LWT - Food Science and Technology 70 (2016) 309–314

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

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

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ARTICLE INFO

Article history:
Received 1 September 2015
Received in revised form
1 March 2016
Accepted 6 March 2016
Available online 9 March 2016

Keywords:
Honey
Phenolic compounds
Flavonoids
Pediococcus pentosaceus
Lactobacillus fermentum

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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http://dx.doi.org/10.1016/j.lwt.2016.03.009
0023-6438/

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