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Effect of *Prosopis* sp. honey on the growth and fermentative ability of *Pediococcus pentosaceus* and *Lactobacillus fermentum*

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ABSTRACT

Honey is widely known for having antimicrobial and antioxidant activity. These abilities are attributed to honey hydrogen peroxide (H_2O_2) and polyphenols. Polyphenols also exert beneficial effect on some species of lactic acid bacteria (LAB). In this study, we evaluate the effect of *Prosopis* sp. honey on the growth and fermentative activity of *Pediococcus pentosaceus* and *Lactobacillus fermentum*. *Prosopis* sp. honey was found to be an important source of phenolic compounds, especially flavonoids, being their average content superior to other honeys. LAB assessed in this study exhibited different responses to the presence of honey. *P. pentosaceus* was able to develop in concentrations of honey up to 25% (w/v), whilst *L. fermentum* showed high sensitivity, being affected both growth and fermentative activity. However, as a result of LAB fermentative capacity, the total phenolic and flavonoid content present in 6.5% (w/v) honey solutions was increased, improving the antioxidant activity of this system.

1. Introduction

Honey has been extensively studied for having effective antimicrobial activity against many pathogens (Al-Waili, 2004; Shamala, Shri Jyothi, & Saibaba, 2000; Taormina, Niemira, & Beuchat, 2001). It is a natural complex system constituted of fructose (38% w/v) and glucose (31% w/v) as major compounds, and of several minor compounds as disaccharides, oligosaccharides, organic acids, amino acids, proteins, enzymes (glucose oxidase, catalase, etc.), and phenolic compounds such as flavonoids and phenolic acids (Gheldof, Wang, & Engeseth, 2002). Some of these constituents are from bee origin, and some others derived from the plant (Ouchemoukh, Schweitzer, Bey, & Djoudad-Kadji, 2010), so the bioactive profile of honeys varies along with the botanical source. The antibacterial activity of most honeys is attributed to hydrogen peroxide (H_2O_2) (Brudzynski, Abubaker, & Miotto, 2012; White, Riethof, Subers, & Kushnir, 1962) and phenolic compounds (Al-Waili, 2004; Isla et al., 2011), which lead to oxidative damage of biomolecules and cells (Smirnova, Samoylova, Muzyka, & Oktyabrsky, 2009) and alter the cytoplasmic membrane and cell walls (Rodriguez et al., 2009), respectively. Hydrogen peroxide is mainly produced by glucose oxidase enzyme when honey is diluted from full strength (Bang, Buntting, & Molan, 2003). Phenolic autooxidation provides an additional source of H_2O_2 in honeys (Brudzynski et al., 2012). In addition, honey phenolic compounds are effective antioxidants able to scavenge free-radicals and reactive oxygen species (Gheldof et al., 2002; Iurlina, Saiz, Fritz, & Manrique, 2009; Kishore, Halim, Syazana, & Sirajudeen, 2011; Küçük et al., 2007). Antioxidants not only play an important role in human health but also in food preservation (Ferreira, Aires, Barreira, & Estevinho, 2009). They can prevent the enzymatic browning of fruit and juices, delay lipid oxidation in meat (Gheldof et al., 2002; de la Rosa et al., 2011), and contribute to the aroma and colour of foods (Burda & Oleszek, 2001; Procházková, Bousosvá & Wilhelmová et al., 2011; Rodriguez et al., 2009). Other honey constituents can modify some organoleptic and functional properties of foods as well. Several authors have reported that glucose oxidase and its reaction product, H_2O_2, improve the quality of baked products by modifying gluten proteins through crosslinking. (Bonet et al., 2006; Rasiah, Sutton,Low, Lin & Gerrard, 2005). In addition, honey organic acids are involved in flavour development as well (Suarez-Luque, Mato, Huidobro, & Simal-Lozano, 2002).

It has been found that phenolic compounds exert beneficial effect on some species of LAB (Rodriguez et al., 2009; Tabasco et al., 2011; Zhao & Shah, 2014). These bacteria constitute a small part of

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the autochthonous microbiota of plant origin products, so they are likely adapted to intrinsic characteristics of the raw materials (Rodriguez et al., 2009). LAB are microorganisms widely used in the food industry, mainly because of their metabolic activities, which have an impact on food products. They are adapted to live in microaerophilic environments rich in nutrients that include sugars and amino acids (Vrancken, Rimaux, DeVuyst, & Leroy, 2008). In this way, honey provides an important energy source for the proper development of these bacteria.

Exploring the behaviour of LAB in presence of honey will be suitable to optimize fermentative processes and to obtain foods with improved nutritional properties. In this work, we evaluate the effect of bioactive compounds naturally occurring in honey on the growth and fermentative activity of two LAB, *Pediococcus pentosaceus* and *Lactobacillus fermentum.*

2. Materials and methods

2.1. Materials

2.1.1. Reagents and standards

All chemical reagents used were of analytic grade. Folin-Cioclateu 2 N solution, catalase enzyme, gallic acid, quercetin, and luteolin, were provided by Sigma–Aldrich (USA). Myricetin was acquired from Fluka (Switzerland). De Man, Rogosa, and Sharpe broth (MRS broth) and MRS agar were obtained from Britania (Argentina). Methanol was of HPLC grade, conserved at −20 °C, and protected from light.

2.1.2. Bacterial strains

Two strains of LAB, *P. pentosaceus* (CRL 922) and *L. fermentum* (CRL 220), provided by Centro de Referencia para Estudios de Bacterias Lácticas (CERELA, Tucumán, Argentina), were used in this study.

2.1.3. Source of honey sample

The honey sample used in this study was directly collected from beehives by natural decantation and kept at 4 °C until its use. The sample came from San Luis Province (33° 17′ S - 66° 22′ W), Argentina. This area is located in the west-centre plains of Argentina; it corresponds to the phytogeographical region known as pampean meadow.

2.2. Methods

2.2.1. Growth conditions

LAB strains were separately grown in 9 ml of MRS broth. Incubation was carried out anaerobically at 32–35 °C for 19 h, using an anaerobic jar and Anaerocult® C. The cultures were standardized in Butterfield's phosphate buffered dilution water (0.25 M KH_2PO_4, pH 7.2, Butterfield, 1932) until a turbidity equivalent 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). This inoculum was also diluted until bacterial concentrations of 10^5 and 10^3 cfu/ml.

2.2.2. Honey sample

2.2.2.1. Pollen analysis. The botanic origin of the honey sample used in this study was determined by microscopic analysis, according to Louveraux, Maurizio, and Vorwhol (1978). The determination of pollen frequency classes in honey was performed by optical microscopy. According to frequency classes, pollen types can be classified as dominant pollen (>45% of total pollen), secondary pollen (45–16%), pollen of minor importance (15–3%), and pollen traces (<3%) (Tellería, 1996). Monofloral honeys are considered as such, whenever the dominant pollen is found over 45% of the total pollen content (Sabatino, Iurlina, Eguaras, & Fritz, 2006).

2.2.2.2. Total phenolic and flavonoid content of honey

2.2.2.2.1. Sample preparation. For total phenolic and flavonoid determination, 0.1 g of honey was diluted in 1 ml of methanol. This solution was homogenized and centrifuged (9000 rpm, 5 min). The supernatant was reserved for further analyses.

2.2.2.2.2. Total phenolic content. Total phenolic content was determined by the Folin-Cioclateu (FC) reagent (Singleton, Orthofer & Lamuela-Raventos, 1999). The FC reagent is reduced by the abstraction of an electron from the antioxidants present in the sample, causing colour changes, which are detected spectrophotometrically. Absorbance was measured at 765 nm with a UV–visible spectrophotometer (Shimadzu, UV-2101-PC). Gallic acid standard solutions were used to construct the calibration curve. Total phenolic content was expressed as mg gallic acid equivalents per 100 g honey (mg GAE/100 g honey).

2.2.2.2.3. Total flavonoid content. Total flavonoid content was determined using the method described by Meda, Lamien, Romito, Millongo, and Nalcouma (2005). Briefly, 1 ml of the supernatant (2.2.2.2.1.) was added to 5 ml of 2% (w/v) aluminium chloride ($AlCl_3$) in methanol, and it was incubated for 10 min. Hydroxyl groups present in the flavonoid molecule react with $AlCl_3$ to form a complex, detected spectrophotometrically. Absorbance was measured at 425 nm with a UV–visible spectrophotometer (Shimadzu, UV-2101-PC). Quercetin standard solutions were used to construct the calibration curve. Total flavonoid content was expressed as mg quercetin equivalents per 100 g honey (mg QE/100 g honey).

2.2.3. Effect of honey on LAB growth and fermentative activity

2.2.3.1. Honey solutions. To evaluate the effect of bioactive compounds present in honey on the growth of LAB, *Prosopis* sp. honey solutions (identified according to 2.2.2.1.) were made in different concentrations (6.5, 25, 50, and 75% w/v). The solutions were prepared dissolving honey in sterile MRS broth and were filtered by sterile Millipore filters (GSWPO25, 0.22 μm of pore, MF-Millipore) to avoid antagonistic or symbiotic interactions due to the presence of other bacteria. To evaluate the effect of honey sugars, 80% (w/v) artificial honey solution was made dissolving 40 g of fructose, 30 g of glucose, 8 g of maltose, and 2 g of sucrose in deionized water. MRS broth solution was prepared as a control.

2.2.3.2. Effect of honey on LAB growth. Honey and artificial honey solutions were separately inoculated with each LAB strain (2.1.2.) in three different concentrations (10^3, 10^5, 10^8 cfu/ml), and were anaerobically incubated at 30 °C for 19 h. The standard pour plate technique, using MRS agar, was employed to determine viable cell counts. Inoculated plates were anaerobically incubated at 32–35 °C for 72 h. Cell counts were performed before (t_0) and after (t_{19}) incubation. The results were reported as logarithm colony forming units per millilitre (log cfu/ml).

2.2.3.3. Effect of catalase-treated honey solutions on LAB growth. The effect of honey bioactive compounds, different from H_2O_2, on LAB growth was studied by treating 25% (w/v) honey solutions with 0.2% (w/v) catalase enzyme (2280 units/mg protein). 1 ml of each LAB strain (2.2.1.) was added to catalase-treated honey solutions. Cell counts were performed before (t_0) and after (t_{19}) incubation. The results were reported as log cfu/ml.

2.2.3.4. Effect of honey on LAB fermentative activity. The fermentative activity of LAB in presence of honey was evaluated by measuring the acidification of honey solutions (prepared according

to 2.2.3.1). Honey solutions were inoculated, incubated at 30 °C for 19 h, and centrifuged (4000 rpm, 10 min). From the supernatant, pH and total titrable acidity (TTA) were measured. pH was determined using a pH-meter (Hanna instruments HI 9321) and TTA was measured by potentiometry, neutralizing the supernatant with 0.1 M NaOH. TTA results were expressed as ml of NaOH 0.1 M. Measurements were performed before (t_0) and after (t_{19}) incubation.

2.2.4. Effect of LAB on the phenolic and flavonoid content of honey

To evaluate the effect of LAB on the phenolic and flavonoid content of *Prosopis* sp. honey, 6.5% (w/v) honey solutions were inoculated with *P. pentosaceus* and *L. fermentum* (1.5×10^8 cfu/ml) and were anaerobically incubated at 30 °C for 19 h. The concentration of honey selected for this assay was chosen according to results obtained in Sections 3.3. and 3.5. The solutions were centrifuged (4500 rpm, 10 min) before (t_0) and after (t_{19}) incubation, and total phenolic and flavonoid content was determined from the supernatant (2.2.2.2.1.).

2.2.5. 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, $p < 0.05$ was considered significant.

3. Results and discussion

3.1. Pollen analysis

The botanical identity of the surveyed honey was determined according to the predominant pollen found on it. Microscopic analysis exhibited dominance of *Prosopis* sp. pollen in a range of 46 and 53%, which enabled its classification as monofloral *Prosopis* sp. honey. In addition, the sample exhibited pollen contribution from *Schinus* sp., with an incidence of 16% (secondary pollen). *Prosopis* sp. is one of the pollen contributors found by Iurlina et al. (2009) in mixed honeys (pollen contribution <32%) from other Provinces of Argentina (Mendoza −32° 52′S-68° 49′W- and Santiago del Estero −27° 46′S-64° 16′W). Geo-climatic features determine the dominance of botanical species in a particular region, so the pollinic and nectar content define a specific bioactive profile. The honey sample used in this study came from a region characterized for having a transitional climate between semi-humid and arid, with average temperature levels of 20 °C, and rain levels between 340 and 1100 mm a year (Cabrera, 1971). It has been reported that sunny and dry climates, as found in this region, provide favourable conditions for phenolic synthesis (Tsanova-Savova & Ribanova, 2002).

3.2. Total phenolic and flavonoid content of honey

Prosopis sp. honey showed significant total phenolic and flavonoid content, with average values of 82.53 ± 3.13 mg GAE/100 g honey and 7.73 ± 0.31 mg QE/100 g honey, respectively. These values were considerably higher than that of other mono- and multifloral honeys, which range from 0.196 to 72.2 mg GAE/100 g honey (Ferreira et al., 2009; Gheldof et al., 2002) and from 2.12 to 6.35 mg QE/100 g honey (Yao et al. 2004), respectively. These spectrophotometric measurements allowed a suitable determination of the total phenolic and flavonoid content. The latter fraction includes the major flavonoids of honey, quercetin, myricetin and luteolin. In unpublished HPLC studies, we quantified the major flavonoid profile of *Prosopis* sp. honey, establishing a correlation between both analytical methods. The content of each flavonoid in monofloral *Prosopis* sp. honey was 0.69 mg quercetin, 0.68 mg myricetin, and 0.27 mg luteolin per 100 g of honey. These values (concentration and relative proportion, 2:2:1) were similar to those reported by Iurlina et al. (2009) for mixed *Prosopis* sp. honeys from Mendoza. Whilst, mixed *Prosopis* sp. honeys from Santiago del Estero exhibited a lower content of flavonoids in a different relative proportion (1:3:2) (Iurlina et al., 2009). The geographical proximity between San Luis and Mendoza suggested that geo-climatic features are essential to determine the phenolic and flavonoid profile of honeys. These results showed that monofloral *Prosopis* sp. honey is an important natural source of compounds known for having antioxidant activity. It has been described that low concentrations of phenolic compounds (~6.8 mg GAE/100 g honey) exert prooxidant activity, promoting the oxidation of other compounds (Brudzynski et al., 2012; Procházková, Bousosvá, & Wilhelmová, 2011); while honeys with higher phenolic content (~45 mg GAE/100 g honey) behave as antioxidants rather than prooxidants, preventing and delaying oxidative processes (Brudzynski et al., 2012). In addition, several authors proposed that phenolic compounds promote the growth of certain LAB (Rodriguez et al., 2009; Tabasco et al., 2011; Zhao & Shah, 2014), while others found that these compounds inhibit LAB development (Tabasco et al., 2011).

3.3. Effect of honey on LAB growth

Three different LAB inoculum concentrations were used to evaluate the growth response of *P. pentosaceus* and *L. fermentum* to honey. LAB initial concentrations of 10^3 and 10^5 cfu/ml showed complete growth inhibition after 19 h of fermentation (data not shown). Meanwhile, bacterial initial concentration of 10^8 cfu/ml exhibited different growth results depending on the honey concentration used and the bacterial strain tested. The growth response of *P. pentosaceus* and *L. fermentum* (10^8 cfu/ml) to honey after 19 h of fermentation is shown in Fig. 1.

At t_0, cell counts of all solutions studied were about 8 log cfu/ml (data not shown). After incubation (t_{19}), the growth of both *P. pentosaceus* and *L. fermentum* was significant in control MRS broth, reaching about 12 log cfu/ml. In addition, the growth of

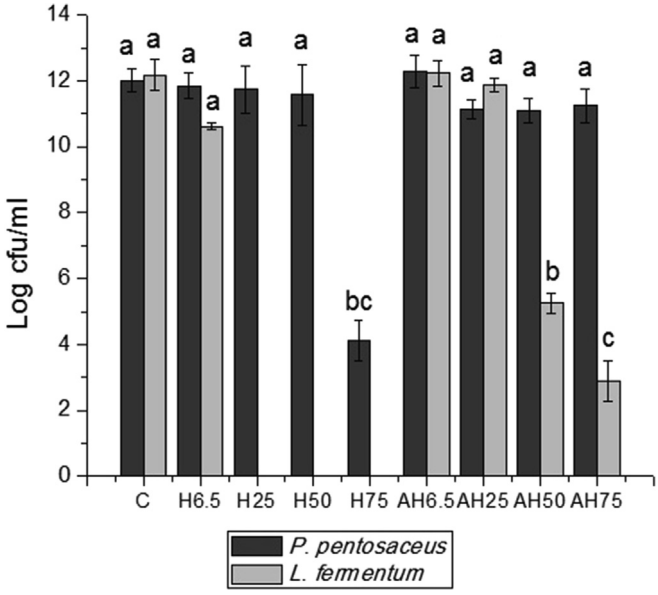

Fig. 1. Growth of *P. pentosaceus* and *L. fermentum* cultured in control MRS broth (C), 6.5–75% *Prosopis* sp. honey (H6.5-75), and artificial honey (AH6.5-75) solutions. Means followed by different letters are significantly different at $p < 0.05$.

P. pentosaceus in 6.5–50% honey solutions was remarkably raised, being cell counts between 11.58 and 11.80 log cfu/ml (no significant differences were registered between honey concentrations).

LAB ferment the main constituents of honey, fructose and glucose, via the heterolactic or homolactic pathway (Corsetti & Settanni, 2007). It has been described that the presence of certain oligosaccharides in honey provides it with potential prebiotic activity, increasing the population of *Bifidobacteria* and *Lactobacilli* (Sanz et al., 2005). In this sense, honey constitutes a significant energetic source for LAB development. Moreover, *Prosopis* sp. honey displayed high phenolic and flavonoid content in comparison with several other Argentinian honeys (3.2.). It has been found that natural extracts rich in flavonoids promote the growth of *Lactobacillus plantarum* and *P. pentosaceus.* The first metabolizes flavonoids, giving rise to compounds that exert significant antioxidant activity and influence the aroma of foods (Rodriguez et al., 2009; Tabasco et al., 2011). Whilst, the latter metabolizes quercetin through absorption or by transport into the cell, when the culture media is dosed with this aglycone (LoCascio, Mills, & Waterhouse, 2006).

The growth of *P. pentosaceus* was only inhibited in 75% honey solutions, being average cell counts about 4.12 log cfu/ml.

On the other hand, the growth of *L. fermentum* was completely inhibited by the presence of honey in concentrations of 25–75%, with no cell counts registered. In contrast, the population of *L. fermentum* was increased (10.62 log cfu/ml) when grown in 6.5% honey solutions.

It is well known that the antibacterial activity of honey is mostly attributable to H_2O_2 produced as a result of the oxidation of glucose by glucose oxidase enzyme (Brudzynski et al., 2012; Isla et al., 2011; Taormina et al., 2001; White et al., 1962). The maximal accumulation of H_2O_2 occurs when honey is diluted to concentrations between 30 and 50% (v/v) (Bang et al., 2003). Moreover, H_2O_2 is formed by autooxidation of phenolic compounds as well (Brudzynski et al., 2012). These compounds constitute the plant-derived components responsible for non-H_2O_2 antimicrobial activity of honey, so this activity varies according to the botanical origin of honey (Molan, 1992). Tabasco et al. (2011) reported that phenolic compounds inhibit the growth of some LAB strains as *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Lactobacillus vaginalis* and *L. fermentum*. The latter has showed sensitivity to grape seed extracts rich flavonoids.

The artificial honey assays were aimed to evaluate the contribution of honey sugars to the growth response of LAB. The growth of *P. pentosaceus* was not restricted at any of the concentrations surveyed (cell counts were ~11.7 log cfu/ml). These results excluded osmolarity as the main inhibitory agent of 75% honey solutions. Enzymatically produced H_2O_2 was excluded as well, because glucose oxidase is not fully active at such honey concentration (Bang et al. 2003). This points out at non-H_2O_2 compounds, like phenolics, for growth inhibition of 75% honey solutions. On the other hand, the growth of *L. fermentum* was remarkably increased when grown in 6.5 and 25% artificial honey solutions (cell counts were ~12.3 cfu/ml), whilst higher concentrations caused partial growth inhibition. Thus, bioactive compounds are the main responsible for growth inhibition of this strain.

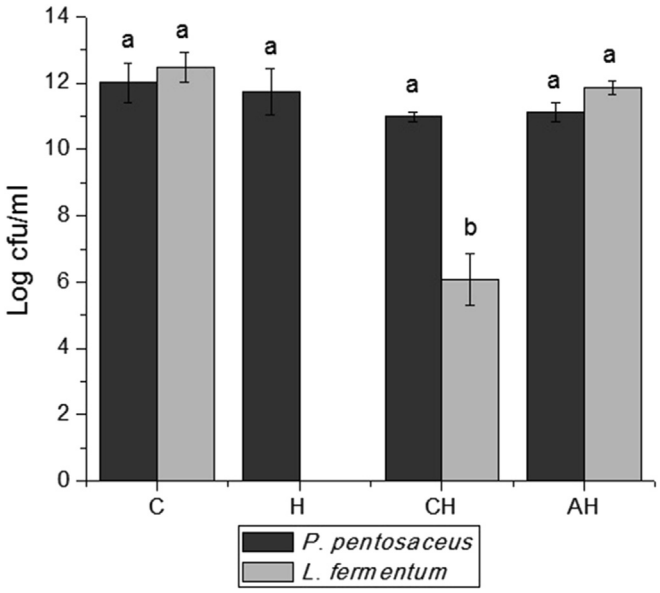

Fig. 2. Growth of *P. pentosaceus* and *L. fermentum* cultured in control MRS broth (C), 25% *Prosopis* sp. honey solutions (H), 25% *Prosopis* sp. honey solutions treated with catalase enzyme (CH), and 25% artificial honey solutions (AH). Means followed by different letters are significantly different at $p < 0.05$.

3.4. Effect of catalase-treated honey solutions on LAB growth

The effect of honey bioactive compounds, different from H_2O_2, on the growth of LAB was studied by treating 25% honey solutions with catalase enzyme. The growth response of *P. pentosaceus* and *L. fermentum* is shown in Fig. 2.

P. pentosaceus population in 25% catalase-treated honey solutions (10.29 log cfu/ml) was approximately the same than that of honey and artificial honey solutions. These results show the ability of *P. pentosaceus* to grown in presence of honey bioactive compounds. This behaviour is likely related to the action of non-heme catalase enzyme, found in 11 strains of *P. pentosaceus* (Whittenburry, 1964) and to the phenolic resistance reported for this stain (LoCascio et al., 2006).

On the other hand, *L. fermentum* was partially inhibited when grown in catalase treated honey solutions (6.7 log cfu/ml). Same concentration of honey showed complete growth inhibition; meanwhile in artificial honey solutions cell counts were about 12 log cfu/ml. This suggests that H_2O_2 was responsible for much of honey inhibitory effect but not for all of it. Many studies reported that the addition of catalase to remove H_2O_2 does not abolish antibacterial activity of honey (Al-Waili, 2004; Taormina et al.,

Table 1
pH and TTA values of 6.5–75% *Prosopis* sp. honey (H6.5-75) and artificial honey (AH6.5-75) solutions inoculated by *P. pentosaceus* (PP) and *L. fermentum* (LF). Means followed by different letters are significantly different at $p < 0.05$.

		Control	H6.5	H25	H50	H75	AH6.5	AH25	AH50	AH75
Initial values	pH	5.9 ± 0.1^{a}	5.4 ± 0.0^{ab}	5.3 ± 0.0^{b}	4.7 ± 0.0^{c}	4.4 ± 0.0^{c}	5.4 ± 0.0^{ab}	6.0 ± 0.1^{a}	5.9 ± 0.1^{a}	5.1 ± 0.0^{b}
	TTA	5.4 ± 0.1^{e}	7.7 ± 1.5^{e}	8.3 ± 0.3^{de}	9.3 ± 0.6^{de}	8.8 ± 0.4^{de}	10.2 ± 0.2^{d}	5.8 ± 0.4^{e}	4.5 ± 0.4^{e}	3.7 ± 0.4^{ef}
PP	pH	3.9 ± 0.0^{cd}	3.8 ± 0.1^{d}	4.3 ± 0.0^{c}	4.9 ± 0.0^{c}	5.1 ± 0.1^{b}	3.7 ± 0.0^{d}	3.9 ± 0.0^{d}	3.7 ± 0.1^{d}	4.65 ± 0.0^{c}
	TTA	19.3 ± 0.5^{b}	19.3 ± 0.3^{b}	15.9 ± 0.3^{bc}	8.8 ± 0.3^{d}	8.7 ± 0.3^{d}	19.8 ± 0.3^{b}	15.0 ± 0.2^{bc}	8.2 ± 0.1^{d}	0.8 ± 0.2^{f}
LF	pH	4.48 ± 0.07^{c}	4.33 ± 0.04^{c}	5.27 ± 0.07^{b}	4.40 ± 0.07^{c}	3.64 ± 0.07^{d}	3.70 ± 0.07^{d}	5.78 ± 0.02^{a}	5.74 ± 0.04^{a}	5.7 ± 0.1^{a}
	TTA	23.30 ± 0.93^{a}	13.33 ± 0.49^{c}	9.50 ± 0.16^{d}	10.30 ± 0.28^{d}	10.80 ± 0.25^{d}	23.23 ± 0.31^{a}	25.90 ± 1.57^{a}	4.67 ± 0.07^{e}	1.47 ± 0.29^{f}

Table 2
Total phenolic and flavonoid content of 6.5% *Prosopis* sp. honey solutions inoculated with *P. pentosaceus* (PP) and *L. fermentum* (LF) at 0 (t_0) and 19 h (t_{19}) of fermentation. Means followed by different letters are significantly different at $p < 0.05$.

Bacterial strain		Total phenolic content (mg GAE/100 g honey)	Total flavonoid content (mg QE/100 g honey)
PP	t_0	81.35 ± 2.81^a	7.01 ± 0.41^c
	t_{19}	91.92 ± 3.45^b	14.42 ± 0.53^e
LF	t_0	80.27 ± 3.72^a	7.48 ± 0.38^c
	t_{19}	89.90 ± 4.01^b	9.72 ± 0.51^d

2001), showing the importance of phenolic compounds in honey antimicrobial activity.

3.5. Effect of honey on LAB fermentative activity

To evaluate the fermentative response of *P. pentosaceus* and *L. fermentum* to honey bioactive compounds, LAB strains were grown in presence of increasing concentrations of honey and fermentative parameters were measured. Results are shown in Table 1.

Honey natural acidity is directly related to its botanical origin (Molan, 1992). Results showed that pH initial values decreased from 5.89 to 4.39, as the concentration of *Prosopis* sp. honey increased from 6.5 to 75%. Acidity of honey is attributed to the presence of organic acids, being gluconic acid the main contributor (Suarez-Luque et al., 2002).

After 19 h of incubation, pH values of control MRS broth solutions decreased 33.4% for *P. pentosaceus* and 23.9% for *L. fermentum* and TTA values increased 3.6 and 4.3 times, respectively.

The presence of honey in concentrations of 6.5 and 25% did not modify the acidic profile of *P. pentosaceus*. pH values decreased between 20 and 30% and TTA values increased about 2 times. Jaziri, Slama, Mhadhbi, Urdaci, and Hamdi (2009) reported that tea extract rich in flavonoids does not affect lactic acid levels of final products. Honey concentrations greater than or equal to 50% negatively affected *P. pentosaceus* fermentative activity, reducing lactic acid production.

On the other hand, *L. fermentum* acidic production was negatively affected by the presence of honey concentrations higher than 6.5%. Acidification of this solution was significantly higher than initial values; pH decreased 20% and TTA was 1.5 times higher. In spite of the high phenolic content of *Prosopis* sp. honey (3.2.), the concentration of these compounds in 6.5% honey solutions was not enough to inhibit the growth of *L. fermentum*. Phenolic concentration in 25% honey solutions represent a threshold value, inhibiting *L. fermentum* population and hence, lactic acid production.

3.6. Effect of LAB on the phenolic and flavonoid content of honey

Total phenolic and flavonoid content was evaluated in 6.5% honey solutions before (t_0) and after (t_{19}) 19 h of fermentation by LAB. Results showed that the growth and/or the fermentative activity of *P. pentosaceus* and *L. fermentum* modified the total phenolic and flavonoid content of *Prosposis* sp. honey (Table 2). Phenolic concentration increased 13 and 12%, respectively. In contrast with the results obtained by LoCascio et al. (2006), who reported that certain LAB are capable of reducing the flavonoid content present in the culture media, we observed a remarkable increase of the total flavonoid moiety after 19 h of incubation. These increments were of 120% for *P. pentosaceus* and 30% for *L. fermentum*.

Flavonoids are widely distributed in vegetal products as O-glycosides, being the aglycon fraction responsible for their bioactivity (Di Gioia, Bregola, Aloisio, Marotti, & Dinelli, 2010). Several microorganisms are able to biotransform flavonoid glycosides by different enzymatic reactions, as hydroxylation, dehydroxylation, deglycosylation, etc. Some of these, especially the latter, increase flavonoid bioavailability by releasing sugars from the aglycone moiety (Cao, Chen, Jassbi & Xiao, 2015). Deglycosilation could be attributed to β-glucoside activity, reported for some LAB strains (Di Gioia et al., 2010). This hydrolysis is favoured under acidic conditions (Ferreres, Tomás-Barberán, Soler, Ortiz, & Tomás-Lorente, 1994), so the fermentative capacity of LAB accentuates biotransformation reactions. We suggest that, as a result of LAB activity, the hydroxyl groups of aglycones become exposed, increasing the bioavailability of these compounds. The higher acidity provided by *P. pentosaceus* could emphasize this hydrolysis, incrementing flavonoid content in *Prosopis* sp. honey.

4. Conclusion

In the present study, we found that *Prosopis* sp. honey is a significant source of phenolic and flavonoid compounds, known for delaying and preventing oxidative processes. Furthermore, it is able to regulate the bacterial population, because not all LAB have the same response to honey. The ability of *P. pentosaceus* to grow in presence of honey was superior to that of *L. fermentum*. The first is capable of growing and produce organic acids in presence of honey concentration up to 25%. In addition, both *P. pentosaceus* and *L. fermentum* modify the total phenolic and flavonoid content, likely due to flavonoid biotransformation. The significant fermentative capacity of *P. pentosaceus* and their ability to grow in high honey concentrations make this LAB an ideal microorganism to increase the bioavailability of flavonoids, and thus, antioxidant activity.

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