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Effect of high hydrostatic pressure applied to a Mexican honey to increase its microbiological and functional quality

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ABSTRACT

High hydrostatic pressure (HHP) applied at 600 MPa (0, 2, 5, 8, 12 and 15 min) was used as an alternative method to reduce the microbial population, preserving bioactivity (antioxidant activity (AOA), vitamin C, total phenolic and carotenoid contents) and other quality factors (diastase activity, 5-hydroxymethylfurfural, fructose and glucose contents and rheological behavior) of a Mexican honey. HHP processing reduces viable microorganisms in honey and the treatment for 15 min reduced the total microbial count below detection limit. AOA and total phenolic content were increased by 30% and 6%, respectively, after 2 min processing. The vitamin C, fructose, glucose and maltose contents in HHP treated honey were retained at all processing times. The total carotenoid content was maintained at processing times up to 8 min, while violaxanthin content was reduced by 56% after 15 min. HHP did not change the contents of 5-hydroxymethylfurfural, while the diastase activity decreased by 10% after 12 min treatment. Honey exhibited a Newtonian behavior and its viscosity was decreased at all HHP processing conditions. HHP treatment at 600 MPa for 2 min offers an opportunity to enhance the antioxidant properties of honey while reducing its microbiological load and preserving its the general quality parameters of honey.

1. Introduction

Honey is a natural product produced from the nectar and exudation of plants by honeybees (*Apis mellifera*). The natural honey contains about 200 substances, mainly fructose and glucose, with phenolic compounds, minerals, proteins, free amino acids and vitamins as minor components (Küçük et al., 2007). The composition of honey varies depending on the floral source, processing, seasonal and environmental factors. It has been used with different purposes and has a great potential to serve as a natural food antioxidant in which phenolic acids and flavonoids, certain enzymes (glucose oxidase, catalase), ascorbic acid, Maillard reaction products, amino acids and proteins contribute to this activity (Alvarez-Suarez et al., 2010a,b; Gheldof and Engeseth, 2002).

Honey is usually heated to 60 °C or above to inactivate microorganisms, facilitate packing and delay crystallization (Kaur Bath and Singh, 1999; Tosi et al., 2004). Heating has a negative effect on honey due to the loss of compounds, which give its specific aroma, flavor and some of its

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biological activity; the degree of losses are proportional to the temperature and duration of the thermal treatment applied (Tosi et al., 2004). The damage caused by heating can be evidenced by measuring diastase activity and hydroxymethylfurfural content (HMF) (Bogdanov et al., 1999). Diastase, one of the most important honey enzymes, can break down glycosidic linkages in oligo- and polysaccharides. The activity of this enzyme decreases with the time of storage and heating, being an important parameter to determine quality of honey. HMF is a cyclic aldehyde formed by fructose and glucose dehydration in an acid media as an intermediate in the Maillard reaction (Rizelio et al., 2012; Tosi et al., 2002). Several factors influence HMF level, such as temperature and time of heating, storage conditions, pH and floral sources, so it indicates overheating and inadequate storage (Fallico et al., 2006).

The consumer awareness for healthier foods, has stimulated the food industry to explore novel potential alternative technologies to inactivating microorganisms avoiding the negative effects of thermal processing on organoleptic, nutritional and, functional properties of foods. The extensive research on HHP processing has originated new opportunities to improve the balance between the safety and quality of food products (Vervoort et al., 2012). Due to the non-thermal nature of HPP processing, it potentially produces high quality food with 'fresh-like' characteristics and improved functionalities (Prasad et al., 2010; Fauzi et al., 2013).

It has been reported that HHP can increase the total phenolic compounds and AOA of Manuka honey (Akhmazillah et al., 2013; Al-Habsi and Niranjan, 2012; Fauzi et al., 2013). Quality changes in terms of color and viscosity when HPP is combined with thermal treatment, have also been reported (Fauzi et al., 2013). Likewise, changes in minor components in Manuka honey such as methylglyoxal and non-peroxide antibacterial activity (MGO) have been studied after HHP application (Al-Al-Habsi and Niranjan, 2012; Grainger et al., 2014).

Honey production in Mexico has very long traditions going back to ancient times; however, its composition and functional properties have not been studied comprehensively. Mexico is the ninth largest producer in the world with an annual production of 56 907 t of honey, and it is also the world's third leading exporter of honey (FAO, 2013).

The aim of this study was to investigate the effect of HHP at 600 MPa after different processing times (0, 2, 5, 8, 12, and 15 min) on the microbial population and contents of total phenolic (TPC), carotenoids, vitamin C, and AOA of a Mexican honey. Quality parameters such as HMF, diastase number, sugar content and rheological behavior were also evaluated.

2. Material and methods

2.1. Raw material

Honey (multifloral) obtained from Guerrero State (25 kg), Mexico (D'Zarzas S.A. de C.V.) was used in this study (pH of 3.80 ± 0.06, 80.0 ± 0.1°Brix, and a_w 0.589 ± 0.070). Honey was placed in plastic bottles (1 kg) and stored at room temperature (25 °C) while processed. The Harmonized Methods of the International Honey Commission (Bogdanov et al., 2009) were used to evaluate pH, free acidity (meq acid/kg honey) and moisture (% on wet basis, wb). Determinations were carried out in triplicate.

The color parameters (L*, a* and b*) were determined in raw sample filled into a clear glass Petri dish and using a Minolta colorimeter (CR-10, JAN). The results were expressed in accordance with the CIELAB system with reference to illuminant D65 and with a visual angle of 2°. Three replicate samples were analysed.

2.2. High hydrostatic pressure treatments

About 40 g of honey was vacuum packed (model EVD 4, Torrey, Monterrey, Nuevo León, Mexico) in 15 × 8 cm polyethylene bags (Filmpack SA de CV, Guadalupe, Nuevo León, Mexico) and processed in a 2 L capacity high-pressure food processor (Avure Technologies, Middletown, OH, USA) employing water as pressurizing medium and operated at 600 MPa, for different holding times (0, 2, 5, 8, 12 and 15 min), where 0 min indicates the time required to reach the pressure level (1.8 min), in where once the pressure level was reached, the samples were immediately depressurized (decompression time was almost instantaneous).

Once pressure level was reached, final average temperatures during processing time were 37 ± 1, 37 ± 1, 35 ± 2, 31 ± 1, 30 ± 2 and 28 ± 1 °C at 0, 2, 5, 8, 12 and 15 min, respectively. Duplicate runs of each treatment were performed.

2.3. Microbial analysis

Microbiological determinations were performed according to the Mexican normativity (NOM-092-SSA1-1994, 1994; NOM-111-SSA1-1994, 1994). Ten grams of each honey sample was homogenized into 90 mL of peptone aqueous solution (10^{-1}). Decimal dilutions were made into the same solution (10^{-2} and 10^{-3}), and 1.0 g (10^{0}) of honey sample or 1.0 mL of each dilution was plated into triplicate plates of appropriate agar. Aerobic mesophilic bacteria were counted on standard plate count agar (PCA) and incubated at 35 ± 2 °C for 48 h, and plates with 25–250 colonies were counted. The detection limit of the method used for aerobic mesophilic bacteria was 25 CFU/g. The molds and yeast were estimated using potato dextrose agar medium (pH 3.5 ± 0.1) and incubated at 25 ± 2 °C for 5 days, and plates with 15–150 colonies were counted. The detection limit of the method used for molds and yeast as stablished for the normativity was 15 CFU/g. Microbial counts were expressed as colony-forming units per gram of honey (CFU/g). Log N was calculated to determine the inactivation effect, where N is the number of viable microorganisms.

2.4. Quality parameters

2.4.1. Hydroxymethylfurfural (HMF) content and diastase activity

The HMF and diastase activity or diastase number (DN) was carried out according to the Harmonized Methods of the International Honey Commission (Bogdanov et al., 2009). HMF content was determined after clarifying samples with Carrez reagents (I and II) and addition of 0.2% sodium bisulphate. Sample solutions were placed in 10 mm quartz cells and the absorbance was measured using UV/vis spectrophotometer (Genesys 10S UV-vis, Thermo Scientific, China) at 284 and 336 nm. The readings were expressed as mg HMF/kg honey. The DN is equivalent to Gothe scale number, to determine it the honey sample was dissolved in acetate buffer (0.1 M, pH 5.3) and sodium chloride solution (0.5 M). The samples were placed in test tubes with starch solution (2%) in a water bath at 40 °C. Changes in color were measured at defined intervals of time in the UV/vis spectrophotometer. The results were expressed in Gothe units. Determinations were performed in triplicate.

2.4.2. Quantification of glucose and fructose

An HPLC with IR detection (Waters 2114, Milford, MA, USA) integrated with an auto sampler including temperature control for the column (Shodex SH1011 CA, Waters, USA) at 60 °C was used to determine glucose and fructose. The mobile phase consisted of 5 mM sulfuric acid at a rate of 0.6 mL/min