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Effect of high hydrostatic pressure on antimicrobial activity and quality of Manuka honey

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ABSTRACT

The antimicrobial activity of Manuka honey is of major interest to beekeepers and the honey industry. In this study, the effect of high hydrostatic pressure and thermal treatments on antimicrobial activity and quality parameters (principally, diastase number and hydroxymethylfurfural levels (HMF)) of Manuka honey were investigated. The honey was subjected to different pressures (100–800 MPa) at 25 °C for a range of holding times (15–120 min). The antimicrobial activity was found to increase with applied pressure for a given holding time, while the diastase number and HMF levels remained, more or less, unaffected. The percentage inhibition in microbial growth correlated linearly (R^2 = 0.94) with methyglyoxal concentration in the honey after treatment over the entire range of pressure, temperature and holding times studied. Maximum percentage inhibition (78.83%) was achieved when honey was subjected to 800 MPa compared to the control (57.93%). Thermal treatments at higher temperatures were found to have a detrimental effect on antimicrobial activity based on percentage inhibition as well as methylglyoxal content. Thus, it can be concluded that the levels of methylglyoxal, and therefore the antimicrobial effect of Manuka honey, can be enhanced by using high pressure processing without adversely affecting honey quality.

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1. Introduction

Honey has been known since ancient times as a traditional remedy for bacterial infections. Manuka honey is derived from Manuka tree (*Leptospermum scoparium*), which originated from New Zealand, and it has been demonstrated to possess significant antimicrobial activity against different types of bacteria (Lee, Churey, & Worobo, 2008; Sherlock et al., 2010; Weston, Mitchell, & Allen, 1999). The antimicrobial properties of different honeys were thoroughly reviewed by Molan (1992b). Two types of antibacterial agents, also known as inhibines, are found in the honeys, which imbues them with antibacterial properties (Bogdanov, 1984). The most common inhibine in all honeys is the hydrogen peroxide (Weston, 2000). However, Manuka honey was reported to exhibit antimicrobial properties from non-peroxide activity reportedly attributed to methyglyoxal (MGO) levels (Bogdanov, 1997; Snow & Manley-Harris, 2004). Adams, Manley-Harris, and Molan (2009), studied the origin of MGO in New Zealand Manuka honey. They found that MGO was formed from diyhydroxyacetone, which in turn originated from the nectar of Manuka flower in varying amounts. They also found that a remarkably small amount of MGO and high level of diyhdroxyacetone were present when the honey was freshly produced by bees. However, storage of honey at 37 °C resulted in an increase of MGO followed by a decrease in dihydroxyacetone content. It was also observed that when the honey was subjected to a temperature of 50 °C and above, both MGO and diydroxyacetone contents decreased. At the same temperatures, there was a marked increase in the hydroxymethylfurfural content which is an indicator of honey quality decrease. Fedoronko and Konigstein (1969), confirmed that in aqueous solution of acetic acid, both, D,L-glyceraldehyde and dihydroxyacetone condense irreversibly to form methyglyoxal.

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism after overnight incubation. This term is commonly used as a measure of the antimicrobial activity and compares the activity of different agents (Reeves, 2007). MIC has been used as an indicator of antimicrobial activity of honey against different microorganisms (Boorn et al., 2010; Henriques, Jenkins, Burton, & Cooper, 2010; Weston et al., 1999). The MIC differs from one Manuka honey to another, depending on the MGO content.

Some beekeepers reportedly prolong the storage time and subject the honey to mild heat in order to manipulate the antimicrobial activity (Adams et al., 2009; Stephens et al., 2010). This approach allows enough time for the condensation of diydroxyacetone, and facilitate the buildup of methyglyoxal (Adams et al., 2009). However, the quality of honey deteriorates due to the

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prolonged storage and the application of heat (Gupta, Kaushik, & Joshi, 1992; Turhan, Tetik, Karhan, Gurel, & Tavukcuoglu, 2008). Diastase number (DN) and hydroxymethylfurfural (HMF) are commonly used parameters to assess the quality of honey (Nanda, Bera, & Bakhshi, 2006; Tosi, Ciappini, Re, & Lucero, 2002; White, 1994). The maximum allowed HMF is 40 mg/kg, with exception of 80 mg/kg if the origin of the honey is from a tropical climate (Fallico, Arena, & Zappala, 2008; Spano et al., 2006). The range permitted for diastase number varies from 3 to 8 on Gothe's scale, depending on the climate prevailing in the place where the honey originates (Schade, Marsh, & Eckert, 1958; Tosi, Martinet, Ortega, Lucero, & Re, 2008).

Thus, there is a need to find ways of maintaining, simultaneously, the quality and antimicrobial functionality of honey. One possible way is to use high hydrostatic pressure (HHP) processing. This process has been known for more than hundred years to destroy and inactivate microorganisms and preserve the quality of food. It has also been suggested as the best alternative for thermal process to maintain the nutritional and functional values of food (Basak & Ramaswamy, 1998; Knorr, 1993; Prasad et al., 2010; Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007). Therefore, this paper aims to investigate the effect of HHP on the antimicrobial activity of Manuka honey, which has not been investigated so far. High hydrostatic pressure is also considered to be one of the thermodynamic factors influencing condensation reactions in general (Isaacs, 1981). This paper, therefore, examines the effects of HHP on the condensation of diyhydroxyacetone to form methyglyoxal in Manuka honey and determines the antimicrobial activity of the resulting product. The paper also reports on the resulting quality changes occurring in honey expressed in terms of Diastase number and HMF.

2. Materials and methods

2.1. Materials

Manuka honey with unique Manuka factor (UMF) (+10) was supplied by Rowse Honey Limited Company (Wallingford, Oxon, UK). All chemicals were obtained from Sigma–Aldrich (United Kingdom) unless otherwise stated.

2.2. High pressure and thermal treatments

High pressure treatment was carried out using a pressure vessel (Model S-FL-085-9-W, Stansted Fluid Power, England) employing a mixture of distilled water and 1,2-propanediol (70:30) as the pressure-transmitting medium; the temperature was maintained at 25 °C using a temperature-controlled water bath. One vacuum packaged plastic bag of honey (80–100 g) was used for each treatment and was repeated in triplicate. The honey was subjected to a range of pressures (100–800 MPa) and held at each pressure for different times (15–120 min). Some of the pressure holding times may appear long in relation to normal application of HHP technology, however, these holding times are still relevant in the context of honey processing where thermal processing times can be as long as 3–4 days (Subramanian, Hebbar, & Rastogi, 2007). The effect of temperature on MGO formation at ambient pressure was also studied. Honey samples were packed in plastic bags and subjected to a range of temperatures (50–70 °C) for different times (15–120 min). A thermostatically controlled water bath was used for this purpose. It may be noted that the pressure range employed in this work is normal for HHP applications (100–800 MPa). As mentioned above, the temperature used for the high pressure experiments was 25 °C. Higher temperatures (up to 70 °C) were only used for experiments conducted at ambient pressure, to ascertain levels of methylglyoxal formation under these conditions.

2.3. Determination of antimicrobial activity

The minimum inhibitory concentration (MIC) of honey was determined using the broth dilution method in 96-well, flat-bottomed microtiter plates (Cellstar®, Greiner bio-one Ltd., UK). The honey was diluted to 50% (w/v) using LB broth, Miller Luria–Bertani (Fisher Scientific, UK). Further dilutions were done to achieve honey concentration of 25%, 12.5%, 6.25%, 3.13%, 1.56%, 0.78%, 0.4%, 0.2% and 0.1% (w/v). The minimum concentration of honey that inhibits the growth of a specific microorganism when compared to a negative control, was considered to be the MIC.

Staphylococcus aureus ATCC 25923 (Oxoid, Basingstoke, UK) was used as the test strain, since it has been used most frequently in honey inhibine studies, and it is sensitive to honey antimicrobial action. A fixed volume of 190 μl of a given honey concentration was used in each well. The wells were inoculated with 10 μl undiluted overnight broth cultures of test bacteria approximately 1×10^8 cfu/ml and incubated at 37 °C for 24 h. Tecan Spectrafluor plus microplate reader (Genios®, Austria) was used to measure the optical density (OD) at 620 nm and related to bacterial growth. The growth inhibition for the test wells at each honey dilution was determined by the following expression (Tan et al., 2009):

$$\text{Percent inhibition} = \left[1 - \frac{(\text{OD test well} - \text{OD corresponding negative control well})}{(\text{OD viability control well} - \text{OD broth only well})}\right] \times 100 \quad (1)$$

2.4. Methylglyoxal content

Methylglyoxal concentration was measured using a derivatisation method with *o*-phenylenediamine (OPD) according to Adams et al. (2008). Honey (ca. 0.6 g) was dissolved in 2 ml HPLC grade water. OPD was dissolved in 0.5 M phosphate buffer, pH 6.5 (2%). 1.5 ml honey solution was treated with 0.75 ml OPD solution for 16 h in the dark at room temperature. Samples were then filtered on 0.45 μm filter and immediately injected in an HPLC (Hewlett Packard, series 1050) with a UV detector. The wavelength range was 100–550 nm and the chromatograms were monitored at 312 nm. The HPLC column was ACE 5 μm AQ, 4 μm, 4.6 × 250 mm. The flow rate was set at 0.3 ml min^{-1}, and injection volume: 5 μl. Eluent A was 0.075% acetic acid in water and eluent B was 80% methanol in water (final solution made up 0.075% acetic acid). Gradient steps were (min, (%B)): 0 (10), 4 (10), 5 (421), 30 (55), 31 (100), 35 (10) and 40 (10). MGO was identified by splitting the peak in honey with a standard MGO and by comparing the spectrum of MGO standard with that of honey samples. The MGO content in honey samples was calculated using a standard calibration curve prepared by plotting MOG concentration versus the peak area.

2.5. Diastase activity (Diastase number)

The determination of Diastase activity or diastase number (DN) was carried out according to Phadebas method recommended by Harmonized methods of the International Honey Commission (Bogdanov, 2009). One unit of diastase activity, also known as Schade or Gothe unit, was defined according to this method as the amount of enzyme which will convert 0.01 g of starch to the prescribed end point in one hour at 40 °C under the condition of the test. A honey sample (1 g) was dissolved in 100 ml acetate buffer solution (0.1 M, pH 5.2). The sample solution (5 ml) was transferred to a test tube and placed in a water bath at 40 °C. Acetate buffer (5 ml) was prepared in another test tube and was treated exactly as the sample solution and was considered as blank. After 10 s from

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