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New bread formulation with improved rheological properties and longer shelf-life by the combined use of transglutaminase and sourdough

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ABSTRACT

The combined use of the protein reticulating enzyme transglutaminase (TGase) and a selected microbial consortium of *Lactobacillus sanfranciscensis* and *Candida milleri* for improving the rheological properties, aroma, and shelf-life of a bakery product was evaluated. A microbial TGase, showing the highest activity over a wide temperature range on different protein substrates, was selected among different types. Results showed that this TGase was able to produce isodipeptide bonds, especially in the gluten fraction, leading to the formation of protein aggregates, which improved the structure of a sourdough bakery product. The microbial TGase in combination with sourdough exhibited a positive synergistic effect allowing the production of flavor-enriched bread, with rheological properties similar to those of standard bread.

1. Introduction

Bread is a fundamental food in the Western world and it is generally viewed as a perishable commodity, due to its fast decrease of freshness features and its rapid staling (Minervini, De Angelis, Di Cagno, & Gobbetti, 2014). Lactic acid bacteria (LAB) and yeasts in the form of sourdough have been reported to have positive effects on wheat bread quality and staling (Clarke, Schober, & Arendt, 2002; Corsetti et al., 2000; Crowley, Schober, Clarke, & Arendt, 2002) as they are responsible for the capacity of dough to leaven, while acidifying it (De Vuyst & Neysen, 2005). Traditional sourdough obtained with selected microorganisms is able to increase bread shelf-life by delaying staling (Chavan & Chavan, 2011) and improve bread properties through enhancing its nutritional value, taste, and aroma profile (Arendt, Ryan, & Dal Bello, 2007; Hansen & Schieberle, 2005; Poutanen, Flander, & Katina, 2009). However, the use of LAB may affect the rheology of leavened bakery products through a strain-dependent proteolytic activity (Gobbetti, Smacchi, & Corsetti, 1996). These rheological properties, besides gas retention, depend on gluten proteins, composed of extensible, viscous gliadins and rigid, elastic glutenins. Chemical agents or

Abbreviations used: acTGase, Activa WM transglutaminase; AEC, 3-amino-9-ethylcarbazole; ANOVA, analysis of variance; AtPng1p, *Arabidopsis thaliana* peptide *N*-glycanase; CFU, colony forming unit; C, control; Cd, control dough; Cb, conventional bread; DY, dough yield; DMC, *N*′, *N*′-dimethyl casein; FN, fibronectin; SPME-GC-MS, gas chromatography-mass spectrometry coupled with solid phase micro-extraction; HRP, horseradish peroxidase; LAB, lactic acid bacteria; PC, principal component; PCA, principal component analysis; Sd, selected sourdough; Sb, sourdough bread; TGase, transglutaminase; TPA, texture profile analysis; MCFA, medium-chain fatty acid; RT, room temperature; SDS-PAGE, sodium dodecyl sulphate poly acrylamide gel electrophoresis; VCs, volatile compounds.

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cross-linking enzymes, such as glucose oxidase, peroxidase, or transglutaminase (TGase), have been reported to improve dough handling properties and to increase fermentation stability, and loaf volume (Caballero, Gómez, & Rosell, 2007; Steffolani, Ribotta, Perez, & Leon, 2010). In particular, TGase (EC 2.3.2.13) is an important enzyme for the food industry (Basman, Koksel, & Ng, 2002) as it catalyses the formation of protein cross-links resulting in extensive nets (Nonaka et al., 1989). The formation of protein polymers, as a result of TGase activity, can modify the rheological properties of gluten (Köksel, Sivri, Ng, & Steffe, 2001) and allow the transformation of a very weak gluten into a very strong one (Larre et al., 2000). In previous studies, the positive effects of TGase application on wheat-based baked products have been described (Renzetti, Behr, Vogel, & Arendt, 2008), (Gerrard et al., 2000). The effects of TGase on empirical rheological properties of dough (Basman et al., 2002; Marco, Perez, Ribotta, & Rosselli, 2007) and on the formulation of sourdough (Arendt et al., 2007; Clarke et al., 2002) in order to obtain good-quality bread have been described. To date, however, the combined use of the two biological agents, i.e., TGase and sourdough, has never been reported.

In the present work, we tested the possibility of improving bread quality through the combined use of sourdough and a protein-reticulating enzyme. TGase was selected in order to improve rheology (Gerrard et al., 1998), while sourdough based on *Lactobacillus sanfranciscensis* and *Candida milleri* was chosen for its ability to improve the aroma profiles and extend the shelf-life of the final product (Scarnato et al., 2016; Vernocchi et al., 2008). *L. sanfranciscensis* is a key organism for sourdough acidification and produces aroma precursors (Gobbetti et al., 1996; Gänzle, Vermeulen, & Vogel, 2007), while *C. milleri* is able to grow in association with hetero-fermentative LAB, enhancing the accumulation of specific aroma compounds, including alcohols, lactones, and medium-chain fatty acids (MCFAs) (Gobbetti, 1998; Gänzle, Ehmann, & Hammes, 1998). Results show that the combination of a protein-reticulating enzyme and sourdough on wheat bread produced a positive synergistic effect.

2. Materials and methods

2.1. Materials

Straight-grade wheat flour was provided by Barilla S.p.A. (Parma, Italy). The TGases tested in this study came from different sources: (i) Activa® WM (acTGase), from *Streptoverticillum mobaraense*, was purchased from Ajinomoto (specific activity: >0.1 U/mg powder preparation whose composition is 1% enzyme and 99% maltodextrin), Mesnil-Saint-Nicaise, France, (ii) a recombinant microbial TGase (zTGase) was purchased from Zedira (specific activity: >25 U/mg, Darmstadt, Germany), (iii) a mammalian TGase, from guinea pig liver, was purchased from Sigma-Aldrich (specific activity: >2.4 U/mg, Milan, Italy), and (iv) a recombinant TGase of plant origin, the *Arabidopsis thaliana* peptide *N*-glycanase (AtPng1p), was purified as previously described (Della Mea, Caparros-Ruiz, & Rigau, 2004) (specific activity: > 0.5 U/mg).

All reagents and solvents (unless otherwise indicated) were of the highest purity and were obtained from Sigma-Aldrich (Milan, Italy).

2.2. Sourdough preparation

Sourdough was prepared by a two-step fermentation process using *L. sanfranciscensis* strain LSCE1 and *C. milleri* strain PFL44, both belonging to the Department of Agricultural and Food Science, University of Bologna (Italy). The dough was prepared by mixing wheat flour and water to reach a dough yield (DY) of 220. Exposure to osmotic stress was performed by adding sucrose (40% of the final dough volume) to the water. Fermentation was performed in a fermentor (BioFlo/CelliGen® 115, New Brunswick, Eppendorf) as previously described (Scarnato et al., 2016). The sourdough obtained was used for the preparation of bread in association or not with TGase.

2.3. Enzymatic treatment with TGase

In order to induce protein cross-links, different amounts of TGase (0.5, 1, 2, and 5 U/g flour) were added to the wheat dough (Cd) obtained using *Saccharomyces cerevisiae* as a conventional leavening agent and the sourdough (Sd) made with the selected microbial consortium described above. The enzyme was mixed to the flour for 15, 60 or 90 min at different temperatures, from 4 to 37 °C.

Cross-linking was evaluated by protein extraction and separation using 15% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Total proteins were extracted under reducing conditions using the buffer described by (Marco et al., 2007). Albumins/globulins (F1), prolamins (F2), and glutelins (F3) were extracted following a sequential extraction method using different solvents (Marco et al., 2007). Globulins 7S and 11S were prepared and purified as previously described (Thanh, Okubo, & Shibasaki, 1975). The protein content of the extracts was determined by the bicinchoninic acid method (Smith et al., 1985).

2.4. Measurement of TGase activity

TGase specific activity was measured by the conjugation of biotinylated cadaverine to protein substrates as previously described (Lilley, Skill, Griffin, & Bonner, 1998) with slight modifications. Protein substrates, such as standard proteins and wheat protein extracts, were covalently attached to the surface of microplate wells. The level of biotinylated cadaverine incorporation was determined according to an established protocol (Lilley et al., 1998) with 10 mM cystamine replacing EDTA in negative controls. The specific activity was expressed as a 0.1 change in A_{450} per h per mg of TGase used (U/mg prot.).

2.5. Dot blot of enzyme reaction products

Fractions F1, F2, and F3 were treated with TGase and the reaction products were blotted onto nitrocellulose. The membrane was incubated with Ab3, a monoclonal antibody raised against soluble TGase (Neomarker, Fremont, CA, U.S.A.) and with 81D4, a monoclonal anti-Nε (γ-glutamyl)-lysine antibody (Covalab, Lyon, France), which is a product of the TGase cross-linking reaction. Dots were revealed using horseradish peroxidase (HRP)-conjugated rabbit anti-mouse IgG and 3-amino-9-ethylcarbazole (AEC).

2.6. Bread preparation

Bread was prepared with a bread maker (Deluxe Princess, 152000) using an industrial recipe (wheat flour, water, sugar, salt, baker's yeast, and extra-virgin olive oil) to obtain a final volume of about 500 g with DY 150. When Sd was used, it was added at a concentration of about 30% of the final weight of dough; the amount of flour and water was reduced accordingly in order to maintain the same DY. TGase was added at different concentrations (0.5, 1, and 2 U/g flour). Doughs were kneaded for 14 min and fermented for 20 min. Then, a second kneading of 8 min was performed, followed by 1 h of fermentation. Dough samples Cd, and Sd with and without TGase were then baked at 180 °C for 30 min in order to obtain conventional bread (Cb) and sourdough bread (Sb).