Food Chemistry 178 (2015) 267–275

Contents lists available at ScienceDirect

Food Chemistry

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

Myo-inositol hexakisphosphate degradation by *Bifidobacterium pseudocatenulatum* ATCC 27919 improves mineral availability of high fibre rye-wheat sour bread

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ARTICLE INFO

Article history:
Received 23 July 2014
Received in revised form 16 December 2014
Accepted 20 January 2015
Available online 28 January 2015

Chemical compounds studied in this article:
Myo-inositol hexakisphosphate (PubChem CID: 890)
Myo-inositol pentakisphosphate (PubChem CID 482)
D-Lactic acid (PubChem CID: 61503)
L-Lactic acid (PubChem CID: 107689)
Acetic acid (PubChem CID 176)

Keywords:
Whole rye flour
Sourdough
Phytates
Phytase
Bifidobacterium
Dietary fibre
Mineral availability
Dietary reference intakes

ABSTRACT

The goal of this investigation was to develop baking products using *Bifidobacterium pseudocatenulatum* ATCC27919, a phytase producer, as a starter in sourdough for the production of whole rye-wheat mixed bread. This *Bifidobacterium* strain contributed to *myo*-inositol hexakisphosphate (phytate) hydrolysis, resulting in breads with higher mineral availability as was predicted by the phytate/mineral molar ratios, which remained below the inhibitory threshold values for Ca and Zn intestinal absorption. The products with sourdough showed similar technological quality as their homologous without sourdough, with levels of acetic and D/L lactic acids in dough and bread baking significantly higher with the use of sourdough. The overall acceptability scores showed that breads with 25% of whole rye flour were highly accepted regardless of the inclusion of sourdough. This work emphasises that the *in situ* production of phytase during fermentation by GRAS/QPS microorganisms constitutes a strategy which is particularly appropriate for reducing the phytate contents in products for human consumption.

1. Introduction

Whole grain cereal foods provide significant health benefits. Epidemiological findings indicate a protective role of whole grain foods against several diseases such as diabetes, certain cancers, cardiovascular disease and obesity, including an improved regulation of blood glucose levels (Laaksonen et al., 2005; McIntosh, Noakes, Royle, & Foster, 2003; Pereira et al., 2002). In addition to dietary fibre, whole grains are source of a wide range of vitamins, minerals and bioactive compounds such as lignans, phenolic acids, phytosterols, tocotrienols and phytic acid (Katina et al., 2005). Compared to wheat, rye is a better source of dietary fibre. Rye bran contains a remarkable amount of soluble fibre, due to its high content in soluble arabinoxylan which has prebiotic properties and also fructans which are known to be bifidogenic (Katina et al., 2005; Lappi et al., 2010). It has been shown that rye fibre consumption is more effective than wheat fibre for the improvement of certain biomarkers of bowel health in humans, such as the reduction of faecal β-glucuronidase activity, postprandial plasma insulin and postprandial plasma, as well as the increase of plasma enterolactone and faecal butyrate (Gråsten et al., 2000; McIntosh et al., 2003). In addition, whole grain rye is characterised by a well-balanced composition of macro and micronutrients. However, whole grains also contain phytic acid, an antinutrient that impairs mineral absorption (Greiner and Konietzny, 1999; Lopez et al., 2001).

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http://dx.doi.org/10.1016/j.foodchem.2015.01.099
0308-8146/

Processing is a prerequisite for consumption of whole grains and it is also important because it may modify the amount and bioavailability of nutrients and antinutrients. Sourdough is a mixture of flour and water that is fermented with lactic acid bacteria, mainly heterofermentative strains, thus producing lactic acid and acetic acid in the mixture, and hence resulting in a sour taste of the end product (Gänzle, 2014). The use of sourdough as the natural starter for bread making is one of the oldest biotechnology processes in food fermentation. Nowadays, sourdough is largely used for the manufacture of wheat and rye breads, crackers, pizza, various sweet baked goods, and gluten-free products (Ercolini et al., 2013). Beneficial effects of sourdough fermentation on bread quality include an increased bread flavour, prolonged self life and delayed staling (Gänzle, Loponen, & Gobbetti, 2008). A sourdough fermentation process also improves texture and palatability due to peptide, lipid and carbohydrate metabolism (Gänzle et al., 2008). The production of exopolysaccharides by lactic acid bacteria in sourdough improves bread volume and texture (Gänzle, 2014). Lactobacilli convert peptides (from depolymerisation of proteins/gluten) to amino acids by strain-specific intracellular peptidases, and convert amino acids to specific metabolites with impact on bread flavour (Gänzle, 2014). Their metabolism can also favour lipidoxidation during fermentation, or exert strong antioxidative effects. Chemical degradation of linoleic acid peroxide forms flavour-active aldehydes which are converted to alcohols by the alcohol dehydrogenase activity of heterofermentative lactobacilli (Gänzle, 2014). Regarding nutritional quality, the use of sourdough may decrease the glycemic index, because of its potential to modify the digestibility of starch, owing to increased lactic and acetic acid levels (Katina et al., 2005). Furthermore, the sourdough could enhance the bioavailability of minerals. Microbial metabolism during sourdough fermentation may produce active compounds, such as bioactive peptides and amino acid derivatives with various functionalities, and potentially prebiotic exo-polysaccharides (Gänzle, 2014; Katina et al., 2005). As was mentioned above, in addition to many nutritional components, whole grain cereals also contain significant amounts of phytic acid (*myo*-inositol (1,2,3,4,5,6)-hexakisphosphate or InsP_6) or its salts (phytates), a well-known inhibitor of mineral, proteins and trace elements bioavailability. Aside from this negative effect, phytic acid is a precursor for the generation of bioactive compounds (Haros et al., 2009). The phytate hydrolysis decreases the negative effects on mineral absorption and generates lower *myo*-inositol phosphates with potential benefits to human health. Phytases are the enzymes that catalyse this hydrolysis and several strategies exist to increase their activity. The enzymatic phytate degradation during the breadmaking process depends on many factors including pH, temperature, water content, bran content, leavening agent, fermentation time, process and exogenous phytase addition (generally from microbial sources). The endogenous phytase from cereals has optimal pHs around 4.5 and therefore the use of sourdough improves the degradation of phytates, due to the decrease of pH (Fretzdorff & Brümmer, 1992; Lopez et al., 2001; Reale et al., 2004). *Lactobacillus* strains typically responsible for sourdough fermentation lack phytase activity and their phytate degrading capacity is limited and based on non-specific acid phosphatases able hydrolyse phytates at a low rate (Haros et al., 2009). However, phytase activity has been described for food-grade strains of the *Bifidobacterium* genus, which are endogenous inhabitants of the gastrointestinal tract, suggesting their utility in producing fermented cereal based products. In fact, phytase-producing bifidobacteria have been applied in both direct and indirect breadmaking processes (Sanz-Penella, Tamayo-Ramos, Sanz, & Haros, 2009; Sanz-Penella, Tamayo-Ramos, & Haros, 2012). Results showed that *Bifidobacterium* strains presented a good adaptation to the dough ecosystem and contributed to acidification resulting in whole wheat breads with significantly lower levels of phytates (Palacios, Haros, Rosell, & Sanz, 2008). The aim in the present study was to develop whole rye-wheat mixed bread, with increased nutritional quality, by using *Bifidobacterium pseudocatenulatum* ATCC27919 from human origin as a sourdough starter for improving phytate hydrolysis and the production of organic acids.

2. Materials and methods

2.1. Materials

Commercial flours were purchased from the local market. The characteristics of wheat and whole rye flours were: moisture, 13.79 ± 0.07% and 12.58 ± 0.02%; protein, 11.32 ± 0.09% and 10.13 ± 0.02% in dry basis; fat, 1.02 ± 0.06% and 1.17 ± 0.12% in dry basis; ash, 0.63 ± 0.01% and 1.56 ± 0.01% in dry basis; and phytate contents were: 1.02 ± 0.03 and 7.64 ± 0.05 μmol/g in dry basis, respectively. Compressed yeast (*Saccharomyces cerevisiae*, Levamax, Spain) was used as starter for the breadmaking process and *B. pseudocatenulatum* ATCC 27919, originally isolated from infant faeces, was used as starter in sourdough fermentation.

2.2. Microbial growth conditions

Bifidobacteria were grown in Garche broth in which inorganic phosphate (KH_2PO_4 and NaH_2PO_4) was replaced by 0.74 g/L phytic acid dipotassium salt (Sigma–Aldrich, St. Louis, MO, USA) and 0.1 M of 3-[N-Morpholino] propanesulphonic acid buffer (MOPS, Sigma–Aldrich, St. Louis, MO, USA) (Haros, Bielecka, Honke, & Sanz, 2007). The medium was inoculated at 5% (v/v) with 18-h old cultures, previously propagated under the same conditions (AnaeroGenTM, Oxoid, England) until the beginning of the stationary phase of growth (~14–18 h). Bacterial growth was monitored by measuring optical density at 600 nm.

The inoculum was prepared by harvesting the bacterial cells by centrifugation (21,000×*g*, 20 min, 4 °C, SLC 1500, Sorvall Evolution), washing the pellets twice and suspending them in 0.085% NaCl solution (Sanz-Penella et al., 2009). The bacterial suspensions were used to inoculate the sourdough. Microbial counts in sourdough and dough samples were determined by plate count on selective media. In the case of bifidobacteria counts, Garche agar was employed by using the double layer technique and a temperature of incubation of 37 °C for 48 h (Haros et al., 2007). Yeast counts were determined in Rose Bengal Agar (Scharlau Chemie, Barcelona, Spain) after aerobic incubation at 30 °C for 72 h (Sanz-Penella et al., 2009).

2.3. Breadmaking process

Five formulations were used for making bread dough: 100% refined wheat flour, 25/75% w/w whole rye-refined wheat flour, 50/50% w/w whole rye-refined wheat flour, 75/25% w/w whole rye-refined wheat flour and 100% whole rye flour. The bread dough formula consisted of one of these formulations (750 g), compressed yeast (2% flour basis), sodium chloride (1.8% flour basis), tap water (up to optimum absorption). A Farinograph (Brabender, Duisburg, Germany) with a 300 g mixer was used to evaluate the impact of formulation on the dough development time (time to reach maximum consistency, in min) and water absorption (water required to yield dough consistency of 500 Brabender Untis, in %), following the official standard method (AACC, 2000). The ingredients were mixed for 10 min, rested for 10 min, divided into 100-g pieces, kneaded and rested again for 10 min. Doughs were manually sheeted, rolled and fermented up to the optimum volume increase at 28 °C and 85% of relative humidity. The samples were backed at