

## CHEMICAL, MICROBIAL AND ANTIOXIDANT PROPERTIES OF SELECTED HONEY VARIETIES FROM SOUTH AFRICA

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### Abstract

In this study, the authors measured chemical properties, antioxidant potentials, and microbial composition of eight commercially available varieties of honey from South Africa. Their pH values ranged from  $3.61 \pm 0.03$  to  $6.65 \pm 0.02$ , while the electrical conductivity varied between  $12 \pm 3$  to  $93 \pm 0.4$  mS m<sup>-1</sup>. Concentrations of mesophilic bacteria ranged from  $(1.4 \pm 0.4) \times 10^1$  to  $(1.55 \pm 0.09) \times 10^3$  CFUs g<sup>-1</sup> wet weight of honey, and there was between  $(2.3 \pm 0.5) \times 10^1$  to  $(3.3 \pm 2.3) \times 10^5$  CFUs·g<sup>-1</sup> wet weight of honey of mesophilic fungi. No threats to human health would result from the consumption of any of the honey varieties studied as the faecal coliform concentrations, and the concentrations of *Salmonella spp.*, were below the detection limit of 1–9 CFUs g<sup>-1</sup> wet weight. All samples had antioxidant activity as supported by results of the ATBS and DPPH assays. At the same time, the square-wave voltammetry showed that this activity resulted from two compounds at half-position potentials between  $0.207 \pm 0.003$  and  $0.293 \pm 0.003$  V; and from  $0.444 \pm 0.001$  to  $0.524 \pm 0.031$  V. Based on the Kruskal-Wallis analysis at 5% level of significance, the average values of all but one of the parameters were different among the honeys (all p-values < 0.0124). Stratification of mesophilic fungi was observed in all the studied samples which probably resulted in no difference among the fungal concentrations, i.e. p-value was equal to 0.0740. Preliminary identification of fungal species is presented.

**Keywords:** honey, South Africa, composition

### Introduction

Saccharides, such as fructose and glucose, have been shown to account for 85 to 95% of honey by weight (Gomes et al., 2010). In South Africa, honey has long been used as the raw materials for fermentation of mead-like alcoholic beverages (Cambray, 2005) and it is also consumed raw as sweetener. Plants used for honey production include *Aloe greatheadii var. davyana* (Human and Nicolson, 2008), *Leucospermum cordifolium* and *Erica species* (Basson and Grobler, 2008). To date, the research activities have focused on the wound-healing properties of honey (Karpelovsky et al. 2007), antimicrobial activity against *Candida albicans*, *Staphylococcus oralis* and *Staphylococcus aureus* (Basson and Grobler, 2008); and *Helicobacter pylori* (Manyi-Loh et al., 2010). A lot of research has been conducted on the biology and diseases of the honey bee (e.g. Dieteman et al., 2006); and beekeeping in South Africa (Charles, 2005). Quality of honey depends on the sensory, chemical, physical and microbiological properties (EU, 2001). Data of this kind is currently missing from the literature in South Africa and so it is the aim of this article to address this knowledge gap.

### Materials and Methods

Eight commercially honeys were purchased from supermarkets and local shops in the following provinces of South Africa: Eastern Cape, Western Cape, and North West. The honey samples were stored at room temperature until the analyses were performed. The following chemicals and consumables were purchased from Merck (Pty.) Ltd. (Johannesburg/Cape Town, South Africa): m-FC agar, sodium chloride, nutrient agar, potato-dextrose agar, XLD agar and tetrathionate broth for *Salmonella enumeration*, ascorbic acid, brilliant green, potassium iodide and iodine. Hanna water testers for measurement of pH and electrical conductivity (EC) were purchased from Sigma-Aldrich (Johannesburg, South

Africa). Chemicals for the antioxidant assays in the DPPH and the ABTS methods were also purchased from this supplier. Sterile 40 mL urine jars for microbial sampling and dilutions were purchased from EC Labs (renamed to Spellbound Inc., Port Elizabeth, South Africa).

Values of pH and EC were measured using the method of Gomes et al. (2010). For microbiological analyses, 5–30 g wet weight of the particular honey sample (accuracy 0.01 g) was dispensed into a 40 mL sterile urine jar. Nine to 15 millilitres of sterile physiological saline solution was added and the sample was homogenised by hand-shaking. The aim was ensure complete visible dissolution of the honey in question. Decimal dilutions in physiological saline of the samples were then performed under aseptic conditions. Concentrations of mesophilic bacteria (MB) were enumerated after spread-plating onto nutrient agar, while the mesophilic fungi (MF) were spread-plated onto potato-dextrose agar. Colonies were counted after incubations at 30 °C for 48 hours for bacteria and after 96 hours at 32 °C for fungi. Faecal coliforms were spread-plated onto m-FC agar and counted as blue colonies after growth at 44.5 °C for 24 hours.

Separate samples were prepared for enumeration of *Salmonella spp.* Two millilitres of the diluted sample was mixed with 20 mL of in tetrathionate broth and incubated at 37 °C for 18 hours. After this 1 mL was re-inoculated onto the XLD agar and *Salmonella spp.* was quantified as the number of red colonies that grew after further 48 hours at 37 °C. All incubations were done in one of the following incubators: the Labcon incubator Model FSIM B (Labmark, Johannesburg, RSA), the TS 606/3-I incubator (WTW, Weilheim, Germany), the Labcon low temperature incubator LTIE 10 (Labmark, Johannesburg, RSA); and/or the Heraeus Model FT 420 (Heraeus Kulzer GmbH, Dormagen, Germany). All sterilisations were conducted using the Model RAU-53Bd REX MED autoclave (Hirayama Manufacturing, Tokyo, Japan). Microbial concentrations are reported as colony forming units  $1 \text{ g}^{-1}$  wet weight of the honey (CFUs·g<sup>-1</sup>).

Antioxidant activity of the individual honey samples was evaluated using the DPPH and the ABTS methods; as well as using square-wave voltametry. For the DPPH assay, a 72 mg m L<sup>-1</sup> DPPH solution was made up in 80% methanol, followed by a 100 µg cm<sup>-3</sup> ascorbic acid stock solution in MilliQ water (Millipore-Microsep, Port Elizabeth, South Africa). The calibration solutions were prepared by diluting the ascorbic acid stock solution with MilliQ water. In this way, a range of solutions with the following ascorbic acid concentrations was obtained the following concentrations (µg cm<sup>-3</sup>): 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45 and 50. Two hundred microlitres of DPPH solution was added to each standard in the microtitre plate and the plate was placed into the Powerwave plate UV/VIS spectrophotometer (BioTek, Winooski, USA). Antioxidant activity was evaluated using Eq. (1) after taking reading every 15 seconds for 5 minutes.

$$\text{Antioxidant activity of honey} = 100 \times \frac{(A_{\text{Standard}} - A_{\text{Honey}})}{A_{\text{Standard}}} \quad (1)$$

In Eq. (1),  $A_{\text{Standard}}$  is the average absorbance at 515 nm for ascorbic acid (dimensionless), and  $A_{\text{Honey}}$  is the average absorbance at 515 nm for the particular honey sample (dimensionless). Antioxidant activity was measured as ascorbic acid equivalents.

For the ABTS method, 7mM ABTS solution was prepared by weighing out 0.3845 g ABTS and dissolving it in 80 cm<sup>3</sup> of MilliQ water. Then 0.0662 g of K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> was added and the volume was made up to 100 cm<sup>3</sup> with MilliQ water. The solution was left to stabilise overnight and the ABTS solution was diluted to obtain a solution with absorbance 0.8–1.0 at 734 nm. A 100 µg cm<sup>-3</sup> stock solution of TROLOX was made up in 100% ethanol and it was further diluted to obtain the following concentrations (µg cm<sup>-3</sup>): 2, 4, 6, 8, 10, 15, 20, 25, 30, 35 and 40. The rest of the procedure was analogical to that of DPPH and results were evaluated according to Eq. (1). To identify the number of antioxidants in each honey sample,

square-wave voltammetry was performed on the Potentiostat/Galvanostat 30 (PGSTAT 30; Eco Chemie, Netherlands) and antioxidation capacity was quantified using the peak potential at half height (half-position potential) and number of electrochemical peaks detected (Harbertson and Spayd, 2006).

If there were statistically significant differences in the properties of individual honey varieties was examined using the one-way Kruskal-Wallis analysis of variance at 5% level of significance (KW analysis; Past statistical software package version 2.0, Paleontological Museum, Oslo, Norway and Geological Museum, Copenhagen, Denmark).

### Results and Discussion

No threats to human health would result from the consumption of any of the honey varieties studied as the faecal coliform concentrations, and the concentrations of *Salmonella spp.*, were below the detection limit of 1–9 CFUs·g<sup>-1</sup> wet weight. The remaining results are summarised in the text below and mainly in Table 1. The pH values of the samples ranged from 3.61±0.03 to 6.65±0.02, while the EC values varied between 12±3 to 93±0.4 mS m<sup>-1</sup>. Concentrations of MB ranged from (1.4±0.4) × 10<sup>1</sup> to (1.55±0.09) × 10<sup>3</sup> CFUs g<sup>-1</sup> wet weight of honey, and there was between (2.3±0.5) × 10<sup>1</sup> to (3.3±2.3) × 10<sup>5</sup> CFUs g<sup>-1</sup> wet weight of honey of MF. Morphological examination of the growth characteristics showed that 8 different species of bacteria and 15 different species of fungi were present in the honey samples. More direct identification is currently underway. Preliminary results indicate that 2 of the bacterial isolates belonged to the genus *Bacillus spp.* while 1 fungal isolate was identified as belonging to the species *Saccharomyces spp.* These species have been reported in honey by Snowdon and Cliver (1996). Additional identification will be conducted using DNA sequencing, but this work will commence once additional funding becomes available.

All samples had antioxidant activity as supported by results of the ATBS and DPPH assays. In both assays, there was no statistical difference in the antioxidant properties of the individual honey varieties (p-values > 0.120 in all cases). On average, the antioxidant activities honeys were 43 and 62% lower than the respective assay standard used in teh ATBS and the DPPH assays. The square-wave voltammetry showed that this activity resulted from two compounds at half-position potentials between 0.207±0.003 and 0.293±0.003 V; and from 0.444±0.001 to 0.524±0.031 V. Based on the Kruskal-Wallis analysis at 5% level of significance, the average values of all but one of the parameters were different among the individual honey samples (all p-values < 0.0124). Stratification of MF was observed in all the studied samples led to this observation (p-value = 0.0740). Maximum values for the other parameters from Table 1 were observed for the following samples: Fynbos for pH and EC, Melior for MB, Blue Gum for MF, Goldcrest for the first peak potential and Champagne for the second square-wave peak.

Table 1

**Chemical, microbial and antioxidant properties of the honey varieties**

Sample	pH	EC mS m <sup>-1</sup>	MB CFUs g <sup>-1</sup>	MF CFUs g <sup>-1</sup>	E <sub>1</sub> (V)	E <sub>2</sub> (V)
<b>Goldcrest</b>	3.61±0.03	12±4	44±7	400±361	0.272±0.036	0.484 ±0.047
<b>Melior</b>	3.75±0.01	40±2	1600±97	33±32	0.208±0.003	0.498±0.003
<b>Fynbos</b>	6.65±0.02	93±0	70±18	300±294	0.217±0.011	0.476±0.006
<b>Champagne</b>	3.70±0.04	33±3	90±21	1000±1040	0.209±0.005	0.524±0.031
<b>Blue Gum</b>	3.90±0.02	13±1	140±13	330000 ±232191	0.293±0.003	0.444±0.001
<b>Ikaros</b>	3.77±0.03	27±0	29±6	4000±3987	0.207±0.003	0.493±0.003
<b>Cape Coast</b>	4.47±0.01	32±1	14±4	23±5	0.266±0.004	0.511±0.004

Concentrations of FC, MB and MF, along with the values of pH, EC, are comparable to the data of Gomes et al. (2010). Likely sources of bacteria include the intestines of the worker bees, pollen, and post-harvest handling by the processing plant staff (Snowdon and Cliver, 1996). Sanitary conditions at the processing plants can be considered within regulatory guidelines, as no faecal coliforms or *Salmonella spp.* cells were detected in either of the honey samples. Highly acidic values of the pH measured in the analysed honey varieties indicate that saccharides present in the samples were probably fermented into organic acids (Gomes et al., 2010). The results of the square-wave analysis indicate that all eight honey samples have strong antioxidation potential, since position potential values are below 0.5 V (Harbertson and Spayd, 2006).

### Conclusions

1. Eight honeys from South Africa showed to be safe for human consumption, and showed to have strong antioxidant potentials.
2. Further research will be focused on the precise identification of the mesophilic bacteria and fungi isolated in this study; and identification of the antioxidant compound.

### Acknowledgements

This work was supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and the Academy of Sciences, registration number 1/0096/11.

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