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Effect of honey supplementation on sourdough: Lactic acid bacterial performance and gluten microstructure

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ABSTRACT

In the present study we evaluate the effect of honey on the growth and fermentative ability of two sourdough fermenting lactic acid bacteria (LAB), *Pediococcus pentosaceus* and *Lactobacillus fermentum*, and the impact that honey and LAB have on gluten microstructure. Growth kinetics and fermentative analyses were carried out through cell viability and potenciometry assays, respectively. Honey supplementation of sourdough increased LAB population. *L. fermentum* exhibited a higher growth rate, while *P. pentosaceus* was more acidifying. The fermentative profile of LAB was not altered by the presence of honey. The microstructure analyses were performed through scanning electron microscopy (SEM) and revealed that the microstructure of dough was modified by the fermenting activity of LAB, being involved in the development of gluten fibrils. In addition, honey induced changes in the microstructure of those dough whose pH value were higher than 4, disclosing a strong association between protein subunits.

1. Introduction

Gluten confers dough with unique functional properties for the development of baked goods (Rizzello et al., 2013). Wheat-flour products, rich in gluten, can be used as experimental models to assess the effect that crosslinking agents have on several foods (Rasiah, Sutton, Low & Gerrard, 2005). Gluten proteins are divided in two groups: gliadins and glutenins. The glutenin moiety forms intra- and inter-chain disulphide bonds, leading to the generation of the glutenin macropolymer (GMP) (Vermeulen, Kretzer, Machaliza & Gänzle, 2006). The hydrolysis of GMP has been linked to proteolysis of the gluten proteins by endogenous flour proteases, which have optimal activity under acidic conditions (Gerez, Dallagnol, Rollán, & Font de Valdez, 2012). Acidification is essential to allow hydrolysis of the various protein fractions in sourdough (Vermeulen et al., 2006). Sourdough, a fermented mixture of water and flour, is extensively used in baked goods because it offers several benefits related to the metabolic activities of lactic acid bacteria (LAB), such as, lactic fermentation, proteolysis, synthesis of flavour compounds, as well as avoidance of microbial contamination (Di Cagno et al., 2003).

In the food industry, exogenous enzymes are intentionally added to the dough formulation because of the improving effect they have on functional properties of foods (Di Cagno et al., 2003). One of these enzymes is glucose-oxidase (GOX), which catalyses the oxidation of α-D-glucose to α-D-gluconolactone and hydrogen peroxide (H_2O_2). The latter, oxidizes thiol groups of gluten proteins to form disulphide bonds (Steffolani, Ribotta, Pérez, & León, 2010) which turns in the covalent crosslinking of proteins (Bonet et al., 2006). GOX has been found in red algae, bacteria, insects, mould (Schepartz & Subers, 1963; Wilson & Turner, 1992), and in the pharyngeal gland of honeybees (Schepartz & Subers, 1963). In consequence, honey constitutes a natural source of GOX. Some other compounds with bioactive properties are present in honey, such as phenolic acids and flavonoids (Isla et al., 2011). Honey's phenolic compounds are efficient antioxidants that play a significant role in human health, by scavenging reactive oxygen species (ROS) (Gheldof, Wang, & Engeseth, 2002; Küçük et al., 2007). They are involved in food preservation processes as well, avoiding or delaying enzymatic browning of fruits and juices and lipid oxidation in meat (Gheldof et al., 2002; de la Rosa et al., 2011). Moreover, it has been reported that flavonoids enhance the growth of certain strains of LAB (Rodríguez et al., 2009).

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In this study, we investigate the effect of honey on sourdough LAB, and the impact that both honey and LAB have on GMP microstructure.

2. Material and methods

2.1. Materials

2.1.1. Bacterial strains

The LAB strains used in this study, *Pediococcus pentosaceus* (CRL 922) and *Lactobacillus fermentum* (CRL 220), were provided by Centro de Referencia para Estudios de Bacterias Lácticas (CERELA). These strains were chosen considering the differences in glucose fermentation pathways. *P. pentosaceus* is a homolactic strain, while *L. fermentum* ferments glucose by the heterolactic pathway.

2.1.2. Source of honey

The honey sample used in this study came from San Luis Province, Argentina (33 ° 17′ S − 66 ° 22′ W). It was identified as monofloral *Prosopis* sp. honey.

2.2. Methods

2.2.1. Growth conditions

LAB strains were grown as described in Nutter, Fritz, Iurlina, and Saiz (2016) and were standardized to 0.5 of Mc Farland Scale, which corresponds to a bacterial concentration of 1.5×10^8 colony forming units per millilitre (CFU/ml). LAB cells were collected by centrifugation at 4500 rpm for 10 min, washed twice, and re-suspended in sterile 0.15 M NaCl solution. This suspension was used for dough inoculation.

2.2.2. Fermentative profile of LAB

The ability of *P. pentosaceus* and *L. fermentum* of using different carbohydrates as metabolic substrates was evaluated using the API 50 CH kit (API systems, BioMérieux, France). The strains were incubated in MRS broth at 32 °C for 19 h, isolated by surface spread in MRS agar plates, and incubated at 32 °C for 19 h. About four colonies of each strain were transferred into the API 50 CHL medium until the turbidity was equivalent to grade 2 of Mc Farland scale. The inoculation and incubation of the API 50 CH kit was performed according the manufacturer's instructions.

2.2.3. Sourdough formulation

Sourdough were prepared by mixing 125 g of wheat flour and 125 g of rye flour, 150 ml deionized water, 3.8 g of salt, 9 ml of standardized suspension of LAB (according to Sec 2.2.1.), with or without the addition of 6.5% (w/w) bioactive monofloral *Prosopis* sp. honey. The ingredients were mixed for 3 min in a kneading machine (Hobart N-50, Ontario, Canada). A control dough was prepared under de same conditions except for the addition of honey and LAB. All dough were incubated at 32 °C for 19 h.

2.2.4. Effect of honey supplementation on sourdough LAB

The behaviour of *P. pentosaceus* and *L. fermentum* on honey supplemented sourdough was evaluated by measuring growth kinetics and fermentative activity of these LAB in sourdough supplemented with *Prosopis* sp. honey. The assays were performed at four selected times: 0 (t_0), 6 (t_6), 12 (t_{12}) and 19 (t_{19}) h since fermentation started.

2.2.4.1. Growth kinetics of LAB in honey supplemented sourdough. At each time (0, 6, 12, and 19 h), 10 g samples of each sourdough were aseptically collected and diluted 10 times into 90 ml of sterile Butterfield's phosphate buffered dilution water (Butterfield, 1932). The standard pour-plate technique, using MRS agar, was employed to determine the viable cell counts. The inoculated plates were anaerobically incubated at 32 °C for 72 h. The logarithm (Log) of CFU/g was used to report the growth results.

2.2.4.2. Fermentative activity of LAB in honey supplemented sourdough. At each time (0, 6, 12, and 19 h), 10 g samples of each sourdough were homogenized with 90 ml of deionized and free of CO_2 water with a magnetic stirrer. pH was measured using a pH-meter (Hanna instruments HI 9321) and total titratable acidity (TTA) was measured by potentiometry, neutralizing the suspension with 0.1 M NaOH until pH value of 8.1.

2.2.5. Effect of LAB activity and bioactive compounds of honey on GMP microstructure

The effect that LAB activity and bioactive compounds of honey have on GMP microstructure was evaluated through scanning electron microscopy (SEM). Sourdough were prepared according to Sec 2.2.3., and were incubated at 32 °C for 19 h. In order to minimize any possible disruption of the samples' microstructure, inner pieces of each dough were frozen by immersion in liquid air (−80 °C). To reduce the humidity content down to 20%, the samples were lyophilized in lyophilizer VIRTIS-Benchtop SLC. Lyophilized samples were fractured, gold-palladium coated, and observed with a Jeol JSM-6460 LV scanning electron microscope with a 15 kV acceleration voltage and magnification of 800 and 1000.

2.3. Statistical analyses

All data presented represent mean values from three replicate experiments ± standard deviation (SD) and were performed with SPSS statistics 15.0 for Windows using ANOVA General Linear Models followed by a Tukey's poshoc test, and $p \leq 0.05$ was considered significant.

3. Results and discussion

3.1. Fermentative profile of LAB

P. pentosaceus and *L. fermentum* were evaluated in their ability to use different carbohydrates as metabolic substrates using the API 50 CH test. The metabolic profiles of LAB are shown in Table 1.

P. pentosaceus exhibited a wider carbohydrate metabolism than *L. fermentum*, using 36% of the sugars that constitute the API 50 CH test; meanwhile *L. fermentum* metabolized 22% of these sugars. Wheat and rye dough are rich in starch and polyfructosans, which are enzymatically hydrolysed into fermentable carbohydrates providing dough with mono-, di- and oligosaccharides (Stolz, Vogel, & Hammes 1995). These sugars include glucose, fructose, sucrose, maltose, raffinose, and maltotriose (Barber, Benedito de Barber, & Martinez-Anaya, 1991). Furthermore, honey supplementation of dough provides them with an extra source of fermentable carbohydrates. Fructose and glucose are the main components; together they comprise about 70% of honey constituents, while the disaccharides sucrose and maltose are found in a 10% (Gheldof et al., 2002). Other saccharides, as isomaltose, turanose, erlose, raffinose, melezitose and trehalose are present in less extent (Ouchemoukh, Schweitzer, Bey, & Djoudad-Kadji., 2010). Our results indicated that *P. pentosaceus* and *L. fermentum* were efficient to metabolize glucose, fructose and maltose. *P. pentosaceus* was also able to use trehalose, while *L. fermentum*, sucrose. In addition, pentosans constitute an important fraction in sourdough systems, especially when rye is present. Rye flour has a larger content of pentosans than wheat flour (Girhammar & Nair, 1992), mainly arabinoxylan and xylan. These pentosans are hydrolysed by