Food Chemistry 126 (2011) 1155–1163

Contents lists available at ScienceDirect

Food Chemistry

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

Storage-induced chemical changes in active components of honey de-regulate its antibacterial activity

Katrina Brudzynski *, Linda Kim

Brock University, St. Catharines, Ontario, Canada L2S 3A1

ARTICLE INFO

Article history:
Received 6 August 2010
Received in revised form 19 October 2010
Accepted 25 November 2010
Available online 2 December 2010

Keywords:
Honey antibacterial activity
Storage
Escherichia coli
Bacillus subtilis
MIC_{90}
Colour
UV-absorbing compounds
Melanoidins

ABSTRACT

To elucidate reasons for the observed variability in the antibacterial activity of honeys, we analysed a causal relationship between (a) honey floral sources and the activity and (b) the effect of honey storage on stability of compounds conferring this activity. Honeys from diverse floral sources were screened against *Escherichia coli* (ATCC 14948) and *Bacillus subtilis* (ATCC 6633) using the broth microdilution method. Among "active" honeys, 37% originated from buckwheat, 18% from clover and 12% from blueberry, indicating that these floral sources produced phytochemical(s) that inhibited bacterial growth. The stability of the putative phytochemical(s) was analysed in "active" honeys (MIC_{90} 6.25% v/v) by measuring the activity every 3–6 months for a period of 1–3 years. A sharp decline in activity against both bacteria was observed in the first 3–6 months of storage. The decline coincided with major changes in chemical composition of honeys which included a significant change in colour ($p < 0.0025$), extremely significant change in concentration of UV-absorbing compounds ($p < 0.0001$) and appearance of melanoidins. While these changes reduced *E. coli* sensitivity to honey, it rendered *B. subtilis* completely insensitive. Thus, the data indicates that the presence of phytochemical(s) conferring the antibacterial activity is sensitive to storage. The de-regulation of the antibacterial activity with the concomitant appearance of melanoidins suggests that the active phytochemical components might be sequestered into melanoidin aggregates, losing their function.

Crown Copyright © 2010 Published by Elsevier Ltd. All rights reserved.

1. Introduction

Honey has been shown to efficiently inhibit bacterial growth *in vitro* and *in vivo*.

This functional property has been found in a variety of honeys of diverse botanical origin and from a variety of geographical locations (Allen, Molan, & Reid, 1991; Ceyhan & Ugur, 2001; Lusby, Coombes, & Wilkinson, 2005; Mundo, Padilla-Zakour, & Worobo, 2004; Wilkinson & Cavanagh, 2005). The first laboratory and clinical studies on antibacterial activity were performed on a specific variety of honey, manuka honey, originated from *Leptospermum scoparium and ericoides* of New Zealand and Australia. These studies uncovered the unexpectedly potent antibacterial activity of *Leptospermum* spp. honeys and allowed for establishment a spectrum of bacteria that were sensitive to their action (Blair, Cokcetin, Harry, & Carter, 2009; Cooper, Molan, & Harding, 2002; Molan, 1992a, 1992b; Willix, Molan, & Harfoot, 1992). The research initiated worldwide search for other honey varieties which display antibacterial activity, specifically among types of ethnic honeys including Indian jambhul (*Syzygium cumini*) honey (Subrahmanyam et al., 2001), Malaysian tualang (Tan et al., 2009) and gelam honeys (Aljadi, Kamaruddin, Jamal, & Yassim, 2000), Turkish honey from Anatolia (Kucuk et al., 2007) and honeys from different geographical regions such as North America and Europe (Brudzynski, 2006; Miorin, Levy, Custodio, Bretz, & Marcucci, 2003; Mundo et al., 2004; Taormina, Niemira, & Beuchat, 2001). Most of honey varieties showed either no activity or activities below that observed in honeys originated from *Leptospermum* spp. The intrinsic characteristic of honeys, e.g. high osmolarity, low water activity, low pH, production of hydrogen peroxide, although involved in antibacterial action, are common properties for all honeys and could not explain the variability in activity between honeys. From surveys of antibacterial activity in different honeys, it became clear that a phytochemical composition of honeys was responsible for the degree of bacteriostatic and bactericidal action (Allen et al., 1991; Ceyhan & Ugur, 2001; Lusby et al., 2005; Mundo et al., 2004; Wilkinson & Cavanagh, 2005.

In the early studies, candidates for the active principal components were search among polyphenols; phenolic acids (Aljadi & Yusoff, 2003), and their derivatives (methyl syringate) (Russell, Molan, Wilkins, & Holland, 1990; Weston, Brocklebank, & Lu, 2000), aromatic acids and flavonoids (Bogdanov, 1997), hydrogen peroxide (Bang, Bunting, & Molan, 2003; Brudzynski, 2006;) and

* Corresponding author. Address: Department of Biological Sciences, Brock University, 500 Glenridge Avenue, St. Catharines, Ontario, Canada L2S 3A1. Tel.: +1 905 688 5550x5035; fax: +1 905 688 1855.
E-mail address: kbrudzynski@brocku.ca (K. Brudzynski).

0308-8146/$ - see front matter Crown Copyright © 2010 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2010.11.151

recently, the Maillard reaction products (Brudzynski & Miotto, 2010a, 2010b). The Maillard reactions between amino groups of amino acids/proteins and reducing sugars, leads to formation of, among other molecules, α-dicarbonyl compounds such as glyoxal and methylglyoxal (Adams et al., 2008; Mavric, Wittmann, Barth, & Henle, 2008; Stephens et al., 2010). The observed strong relationship between the concentration of methylglyoxal and antibacterial activity of honeys of *Leptospermum* spp. origin pointed to this molecule as being the potential active principle for manuka honey.

However, variability in the antibacterial activity that has been observed among honeys can not be solely related to the differences in the phytochemical composition. For example, differences in the antibacterial activity were observed within manuka honeys, ranging from totally inactive to highly active honeys, although these honeys all derived from the same *Leptospermum* spp. Thus, other factors and/or chemical processes might play a role in a final antibacterial potency. It has been long recognised that seasonal variations, post-harvest handling of honeys and their storage conditions influence quality of honey.

In this study we assessed contribution of two factors to the variability in the antibacterial activity of Canadian honeys: botanical origin of honey and storage time.

2. Materials and methods

2.1. Honeys

Honeys were donated by beekeepers and included both commercial (pasteurised) and apiary (raw) samples. Over 174 samples of honey were collected from the following Canadian provinces: Ontario-, Manitoba-, Alberta-, British Columbia during the 2006–2008 seasons. Upon arrival at the laboratory, honey samples were assigned a number and analysed for colour, Brix, and pH. Honey samples were divided into portions and stored at room temperature in the dark.

2.2. Determination of honey colour

Honey colour was determined spectrophotometrically by measuring the net absorbance at ($A_{560}-A_{720}$) (Huidobro & Simal, 1984).

2.3. Determination of Maillard reaction content

The melanoidin content was assessed spectrophotometrically as net absorbance at ($A_{450}-A_{720}$) (Ramonaityte, Keriene, Adams, Tehrani, & De Kimpe, 2009) using Ultrospec 3100 Pro, GE Healthcare. The melanoidin content was in absorption units (au).

2.4. Determination of the content of UV-absorbing compounds in honey

The quantitative and qualitative data on the levels of UV-absorbing compounds were obtained from the absorbance spectrum profiles of honey scanned at wavelengths 200–400 nm. Honeys were two-fold serially diluted with water and the concentration of UV-absorbing compounds was determined from the area-under the curve (AUC) for each dilution. Absorbencies of the honey dilutions were analysed in 1 cm path-length quartz cells using a spectrophotometer Ultrospec 3100 Pro equipped with SWIFT II wavescan software. The concentrations were expressed in arbitrary units as the AUC units.

2.5. Antibacterial activity

Standard strains of *Bacillus subtilis* (ATCC 6633) and *Escherichia coli* (ATCC 14948) were purchased from Ward's Natural Science Ltd. (St. Catharines, ON, Canada).

2.5.1. Preparation of working inoculum

Each of the bacterial strains was inoculated into Mueller–Hinton Broth (MHB)(Difco Laboratories) and incubated overnight in a shaking water bath at 37 °C. Overnight culture were diluted with the broth to the equivalent of 0.5 MacFarland standard (approximately.10^8 cfu/ml) measured spectrophotometrically at A_{600} nm.

2.5.2. Antibacterial assay

The antibacterial activity of honeys was determined using a broth microdilution assay in a 96-well microplate format. Serial two-fold dilutions of honey were prepared by mixing 110 ul of honey with 110 ul of inoculated broth (10^6 cfu/ml final concentrations for each of microorganisms) and transferring from row A to row H of a microplate. Row G contained only inoculum and served as a positive control. Row H contained sterile MHB and served as a blank.

After overnight incubation of plates at 37 °C in a shaking waterbath, bacterial growth was measured at A_{595} nm using the Synergy HT multi-detection microplate reader (Synergy HT, Bio-Tek Instruments, Winooski, Vt).

The contribution of colour of honeys to the absorption was corrected by subtracting the absorbance of wells before (zero time) and after overnight incubation.

Statistical analysis and dose response curve were obtained using K4 software.

2.5.3. Determination of minimum inhibitory concentration (MIC)

The absorbance readings obtained from the dose-response curve were used to construct growth inhibition profiles (GIPs) using the following formula:

$$\%\text{growth inhibition} = \frac{A_{\text{control}} - A_{\text{experimental}}}{A_{\text{control}}} \times 100$$

The minimal inhibitory concentrations (MIC_{90}) were determined from the growth inhibition profiles curves and represented the lowest concentration of the honeys that inhibited the bacterial growth by 90% as measured by the absorbance at A_{595} nm.

2.6. Monitoring changes in honeys during storage

Upon arrival to the laboratory, honeys were divided into portions and stored at room temperature (approximately 24 °C) in a dark, dry place for 1–3 years. Every 3–6 months, a portion of honey was analysed for its antibacterial activity, honey colour, the content of UV absorbing compounds and the content of the Maillard reaction products.

2.7. Statistical analysis

Analyses were performed using the statistical program GraphPad Instat version 3.05. (GraphPad Software Inc.). Data were analysed using a one-way ANOVA with subsequent Tukey–Kramer Multiple Comparison test or an unpaired *t*-test. Differences between means were considered to be significant at $p < 0.05$.

Download English Version:

https://daneshyari.com/en/article/10540618

Download Persian Version:

https://daneshyari.com/article/10540618

Daneshyari.com