LWT - Food Science and Technology 77 (2017) 119–125

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

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

Julia Nutter [a, b, *], Rosalia Fritz [a], Amelia I. Saiz [a], Miriam O. Iurlina [a]

[a] Departamento de Química, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Funes 3350, C.P. 7600, Mar del Plata, Provincia de Buenos Aires, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

ARTICLE INFO

Article history:
Received 21 June 2016
Received in revised form
19 September 2016
Accepted 13 November 2016
Available online 14 November 2016

Keywords:
Honey
Lactic acid bacteria
Sourdough
Gluten microstructure

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).

* Corresponding author. Departamento de Química, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Funes 3350, C.P. 7600, Mar del Plata, Provincia de Buenos Aires, Argentina.
E-mail address: jnutter@mdp.edu.ar (J. Nutter).

http://dx.doi.org/10.1016/j.lwt.2016.11.040
0023-6438/

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

Table 1
API 50 CH profiles of *P. pentosaceus* and *L. fermentum*.

Carbohydrate	*P. pentosaceus*	*L. fermentum*
Control	–	–
Glycerol	–	–
Eritrythol	–	–
D-arabinose	d	–
L-arabinose	+	+
Ribose	+	+
D-xylose	+	–
L-xilose	–	–
Adonitol	–	–
β-metil-xyloside	–	–
Galactose	+	+
D-glucose	+	+
D-fructose	+	+
D-mannose	+	d
L-sorbose	–	–
Rhamnose	–	–
Dulcitol	–	–
Inositol	–	–
Mannitol	–	–
Sorbitol	–	–
α-metyl-D-mannoside	–	–
α-metyl-D-glucoside	–	–
N acetylglucosamine	+	–
Amygdalin	+	–
Arbutin	+	–
Esculin	+	–
Salicin	+	–
Cellobiose	+	–
Maltose	+	+
Lactose	–	+
Melibiose	–	+
Sucrose	–	+
Trehalose	+	–
Inulin	–	–
Melizitose	–	–
D-raffinose	–	+
Starch	–	–
Glucogen	–	–
Xylitol	–	–
β-gentiobiose	+	d
D-turanose	–	–
D-lyxose	–	–
D-tagatose	+	–
D-fucose	–	–
D-arabytol	–	–
L-arabytol	–	–
Gluconate	+	+
2-keto-gluconate	–	–
2-keto-gluconate	–	–

endogenous wheat flour enzymes, increasing the bioavailability of the soluble carbohydrates arabinose and xylose. The API test showed that *P. pentosaceus* metabolized both xylose and arabinose, while *L. fermentum* only metabolized arabinose. As result of this fermentation, larger amounts of acid are produced (Rocken, Rick, & Reinkemeyer, 1992).

3.2. Growth kinetics of LAB in honey supplemented sourdough

To evaluate growth kinetics of *P. pentosaceus* and *L. fermentum* in honey supplemented sourdough, dough with and without honey were separately inoculated with each LAB strain and incubated at 32 °C for 19 h. At 0, 6, 12 and 19 h cell counts were made.

Fig. 1 shows the growth kinetics of LAB during sourdough fermentation. Throughout the 19 h fermentation period, a progressive increase in the number of cells was observed for all sourdough. Both *P. pentosaceus* and *L. fermentum* populations of honey supplemented sourdough were statistically ($p < 0.05$) superior than sourdough without honey. Cell counts of honey supplemented

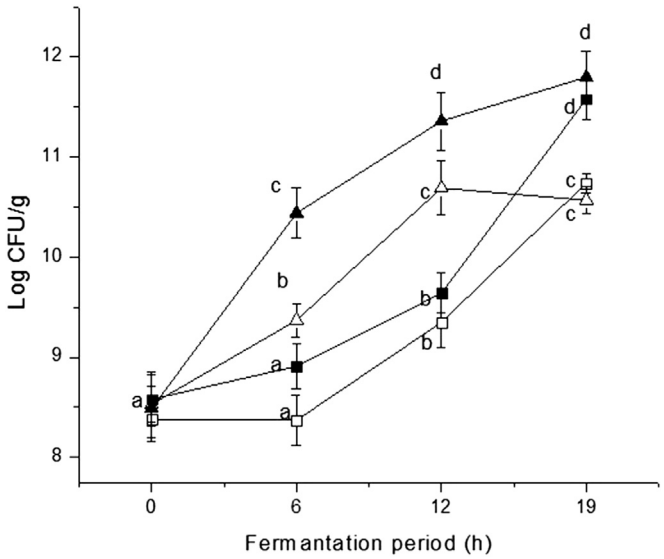

Fig. 1. Growth kinetics. Cell counts (CFU/g) of sourdough inoculated with *P. pentosaceus* with (■) and without honey (□) and sourdough inoculated with *L. fermentum* with (▲) and without honey (Δ) after 19 h of incubation at 32 °C. Different letters are significantly different at $p < 0.05$.

sourdough increased from 8.5 (t_0) to 11.4 log CFU/g (t_{19}), with no statistical differences between strains; whereas the number of cells in sourdough without honey increased to 10.7 log CFU/g (t_{19}), with no statistical differences between strains. These findings indicate that the presence of honey promoted the growth of both *P. pentosaceus* and *L. fermentum*.

In section 3.1. we presented that LAB use some of wheat and rye sugars like glucose, fructose, maltose, arabinose and xylose as metabolic substrates. The energy provided by these carbohydrates is generally used for preservation of cell viability and cell division (Passos, Fleming, Ollis, Felder, & Mc Freeters, 1994), increasing LAB population throughout the fermentative period. Moreover, because honey is mostly constituted upon fructose and glucose, its inclusion into the dough formulation represents a metabolic advantage for the development of both strains.

In addition, honey provides sourdough with compounds known for their antioxidant properties, such as phenolics and flavonoids (Gheldof et al., 2002), which play an important role in human health and food preservation (Ferreira, Aires, Barreira, & Estevinho., 2009), scavenging free radicals and reactive oxygen species (Küçük et al., 2007). It has been reported that phenolic compounds and flavonoids exert prebiotic effects on certain LAB strains (Tabasco et al., 2011). *Lactobacillus plantarum* and *P. pentosaceus* are able to grow in presence of these compounds, and even metabolize them into other components that influence the aroma of foods (Rodríguez et al., 2009; Tabasco et al., 2011).

Despite the fact that populations of both LAB strains were similar at the end of the fermentative period (t_{19}), growth kinetics differed between these bacteria. *L. fermentum* reached maximal growth before *P. pentosaceus*. A clear exponential phase was observed for *L. fermentum* until 12 h of growth. Then, during the following 7 h of fermentation (t_{19}), the population remained approximately constant. Stolz et al. (1995) reported that when fructose was included in the culture medium, *L. fermentum* population reached maximal growth around 12 h of fermentation, when this sugar was absent the time needed for reaching maximal growth doubled. Fructose is used by heterolactic strains as an energetic source; moreover, when glucose is also present in the media, fructose is preferentially used as external electron acceptor

without entering the heterolactic pathway, and increasing LAB growth rate (Richter, De Graaf, Hamann, & Unden, 2003). After 19 h of fermentation, *P. pentosaceus* reached a similar cell number than *L. fermentum* did after 12 h of incubation, showing a slower growth rate.

3.3. Fermentative activity of LAB in honey supplemented sourdough

To investigate the acidic profile of *P. pentosaceus* and *L. fermentum* in honey supplemented sourdough, dough with and without honey were separately inoculated with each LAB strain and incubated at 32 °C for 19 h. At 0, 6, 12 and 19 h pH and TTA were measured.

Fig. 2 illustrates the acidic profile of LAB during sourdough fermentation. The fermentative parameters of non-inoculated dough remained approximately constant throughout incubation. Results showed that acidification of all inoculated sourdough was progressive throughout the fermentative period. Moreover, the presence of 6.5% (w/v) *Prosopis* sp. honey did not significantly ($p < 0.05$) modify the acidification profile exhibited by LAB, indicating that the addition of honey does not interfere with lactic acid production by *P. pentosaceus* and *L. fermentum*. pH values of sourdough fermented by *P. pentosaceus* dropped from 6 (t_0) to 3.8 (t_{19}), whereas pH levels of sourdough fermented by *L. fermentum* reached values of 4.1 (t_{19}) (Fig. 2a). TTA values showed different acidification profiles between LAB strains (Fig. 2b), meaning that pH alone cannot be used as sourdough fermentative activity indicator. TTA measurements showed that acidic production of sourdough fermented by *P. pentosaceus* was significantly ($p < 0.05$) higher than that of *L. fermentum*. The first required a net volume of 9 ml of NaOH 0.1 M to neutralize the system, whilst *L. fermentum* needed about 5 ml to reach that point, consistent with the fact that lactic acid (pKa 3.86) is a stronger acid than acetic acid (pKa 4.78). In addition, between 6 and 12 h of fermentation the slopes of the curves were steeper, meaning that acidification was more pronounced during this period, which is also coincident with a notorious increase in the cell number of both strains (Fig. 1). After 12 h of fermentation, acidic production remained approximately constant.

The different metabolic pathways carried out by these LAB, together with the wider fermentative profile seen for *P. pentosaceus* (Sec 3.1.) contributes to the understanding of the differences in acidic production between these strains, being *P. pentosaceus* the most acidifying microorganism. This strain ferments hexoses by the homolactic pathway, producing 2 mol of lactate. In contrast, heterolactic bacteria, as *L. fermentum*, metabolize hexoses to yield 1 mol of lactate, 1 mol of ethanol or acetate, 1 mol of carbon dioxide (CO_2) (Corsetti & Settanni, 2007). In addition, *P. pentosaceus* is able to ferment flour pentoses (Dobrogosz & DeMoss, 1963), contributing to the acidic production.

3.4. Effect of LAB activity and honey on GMP microstructure

To evaluate possible modifications caused by the fermentative metabolism of LAB and honey's bioactive compounds on GMP microstructure, dough with and without honey were inoculated with *P. pentosaceus* and *L. fermentum*, and incubated at 32 °C for 19 h. The samples were lyophilized and prepared for SEM analyses. Micrographs are shown in Fig. 3.

All sourdough contained small (5 μm) and large (20 μm) starch granules of spherical shape distributed along the protein matrix, as usual in wheat dough.

Micrographs of control dough, without LAB nor honey, showed starch granules immersed in the gluten matrix (Fig. 3a). During the process of dough mixing, certain molecular changes between wheat proteins take place. These modifications involve the formation and breakage of covalent and non-covalent bonds, such as disulphide interchange and hydrogen bonds (Sivam, Sun-Waterhouse, Waterhouse, Quek, & Perera, 2011). Wheat flour contains low molecular weight compounds with thiol (SH) groups, as cysteine and glutathione, which are able to react with the SH groups present in gluten proteins, preventing their oxidative crosslinking (Vermeulen et al., 2006). These activities will influence dough rheology, and thus will have impact on bread quality.

The microstructure of non-inoculated dough with honey addition revealed a strong association between protein subunits, resulting in a more compact gluten network (Fig. 3b). The appearance of dough were consistent with Steffolani et al. (2010) findings, who reported that the microstructure of dough with GOX addition exhibits a continuous and closed gluten network. GOX is one of the enzymes present in honey; whose reaction product, H_2O_2, oxidizes

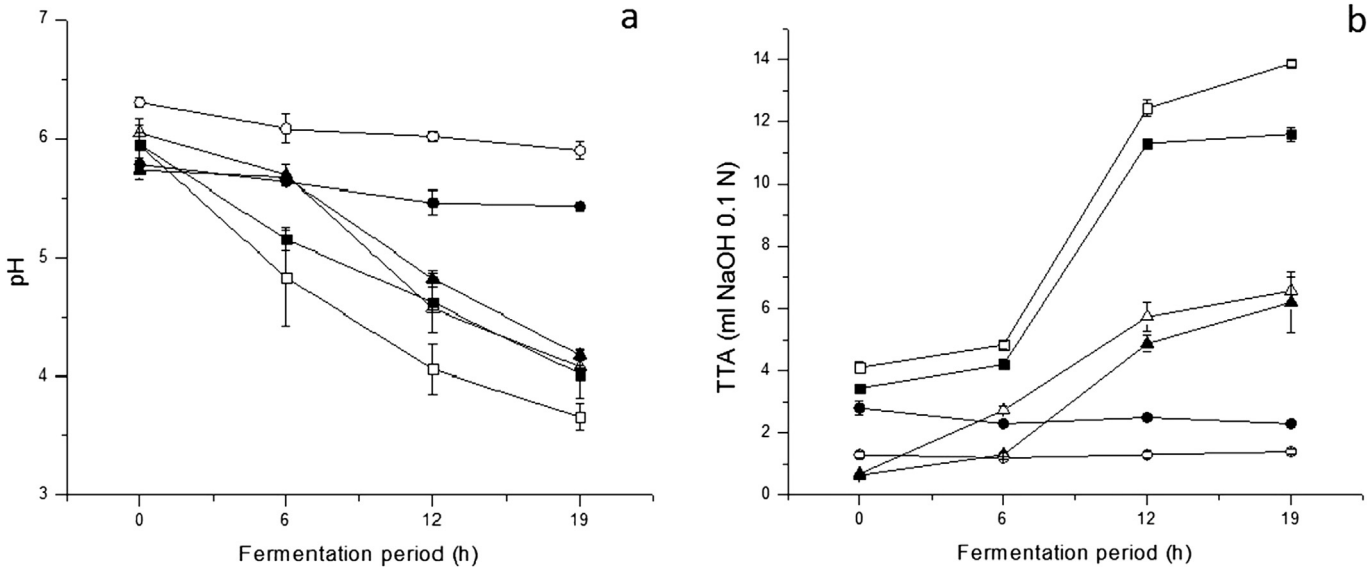

Fig. 2. Fermentative activity of LAB: a) pH and b) TTA values of non-inoculated dough with (●) and without honey (○), sourdough inoculated with *P. pentosaceus* with (■) and without honey (□) and sourdough inoculated with *L. fermentum* with (▲) and without honey (Δ) after 19 h of incubation at 32 °C.

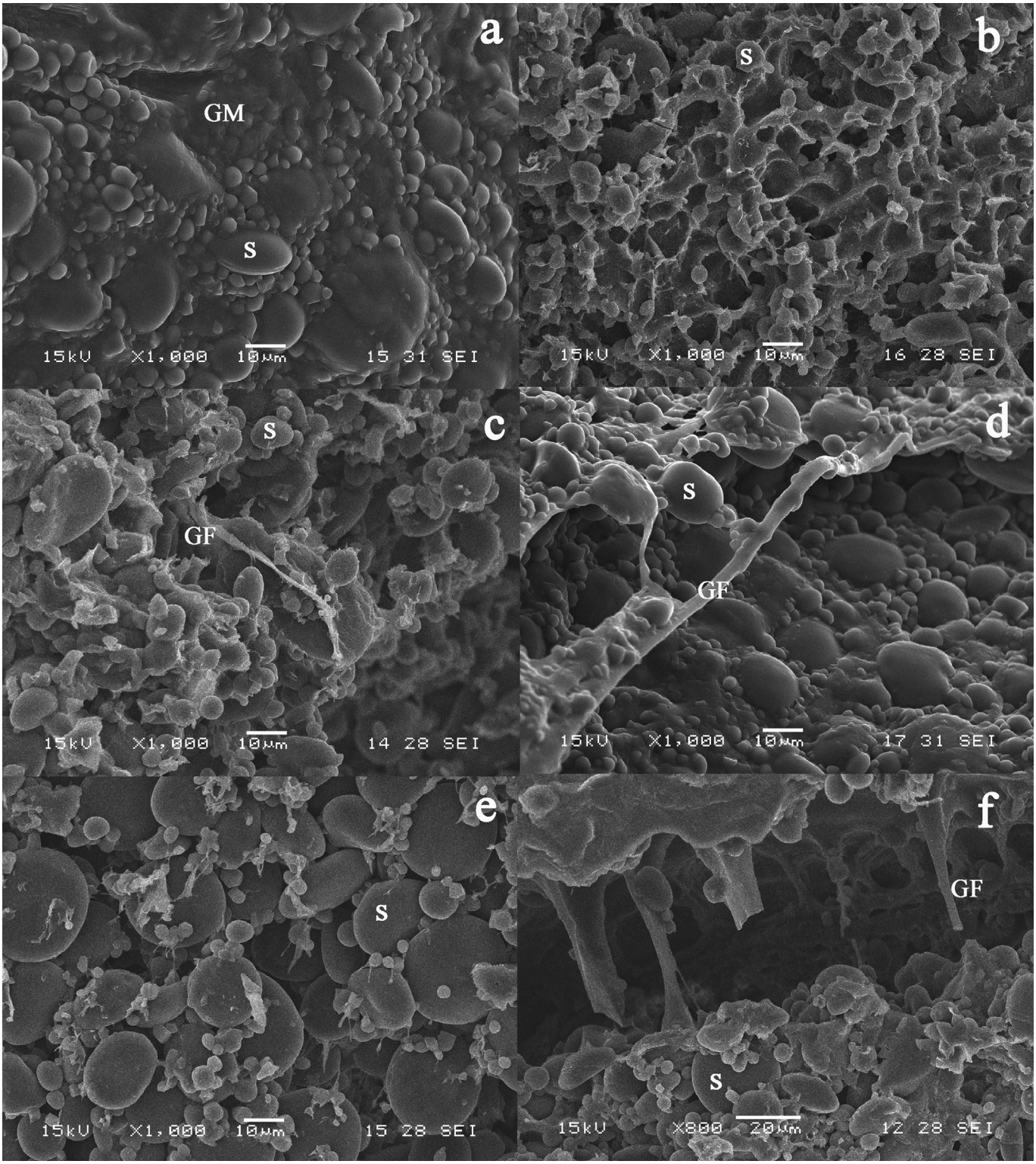

Fig. 3. Scanning electron microscopy (SEM) micrographs of dough a) non-inoculated without honey, b) non-inoculated with honey, c) inoculated with *P. pentosaceus*, d) inoculated with *P. pentosaceus* with honey, e) inoculated with *L. fermentum*, d) inoculated with *L. fermentum* with honey, after 19 h of incubation at 32 °C. S: starch granule, GM: gluten matrix, GF: gluten fibril.

SH groups of two cysteine residues to form disulphide bonds (Joye, Bert, & Delcour., 2009; Rasiah et al., 2005), which lead to protein crosslinking (Bonet et al., 2006). It has been found that the addition of GOX improves dough rheology; however high levels of this enzyme may cause over-oxidation, affecting bread quality (Bonet et al., 2006; Joye et al., 2009).

pH values of non-inoculated dough, with and without honey addition, remained approximately constant throughout the

incubation period (5.37 and 6.1, respectively). At such pH levels, wheat proteases are not active, whereas GOX activity is favoured. This suggests that honey could play a role in glutenin reorganization during dough kneading, being responsible for the modifications observed in dough microstructure.

Sourdough fermented by *P. pentosaceus* showed an organized gluten matrix, in which fibrils appeared associated to the starch granules (Fig. 3c). During their proliferation, LAB produce organic acids that lower pH values of sourdough (Németh, Adányi, Haláz, Váradi, & Szendró, 2007). Under such acidic conditions, endogenous proteases of wheat are able to hydrolyse gluten proteins (Bleukx & Delcour, 2000; Gerez et al., 2012), allowing GMP depolymerisation (Vermeulen et al., 2006), which is essential to promote the development of a gluten network, and thus improve the viscoelastic properties of dough. Sourdough fermented by this strain reached pH values that are optimal for wheat protease activity (4.06 after 12 h of fermentation), in accordance with the generation of gluten fibrils observed in Fig. 3c.

The microstructure of honey supplemented sourdough fermented with *P. pentosaceus* showed numerous protein aggregates, consistent with a less depolymerised gluten matrix (Fig. 3d). These dough had a lower degree of acidification than dough without honey (pH 4.62 after 12 h of fermentation), shifting away from the optimal pH value necessary to ensure a good activity of wheat proteases, which limits the degree of gluten depolymerisation. Moreover, honey did not show an impact on the microstructure of these sourdough, the pH value reached in this system is too low to allow GOX activity.

On the other hand, the microstructure of sourdough inoculated by *L. fermentum* presented cavities corresponding to CO_2 bubbles, generated as metabolic subproduct during heterolactic fermentation. Sourdough fermented by this strain, without honey addition, exhibited a highly disrupted structure that inhibited the development of fibrils. The GMP appears broken with no cohesive structure associated (Fig. 3e). Heterofermentative lactobacilli are able to express glutathione reductase enzyme, capable of reducing the oxidized glutathione, preventing gluten protein crosslinking (Vermeulen et al., 2006). It has been suggested that glutathione activity unfolds gluten proteins, making them more accessible for the hydrolysis of wheat flour proteases.

Honey supplemented sourdough inoculated with *L. fermentum* presented thick oriented gluten fibrils (Fig. 3f), showing a more cohesive structure in comparison to inoculated dough without honey. The microstructural appearance of these dough was similar to those obtained for non-inoculated dough with honey addition (Fig. 3b). The acidification of sourdough fermented by this strain was lower than that of *P. pentosaceus*, therefore we can suggest that GOX activity minimizes or prevents the glutathione effect on GMP.

4. Conclusions

In order to promote human health, the interest of using natural food products with functional properties has been increased. The results of microbiological, chemical, and microstructural analyses presented in this study describe the effect of honey on sourdough fermenting LAB, and the impact that LAB and honey have on gluten microstructure. The growth kinetics of *P. pentosaceus* and *L. fermentum* was positively affected by the presence of 6.5% (w/v) *Prosopis* sp. honey. In spite of having similar population number after 19 h of fermentation, *P. pentosaceus* was more acidifying than *L. fermentum*. The latter, on the other hand, exhibited a higher growth rate than *P. pentosaceus*. In reference to sourdough SEM analysis, honey did not introduce substantial changes in the microstructure of *P. pentosaceus* fermented sourdough; however, it had an impact in those inoculated with *L. fermentum*, improving their cohesiveness, and thus, their quality. In this study, in order to enhance the crosslinking of proteins, we present an alternative to the use of purified enzymes in sourdough formulation. We provide evidence of the potential that a natural and traditional product as honey has to modify the microstructure of wheat proteins, without preventing acidic production by LAB, which is essential to provide the organoleptic characteristics for what sourdough is known for.

Acknowledgments

We gratefully acknowledge the financial support of the Universidad Nacional of Mar del Plata (15/E659 - EXA 709/14) for the research project Mechanisms of antioxidant and antibacterial activity of honey Part II.

References

Barber, S., Baguena, R., Benedito de Barber, C., & Martinez-Anaya, M. A. (1991). Evolution of biochemical and rheological characteristics and breadmaking quality during a multistage wheat sour dough process. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 192*, 46–52.

Bleukx, W., & Delcour, J. A. (2000). A second aspartic proteinase associated with wheat gluten. *Journal of Cereal Science, 32*, 31–42.

Bonet, A., Rosell, C. M., Caballero, P. A., Gómez, M., Pérez-Munuera, I., & Lluch, M. A. (2006). Glucose oxidase effect on dough rheology and bread quality: A study from macroscopic to molecular level. *Food Chemistry, 99*, 408–415.

Butterfield, C. T. (1932). The selection of a dilution water for bacteriological examinations. *Journal of Bacteriology, 23*, 355–368.

Corsetti, A., & Settanni, L. (2007). Lactobacilli in sourdough fermentation. *Food Research International, 40*, 539–558.

Di Cagno, R., De Angelis, M., Corsetti, A., Lavermicocca, P., Arnault, P., Tossut, P., et al. (2003). Interactions between sourdough lactic acid bacteria and exogenous enzymes: Effects on the microbial kinetics of acidification and dough textural properties. *Food Microbiology, 20*, 67–75.

Dobrogosz, W. J., & DeMoss, R. D. (1963). Pentose utilization by Pediococcus pentosaceus. *Journal of Bacteriology, 85*, 1356–1364.

Ferreira, I. C. F. R., Aires, E., Barreira, J. C. M., & Estevinho, L. M. (2009). Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food Chemistry, 114*, 1438–1443.

Gerez, C. L., Dallagnol, A., Rollán, G., & Font de Valdez, G. (2012). A combination of two lactic acid bacteria improves the hydrolysis of gliadin during wheat dough fermentation. *Food Microbiology, 32*, 427–430.

Gheldof, N., Wang, X. H., & Engeseth, N. (2002). Identification and quantification of antioxidant components of honeys from various floral sources. *Journal of Agricultural and Food Chemistry, 50*, 5870–5877.

Girhammar, U., & Nair, B. M. (1992). Isolation, separation and characterization of water soluble non-starch polysaccharides from wheat and rye. *Food Hydrocolloids, 6*, 285–299.

Isla, M. A., Craig, A., Ordoñez, R., Zampini, C., Sayago, J., Bedascarrasbure, E., et al. (2011). Physicochemical and bioactive properties of honeys from Northwestern Argentina. *LWT-Food Science and Technology, 44*, 1922–1930.

Joye, I. J., Bert, L., & Delcour, J. A. (2009). Use of chemical redox agents and exogenous enzymes to modify the protein network during breadmaking: A review. *Journal of Cereal Science, 50*, 11–21.

Küçük, M., Kolayli, S., Karaoglu, S., Ulusoy, E., Baltaci, C., & Candan, F. (2007). Biological activities and chemical composition of three honeys of different types from Anatolia. *Food Chemestry, 100*(2), 526, 253.

Németh, E., Adányi, N., Haláz, A., Váradi, M., & Szendró, I. (2007). Real-time study of the effect of different stress factors on lactic acid bacteria by electrochemical optical waveguide lightmode spectroscopy. *Biomolecular Engineering, 24*, 631–637.

Nutter, J., Fritz, R., Iurlina, M. O., & Saiz, A. I. (2016). Effect of Prosopis sp. honey on the growth and fermentative ability of *Pediococcus pentosaceus* and Lactobacillus fermentum. *LWT- Food Science and Technology, 70*, 309–314.

Ouchemoukh, S., Schweitzer, P., Bey, B. B., & Djoudad-Kadji, H. (2010). HPLC sugar profiles of Algerian honeys. *Food Chemistry, 121*, 561–568.

Passos, F. V., Fleming, H., Ollis, D. F., Felder, R. M., & Mc Freeters, R. F. (1994). Kinetics and modeling of lactic acid production by *Lactobacillus plantarum*. *Applied and Envirnomental Microbiology, 60*, 2627–2636.

Rasiah, I. A., Sutton, K. H., Low, F. L., Lin, H. M., & Gerrard, J. A. (2005). Crosslinking of wheat dough proteins by glucose oxidase and the resulting effects on bread and croissants. *Food Chemestry, 89*, 325–332.

Richter, H., De Graaf, A. A., Hamann, I., & Unden, G. (2003). Significance of phosphoglucose isomerase for the shift between heterolactic and mannitol fermentation of fructose by *Oenococcus oeni*. *Archives of Microbiology, 180*, 465–470.

Rizzello, C. G., Curiel, I. A., Nionelli, L., Vicentini, O., Di Cagno, R., Silano, M., et al. (2013). Use of fungal proteases and selected sourdough lactic acid bacteria for making wheat bread with an intermediate concent of gluten. *Food Microbiology, 30*, 1–10.

Rocken, W., Rick, M., & Reinkemeyer, M. (1992). Controlled production of acetic acid in wheat sour doughs. *Zeitschriftfür Lebensmittel Untersuchungund Forschung, 195*, 259–263.
Rodríguez, H., Curiel, J. A., Landete, J. M., de las Rivas, B., López de Felipe, F., Gómez-Cordovés, C., et al. (2009). Food phenolics and lactic acid bacteria. *International Journal of Food Microbiology, 132*, 79–90.
de la Rosa, L. A., Alvarez-Parrilla, E., Moyers-Montoya, E., Villegas-Ochoa, M., Ayala-Zavala, J. F., Hernández, J., et al. (2011). Mechanism for the inhibition of apple juice enzymatic browning by Palo Fierro (desert ironweed) honey extract and other natural compounds. *LWT-Food Science and Technology, 44*, 269–276.
Schepartz, A., & Subers, M. H. (1963). The glucose oxidase of honey: Purification and some general properties of the enzyme. *Biochimica et Biophysica acta, 85*, 22–237.
Sivam, A. S., Sun-Waterhouse, D., Waterhouse, G. I. N., Quek, S. Y., & Perera, C. O. (2011). Physicochemical properties of bread dough and finished bread with added pectin fiber and antioxidants. *Journal of Food Science, 76*, 97–107.
Steffolani, M. E., Ribotta, P. D., Pérez, G. T., & León, A. E. (2010). Effect of glucose oxidase, transglutaminase, and pentosanase on wheat proteins: Relationship with dough properties and bread-making quality. *Journal of Cereal Science, 51*, 366–373.
Stolz, P., Vogel, R. F., & Hammes, W. P. (1995). Utilization of electron acceptors by lactobacilli isolated from sourdough. *Zeitschrift fur Lebensmittel-Untershung und-Forschung, 201*, 91–96.
Tabasco, R., Sánchez-Patán, F., Monagas, M., Bartolomé, B., Moreno-Arribas, M. V., Peláez, C., et al. (2011). Effect of grape polyphenols on lactic acid bacteria and bifidobacteria growth: Resistance and metabolism. *Food Microbiology, 28*, 1345–1352.
Vermeulen, N., Kretzer, J., Machaliza, H., Vogel, R. F., & Gänzle, M. G. (2006). Influence of redox-reactions catalysed by homo- and hetero-fermentative lactobacilli on gluten in wheat sourdough. *Journal of Cereal Science, 43*, 137–143.
Wilson, R., & Turner, A. P. F. (1992). Glucose oxidase: An ideal enzyme. *Biosensors and Bioelectronics, 7*, 165–185.