tubers were purchased from local producers in a rural cooperative from Quebrada de Humahuaca (Jujuy, Argentina) and stored in a cold chamber prior to use. The Spunta (SP) (*S. tuberosum*) variety was procured in a local market and used as a reference because it is the most widespread commercially. Tubers were washed and cooked in water for 20 min. Unpeeled tubers were mashed with a commercial food processor to prepare the purees.

2.2. Strain and fermentation procedure

The microorganism used in this study is part of the Jujuy National University collection and was previously isolated from a goat's cheese of the Andean region. It was identified as *L. brevis* CJ25 using 16S rRNA gene sequencing. Genomic DNA was used for amplification using the primers 616 Valt and 630R (Chenoll, Macián, Elizaquivel, & Aznar, 2007). The resulting PCR products were purified using the NucleoSpin Extract II Kit (Macherey–Nagel, Düren, Germany) and sequenced using the ABI-PRISM 377 (PE-Applied Biosystems, Foster City, CA, U.S.A.) automated sequencer and the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE-Applied Biosystems, Foster City, CA, U.S.A.). The 16S rDNA sequence was compared with the NCBI database using the Sequence Comparison BLAST tool (http//:www.ncbi.nlm.nih.gov) showing sequence similarity level of 99%. The strain was subcultured twice for 12 h at 32 °C in MRS broth (Britania Co., Buenos Aires, Argentina) and used to inoculate (1.5% v/w; initial cell number corresponding to ca. 6.0 log CFU/g of puree) each of the purees, under strictly sterile conditions. The fermentations were carried out for 72 h at 32 °C in vacuum sealed plastic bags containing 50 g of each puree. Unstarted purees were used as controls.

2.3. Chemical, physical and microbiological analyses

Samples y controls were taken at 24 h intervals for physical and chemical analyses. pH, total titratable acidity (TTA) and total reducing sugars (TRS) were performed in solutions containing 10 g of each puree and 90 ml of distilled water. TTA was determined tritating with 0.1 M NaOH and expressed as g lactic acid/g puree. The values of pH were measured using a digital pH meter (DALVO, MHS 400). To determine TRS the 3.5-Dinitrosalicilic acid (DNS) method was used (Miller, 1959) and results were expressed as g glucose/g puree. To measure the buffering capacities, 100 ml of each media was titrated with HCl (Pai, Tsau, & Yang, 2001) and the values were expressed as the amount of HCl (mmol) needed to drop 1 pH unit per unit volume (1 L). Viable cell counts (log CFU/ml) were also determined at 8 and 16 h of fermentation, by the standard plate method with MRS agar after 48 h of incubation at 32 °C.

2.4. Manufacture and characterization of a probiotic product

CH potato was selected to develop a solid potentially probiotic product. 1.5% NaCl and 0.8% oregano were added to improve organoleptic characteristics. Proximate composition of products and raw materials was determined using AOAC (1998) methods: Moisture (964.22), samples were drained in a conventional oven at 105 °C to constant weight; Proteins (984.13), by Kjedahl method using 6.25 as the nitrogen-to-protein conversion factor; Ash (923.03), samples were burned in a muffle furnace at 550 °C to obtain white ash; Lipids (920.39), by Soxhlet technique. Total carbohydrates were calculated as difference [100 − (proteins + lipids + ash + moisture)].

Water activity was measured using a Water Activity Detector (Aqualab 3TE) at 25 °C. The effect of NaCl and oregano on total count was assessed by determining viable LAB cells in the puree with and without additives at 0, 8, 16, 24, 48 and 72 h of fermentation.

2.5. Effect of cold storage on cell counts

The product was stored 4 weeks at 4 °C and samples were collected weekly. In order to evaluate the growth of *L. brevis*, pH and viable cell counts were estimated. Enterobacteria content was estimated using Violet Red Bile Glucose Agar media (Britania) and incubated at 32 °C for 24 h. The number of yeasts was estimated on Sabouraud Dextrose Agar media (Britania), supplemented with chloramphenicol (0.1 g/L) at 30 °C for 48 h.

2.6. In vitro gastrointestinal tolerance assay

Tolerance to gastrointestinal conditions of *L. brevis* contained in the final product was studied *in vitro* with the technique described by Kim et al. (2012) with modifications.

Simulated GI juices were prepared with phosphate buffered saline (PBS) buffer solution, adjusted to different pH conditions.

The PBS pH was set at 2.5 and 6.4 (control) by the addition of 6 N HCl solution to simulate gastric stress. 0.3% (w/v) of bile salt (Oxoid, Hampshire, UK) was added to PBS and pH was adjusted to 8.0 with NaOH 1 M solution to mimic enteric conditions. Solutions were autoclaved at 121 °C for 15 min, brought to 32 °C and then inoculated with 10% (v/v) of the BAL contained in the product (5 g of fermented product were homogenized in 45 ml of distilled water) and incubated for 3 h at 32 °C. A combined treatment was carried out and included 3 h of gastric phase followed by 3 h of enteric phase. Tolerance to GI simulated conditions was determined by subsequent growth on MRS agar as described previously.

A control was prepared at investigating the intrinsic tolerance of *L. brevis* grown in MRS to GI conditions. LAB cells were cultivated in MRS broth at 32 °C for 12 h, centrifuged (5000 rpm for 5 min), washed 2 times in PBS (pH 6.4) and the assay was performed according to the method described above.