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Chemical and nutritional properties of white bread leavened by lactic acid bacteria

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ABSTRACT

Four LAB strains belonging to the *Lactobacillus* and *Leuconostoc* genera were investigated in experimental bread-making in comparison with *Saccharomyces cerevisiae* (SC). The derived breads were evaluated for microbiological and technological characteristics. Functional features associated to degradation of starch, release of gluten exorphins were investigated ante and post *in vitro* gastrointestinal digestion. Digestates were also assessed for rapidly digestible starch (RDS) through Englyst's method and for secretion of the satiety hormone PYY when administered to an *in vitro* human intestinal cell co-culture. A clear separation emerged between LAB and SC samples, being the highest difference affected by TTA and reduction of phytate. The SC sample was also distinguishable by its highest levels of simple sugars, in particular glucose, post *in vitro* digestion and PYY secretion. Breads from LAB strains differed with regard to volume and moisture, TTA, phytate reduction, exorphins release, total sugars and glucose contents post *in vitro* digestion.

1. Introduction

Bread is one of the most consumed foodstuffs worldwide and represents a primary source of carbohydrates in most European countries, where its average consumption was 61.7 kg per capita in 2015 (Bo et al., 2017). Consequently, people are regularly exposed to baker's yeast that consists of cells of one or more selected strains of *Saccharomyces cerevisiae* (SC), the gold standard in the baking industry to leaven dough. There is no denying that SC has profound implications for food technology thanks to its ability to transform cheap raw materials into tasty and value-added products (El-Helow, Elbahloul, El-Sharouny, Ali, & Ali, 2015; Rinaldi, 2014). Nonetheless, the continuous exposure to baked goods has been correlated to sensitisation to baker's yeast. In particular, allergic symptoms might be elicited in susceptible individuals after consumption of food containing SC. Hence, yeast allergy should be taken into account in patients presenting reactions after exposure to SC in yeast-fermented products (Bansal, Tadros, & Bansal, 2017; Pajno et al., 2005; Rinaldi, Perricone, Blank, Perricone, & Shoenfeld, 2013). As management of yeast allergy, complete exclusion of all products that might contain yeast, comprising baked goods, has been suggested (Bansal et al., 2017). This dietary restriction represents an effective solution without medication to mediate chronic symptomatology linked to the anti-SC autoantibodies (ASCAs) levels in response to the diet (Colboc, Fite, Cannistra, Chaby, & Maillard, 2016; Cunningham, 2013). Anyway, the exclusion diet is not readily feasible and tolerable for people whose food culture is largely based on bread and other leavened bakery commodities.

Sourdough is a mixture of flour and water fermented using yeast and lactic acid bacteria (LAB) for dough leavening. In sourdough, LAB and yeast occur in high numbers (LAB $\geq 10^8$ CFU/g and yeasts $\leq 10^7$ CFU/g) and typical yeasts are *Candida humilis, Kazachstania exigua* and *S. cerevisiae*. Moreover, the addition of baker's yeast SC is generally required for leavening in powdered sourdough with defined starter cultures (De Vuyst & Neysens, 2005; De Vuyst et al., 2014). Thanks to their potential in acidification, flavor formation and dough leavening, heterofermentative LAB could represent an alternative to SC in dough

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fermentation. In this regard, lactobacilli represent the typical LAB associated to sourdough fermentation, but recent studies highlighted the suitability of *Leuconosotc* and *Weissella* strains for application in sourdough bread production (Corona et al., 2016). Up to date, no study has been reported on bread leavened exclusively by LAB. This technological approach represents a strategy for developing bread tailored for individuals suffering from adverse response to baker's yeast and for obtaining bread with other specific nutritional and functional properties. In this regard, the role played by LAB in hydrolyzing the gluten during fermentation has been studied in bakery sourdoughs (Gobbetti, Rizzello, Di Cagno, & De Angelis, 2007). For instance, this activity has been reported to degrade immunogenic peptides by the action of the peptidases portfolio of different mixed cultures of LAB strains. Nonetheless, the proteolytic and peptidolytic systems of LAB and gastrointestinal tract could be involved in the release or degradation of other bioactive peptides implicated in health issues. Recently, LAB were investigated for the capacity to release antioxidant, anti-hypertensive peptides and antitumoral peptides during fermentation of cereal flours (Coda, Rizzello, Pinto, & Gobbetti, 2012, Rizzello, Cassone, Di Cagno, & Gobbetti, 2008; Rizzello, Nionelli, Coda, & Gobbetti, 2012). On the contrary, to best of our knowledge, no study investigated the effect of lactic acid fermentation of dough on the occurrence of gluten exorphins (GE) in bread. Similarly, the combined effect of souring of wheat flour dough and *in vitro* digestion of the derived bread on GE release was not examined. These peptides have been reported to exert opioid effects and to mask the toxic effects of gluten at gastrointestinal level (Pruimboom & de Punder, 2015). Recently, their presence was ascertained in digestates of pasta and yeast's leavened bread and they were demonstrated to survive *in vitro* gastrointestinal digestion and to partially cross a monolayer of 70% Caco-2/30% HT-29 co-culture, used as a model of the human intestinal epithelium (Maggioni et al., 2016; Stuknytė et al., 2015).

The possibility of improving health status using LAB strains could also depend on the satiety capacity of the ingested bread, a feature which could contribute to control the appetite and, as consequence, the weight gain. This perspective takes into consideration the production of the anorectic hormones by intestinal cells at the end of a meal, which are able to exert their function at two different levels: (i) in the gastrointestinal tract *via* inhibition of gastric empty and an increased secretion of pancreatic enzymes which results in an improved digestion and absorption nutrient capacity; (ii) in the Central Nervous System where they acts on the appetite center inducing the production of other anorectic factors (Bruen, O'Halloran, Cashman, & Giblin, 2012; Hameed, Dhillo, & Bloom, 2009). Taken together, these two effects induce long-term effects on satiety and, consequently, reduce food consumption. The anorectic hormones together with the glycemic index, through the associated insulin level, are strong determinant of the resulting overweight and/or obese phenotype (Yazıc & Sezer, 2017). In addition, LAB starter can increase mineral bioavailability of bread because of their phytate degrading enzymes (De Angelis et al., 2003). Phytic acid forms precipitated complexes with divalent cations, such as iron, magnesium and calcium that strongly reduce the absorption of essential minerals (Greiner & Konietzny, 2006). Moreover, phytate complexes are not accessible to enzymatic hydrolysis above pH 5.0 (Fretzdorff & Brümmer, 1992).

Based on these assumptions, in this study four different heterofermentative LAB strains belonging to *Lactobacillus* and *Leuconostoc* genera were used as the only leavening agents for the production of white bread. Some chemical and nutritional properties of derived baked breads were studied and compared to those of baker's yeast bread ante and post *in vitro* gastrointestinal digestion. Particularly, degradation of starch and phytates, occurrence of GEs, and production of the satiety hormone PYY by an *in vitro* intestinal cell co-culture were studied.

2. Materials and methods

2.1. Bacterial strains

The study was performed using a commercial baker's yeast, SC (AB Mauri, Casteggio, Italy) and the LAB strains: *Lactobacillus reuteri* LR12 and *Lb. fermentum* LF16 provided by Centro Sperimentale del Latte (Zelo Buon Persico, Italy), *Lb. parabuchneri* BT117 and *Leuconostoc lactis* BT124 from the bacterial collection of the Institute of Sciences of Food Production – Italian National Research Council (Milan, Italy). The four LAB cultures were routinely activated by sub-culturing aerobically in MRS broth (Biolife Italiana, Milano, Italy) at 30 °C (BT117 and BT124), 38 °C (LF16) and 44 °C (LR12). Prior to baking experiment, for each bacterial culture an initial suspension of 1×10^9 CFU/mL was reached by measuring the absorbance at 625 nm with a microplate reader (Infinite F200 PRO, Tecan, Männedorf, Switzerland). Thereafter, the concentration of the bacterial suspensions was confirmed by plating on appropriate culture media (see Section 2.4). Ten milliliters of LAB cell suspension were transferred into a centrifuge tube (50 mL), the cell pellets were harvested twice by centrifugation at 3.000 rpm at 4 °C for 10 min, washed with sterile quarter-strenght Ringer solution (Scharlau Microbiology, Barcelona, Spain), and aseptically re-suspended in the tap water used to prepare dough. A final yeast and LAB inoculum of 10^{10} CFU in the dough (200*g*) was used.

2.2. Bread-making

The fermented dough was prepared in the KitchenAid® mixing bowl (model 5KSM150, KitchenAid, Benton Harbor, MI, USA) equipped with a dough hook, fixing the mixing speed at level 2. Each fermented dough sample was prepared from a 125 g commercial wheat flour mixed with 75 mL tap water containing 2 g NaCl. At this point, baker's yeast or LAB were inoculated properly (10^{10} CFU in the dough) as previously described. After 2 min mixing, 2.5 mL of virgin olive oil were added and mixing was continued for other 4 min. The dough was maintained for 15 h at 22 °C to promote fermentation. An aliquot (140 g) of each fermented dough was used as inoculum and mixed with the remaining part of the ingredients (700 g flour; 420 mL water; 14 g of virgin olive oil; 10.5 g salt) applying the all-in-one process. After a final mixing of 6 min, the dough was left to rest for 10 min at room temperature, then divided into four portions of 250 g each, shaped into cylinder form and put into a baking pan (8 cm height); the final proofing was carried out for 5 h at 37 °C and 60% RH. The leavened dough samples were baked at 220 °C for 30 min in a oven (Rational AG, Mestre, Italy). The leavening performances of LAB were compared with those of yeast sponge dough prepared in the same conditions used to obtain the LAB fermented dough samples.

2.3. Chemical and physical characteristics of final dough and bread samples

Total Titratable Acidity (TTA) and pH of final leavened dough were evaluated by using a T50 automatic titrator (Mettler-Toledo AG, Greifensee, Switzerland). An aliquot of 20 g of leavened final dough was suspended in 200 mL of distilled water and titrated by adding 0.1 M NaOH solution and measuring the pH until pH 8.4 was reached. The TTA was expressed in mL NaOH/10 g sample.

The apparent volume of bread loaf (n = 4 for each fermentative microorganism) was determined as described by (Marti, Cardone, Nicolodi, Quaglia, & Pagani, 2017). The bread weight was recorded with an electronic balance Europe 1700 (Gibertini Elettronica Srl, Novate Milanese, Italy) and the specific volume was determined through the volume/mass ratio and expressed in mL/g. Moisture content was evaluated in triplicate by an infrared balance (MA 210.R, Radwag-Wagi Elektroniczne, Chorzòw, Poland) after drying the sample at 130 °C until the weight remained constant for 60 s. Phytic acid content was measured using the phytic acid (phytate)/total phosphorus the K-PHYT 05/