Analytical and Aroma Profiles of Slovak and South African Meads

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Abstract

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The basic analytical parameters and aroma compounds of three Slovak and two South African meads were summarised. The ethanol concentration of the Slovak meads was about 13.5% (v/v), the residual sugars, depending on the mead style, ranged approximately from 140 g/ to 200 g/l. In the South African meads, the average ethanol concentration was 12% and the average residual sugar content about 70 g/l. The residual sugar content in all types of the Slovak meads was significantly higher. The acidity of the South African meads was slightly higher than that of the Slovak ones, while the extract and protein contents were higher in all of the Slovak meads. No significant differences were found in the total polyphenol content, which ranged from 178 mg/l to 242 mg/l of gallic acid equivalents. Ethyl acetate represented the main component of all volatile compounds across all the samples tested, with a significantly higher concentration in the Slovak meads (46.36–60.03 mg/l) compared to the South African ones (16.35 mg/l–16.97 mg/l). Higher alcohols were more prevalent in South African meads.

Keywords: mead; honey wine; fermentation; volatile compounds

Mead, also referred to as honey wine or honey beer, is a traditional alcoholic beverage containing from 9% to 18% volumetric of ethanol, produced by the fermentation of diluted honey. References to mead have been found in many sources of the ancient history throughout Europe, Africa, and Asia, thus mead can be regarded as the ancestor of all fermented drinks. Mead may be flavoured with various spices such as hop, nutmeg, cinnamon, or fruit juice, depending upon local traditions. Different meads are recognised based upon the rate of dilution of honey with water, and the type of honey. Honey is mainly composed of a complex mixture of carbohydrates and other minor substances, such as organic acids, amino acids, proteins, minerals, vitamins, and lipids. Fructose and glucose predominate in almost all honey types. These two saccharides account for nearly 85–95% of the total honey carbohydrates. However, the composition of honey is rather variable and primarily depends on the floral source; in addition, certain external factors also play an important role,

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such as seasonal and environmental factors, and so does the processing method. In many places in Africa and South America, tropical honey is produced, which is very liquid and speedy to ferment (Navratil *et al.* 2001; Finola *et al.* 2007; Sroka & Tuszynski 2007).

In Slovakia, the controlled fermentation process of honey is performed by free yeast cells of the *Saccharomyces* genera, typically at temperatures between 15°C and 22°C. Low fermentation temperature helps to achieve a steady fermentation and a better transformation of the aromatic and taste qualities of the ingredients into the final product. Depending on the fermentation condition and dilution of honey, mead is usually fermented for 2–3 months. The maturation time varies from 9 months to 2 years. Most Slovak meads are produced using only honey, water and yeast, without the addition of any other flavours or aromas.

A unique fermented honey beverage, produced within the Eastern Cape region of South Africa, is called iQhilika. The Xhosa people, and the descendants of the Khoi-San group of peoples who inhabit this region, produce the beverage by taking specially prepared roots of succulents of the *Trichodiadema* genus and mixing this with a saccharide source such as honey and certain fruits. Sometimes pollen or an extract of bee larvae similar in nature to royal jelly is added to the brew.

The aim of this contribution was to compare the basic analytical parameters and aroma compounds of three Slovak and two South African meads, such as acidity, ethanol, residual sugars, extract, proteins, polyphenols, and volatile compounds.

MATERIAL AND METHODS

Mead samples. Slovak Herbal (made from cherry floral apian honey, flavoured with herbs and spices extract), Slovak Pale (made from acacia apian honey), and Slovak Dark (made from honeydew forest apian honey) were obtained from Slovak local meadery. iQhilika Herbal (made from wild natural plants of Eastern Cape apian honey, flavoured with rooibos, honeybush tea, cinnamon, and apple) and iQhilika Chilli (made from wild natural plants of Eastern Cape apian honey, flavoured with African Birds Eye Chilli) were obtained from a South African local meadery.

Analytical methods. The analysis was performed on mead immediately upon opening; the determination of free and bound sulphur dioxide was performed by iodometric titration (Joslyn 1955). The ethanol, extract, residual sugar content, and total and volatile acidity were determined using official methods (OIV 2009). The protein content was determined using the Bradford method (Bradford 1976).

The total polyphenol content was analysed using a modification of the method described by Slinkard and Singleton (1977). All samples were analysed in triplicate and determined using a gallic acid standard curve (at concentrations of 50, 100, 150, 250, and 500 mg/l in water) as mg/l gallic acid equivalents.

Volatile compounds were analysed by the solid-phase microcolumn extraction (SPMCE) method using a GC 8000 Top Series, CE Instruments (Rodano, Milan, Italy) equipped with a modified inlet (Hrivnak *et al.* 2010). For the analysis, a 100-ml aliquot of a mead sample was transferred into a 500-ml volumetric flask containing 20 g NaCl, and the flask was vigorously shaken by hand for approximately 30 s at room temperature. After a 10–15 min equilibration, 10 ml of the headspace from a distance of about 1–2 cm above the liquid phase was withdrawn. The headspace content in the syringe was immediately pushed through the microcolumn (1 mm *i.d.* packed with 5.0 mg of 60–80 mesh Tenax TA). The loaded microcolumn was transferred into a modified GC inlet. The trapped aroma compounds were desorbed at 10 kPa by heating the microcolumn to 230°C for minute. After desorption, the carrier gas pressure was increased to 60 kPa and the temperature program was started. During the run, the microcolumn remained in the GC inlet.

The chromatograph was equipped with an FID and fused silica VF-WAXms capillary column of 30 m in length × 0.25 *i.d.* × 0.5 µm of film thickness (Varian, Lake Forest, USA). The chromatographic elution was temperature programmed as follows: isothermal at 30°C (4 min), then increased at a rate of 5°C/min to 200°C and held for 10 minutes. The temperature of the inlet chamber was 230°C; helium was used as a carrier gas.

For quantitative analysis, five-point calibration curves over the range of appropriate concentrations in the 12% (v/v) of ethanol were measured.

All values in this work are the averages of the results obtained in triplicate, while the data variation was less than 5% of RSD (percentage relative standard deviation).

RESULTS AND DISCUSSION

As shown in Table 1, the ethanol concentration in all three samples of Slovak meads was slightly higher (it varied from 13.26% to 13.56%, v/v), and the residual sugar content, depending on the mead type, was significantly higher (it ranged from 136.6 g/l to 199.6 g/l), than in the South African meads (ethanol from 11.92% to 12.03% (v/v); residual sugars from 67.3 g/l to 76.9 g/l).

Conversely, the titrable acidities of the South African meads were slightly higher. However, the volatile acidities were lower in the South African meads (0.75 g/l and 0.79 g/l of acetic acid equivalent), whereas in the Slovak meads, they varied from 0.99 g/l to 1.49 g/l. The values found in the Slovak meads were in agreement with those reported in Polish meads (Sroka & Tuszynski 2007) and those reported in the meads produced by Mendes-Ferreira *et al.* (2010) – from 0.51 g/l to 0.84 g/l. Pereira *et al.* (2009) verified that the concentrations of volatile acids in mead are similar to those reported for *S. cerevisiae* in wine must fermentations by Nikolaou *et al.* (2006). The levels of medium-molecule fatty acids determined in mead by Sroka and Tuszynski (2007) were markedly higher than those obtained in the study of Mendes-Ferreira *et al.* (2010). The content of volatile acids in mead is influenced by organic acids contained in honey. The main acids to form during fermentation are acetic and succinic acids, which reduce the final pH of mead.

South African meads had considerably higher sulphur dioxide levels than Slovak meads, ranging from 184.1 mg/l to 190.1 mg/l as compared to 35.6 mg/l to 41.6 mg/l of SO_2 in the Slovak ones. The free sulphur dioxide content followed a similar pattern. All meads fell within the allowed limits of the sulphur dioxide concentration and volatile acidity. The extract and protein content were higher in all of the Slovak meads.

Volatile compounds – esters, higher alcohols, acetaldehyde and acetone – assayed in the Slovak and South African meads are presented in Table 2.

Esters are synthesised during fermentation as a result of yeast activity and contribute several "fruitlike"' and "floral" aromas. Ethyl acetate, a compound responsible for a pleasant fruity aroma, predominated in the volatile profile of both the Slovak and South African meads, with a significantly higher concentration in the Slovak meads (from 46.36 mg/l to 60.03 mg/l) compared to the South African ones (16.35 mg/l and 16.97 mg/l). The concentrations of this compound in the Slovak meads corresponded with the results obtained by Mendes-Ferreira *et al.* (2010), as ethyl acetate concentrations in their meads were between 20 mg/l and 50 mg/l. Conversely, the concentrations of ethyl acetate (from 14.62 mg/l to 23.21 mg/l) in meads, as published by Vidrih and Hribar (2007), were similar to the concentrations of this compound found in South African meads. The second predominant ester with a fruity odour was ethyl butyrate. Its concentration in one of the Slovak meads (Slovak Herbal) was 5.75 mg/l. All other meads contained ethyl butyrate in lower concentrations (from 2.60 mg/l to 3.72 mg/l). The concentration of methyl acetate was slightly higher in one of the Slovak meads (Slovak Dark – 1.82 mg/l) than in the South African meads (1.20 and 1.27 mg/l). Two other Slovak meads contained 0.68 mg/l and 0.98 mg/l of this ester. The concentration of ethyl decanoate was higher in one of the Slovak meads (Slovak Herbal – 1.00 mg/l) than in all the other meads (from 0.50 mg/l to 0.69 mg/l). The concentrations of other esters, including *iso*-butyl acetate, amyl acetate, *iso*-amyl acetate, ethyl hexanoate, ethyl octanoate, and diethyl succinate, did not differ significantly between the Slovak and South African meads. Mendes-Ferreira *et al.* (2010) reported slightly lower concentrations of these esters, with the exception of *iso*-amyl

Table 1. Basic analytical parameters of the Slovak and South African meads

Mead	Residual sugars (g/l)	Free SO_2 (mg/l)	Total SO_2 (mg/l)	Volatile acidity (g/l acetic)	Titrable acidity (g/l tartaric)	Extract (g/l)	Ethanol (% v/v)	Proteins (mg/l)
Slovak Herbal	136.6	4.5	35.6	0.99	4.35	138.9	13.26	17.9
Slovak Pale	199.6	5.9	38.6	1.29	3.17	198.2	13.12	10.4
Slovak Dark	165.5	7.4	41.6	1.49	4.66	194.1	13.56	16.0
iQhilika Herbal	76.9	17.8	184.1	0.75	6.83	109.3	11.92	3.6
iQhilika Chilli	67.3	19.3	190.1	0.79	7.02	94.3	12.03	3.7

Table 2. Volatile compounds in the Slovak and South African meads (in mg/l)

Compound	Slovak Herbal	Slovak Pale	Slovak Dark	iQhilika Herbal	iQhilika Chilli
Methyl acetate	0.98	0.68	1.82	1.20	1.27
Ethyl acetate	46.36	60.03	53.53	16.35	16.97
Butyl acetate	< 0.01	< 0.01	< 0.01	< 0.01	nd
i-Butyl acetate	0.56	0.57	0.56	0.56	0.56
Amyl acetate	0.89	0.89	0.89	0.89	0.90
i-Amyl acetate	0.93	0.903	0.903	0.90	0.91
Ethyl butyrate	5.75	2.80	3.07	2.60	3.72
Ethyl *i*-valerate	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Ethyl hexanoate	0.91	0.88	0.88	0.87	0.88
Ethyl octanoate	0.41	0.40	0.40	0.40	0.40
Ethyl decanoate	1.00	0.61	0.50	0.60	0.69
Diethyl succinate	0.53	0.53	0.53	0.53	0.53
Acetaldehyde	7.11	8.78	15.01	1.56	1.66
Acetone	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Propanol	5.69	9.73	7.04	9.36	9.65
Butanol	0.15	0.10	0.12	0.23	0.22
2-Butanol	< 0.01	nd	nd	< 0.01	nd
i-Butanol	5.88	10.32	6.65	11.98	11.64
i-Amyl alcohol	11.20	2.39	2.15	11.41	10.26
Hexanol	0.53	0.13	0.34	1.19	1.12
2-Phenylethanol	1.24	nd	nd	0.86	nd

nd – not detected

acetate, whose concentration corresponded with those found in our meads.

Acetaldehyde is formed as a metabolic branch point in the pathway leading from carbohydrate to ethanol, synthesised during the initial phase of fermentation. Its level varies during fermentation and ageing of fermented beverages. At concentrations of 20–25 mg/l, acetaldehyde causes "green vegetation" or "vegetable" flavour (Smogrovicova & Domeny 1999). The concentration of acetaldehyde was significantly higher in the Slovak meads (7.11–15.01 mg/l) than in the South African ones (1.56–1.66 mg/l). However, its concentrations were lower than the threshold concentration causing the unpleasant flavour mentioned.

Higher alcohols constitute an important part of the by-products formed during fermentation, but like the major product of fermentation, ethanol, have a small impact on the flavour of the final beverage. Fusel alcohols, such as butyl, amyl, and *iso*-amyl alcohols, contribute to the general alcohol warming sensation in the mouth (Smogrovicova & Domeny 1999).

The most predominant higher alcohols in the studied meads were *iso*-butanol, with a characteristic mild, sweet-musty flavour, and isoamyl alcohol, with a rather bitter fruity flavour. Both were more prevalent in the South African meads (isobutanol – 11.64 mg/l and 11.98 mg/l, isoamyl alcohol – 10.26 mg/l and 11.41 mg/l) compared to two of the Slovak meads (isobutanol – 5.88 mg/l and 6.65 mg/l, isoamyl alcohol – 2.15 mg/l and 2.39 mg/l). However, one of the Slovak meads (Slovak Pale) contained a similar concentration of *iso*-butanol (10.32 mg/l), and another one (Slovak Herbal) contained a similar concentration of isoamyl alcohol (11.20 mg/l) as the South African ones. Propanol was the second most predominant alcohol, its concentration ranging from 9.36 mg/l to 9.65 mg/l in the South African meads and from 5.69 mg/l to 9.73 mg/l in the Slovak meads. 2-Phenylethanol, with a characteristic sweet rose odour,

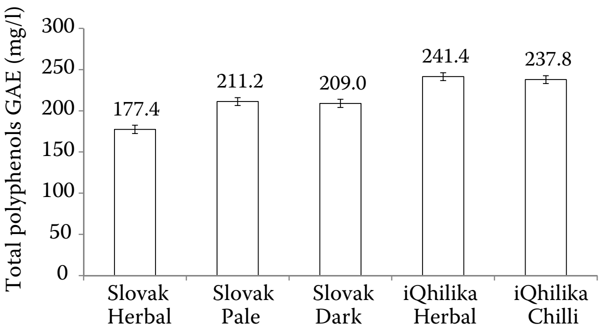

Figure 1. Total polyphenol content in the Slovak and South African meads

was detected only in one of the Slovak and one of the South African meads in concentrations of 0.86 mg/l and 1.24 mg/l, respectively. Hexanol was found in concentrations ranging from 1.12 mg/l to 1.19 mg/l in the South African meads and in concentrations from 0.13 mg/l to 0.53 mg/l in the Slovak meads.

Vidrih and Hribar (2007) reported higher concentrations of propanol (20.79–39.10 mg/l), *iso*-butanol (24.92–30.46 mg/l), *iso*-amyl alcohol (59.45–112.95 mg/l), and 2-phenylethanol (4.20– 6.26 mg/l) depending upon the honey type used. However, the authors used also chestnut honey with insoluble solids, which was reported to be the main cause of an increased production of higher alcohols. Mendes-Ferreira *et al.* (2010) reported higher concentrations of *iso*-amyl alcohol and 2-phenylethanol as compared to our meads. An increased production of higher alcohols worsens the sensorial properties of fermented beverages in general.

One of the South African meads *(*iQhilika Herbal*)* contained an unidentified compound in a relatively high concentration (11.83% of the total peak area of chromatogram), which was not detected in any other mead.

The total polyphenol content was similar across all mead samples tested, ranging between 177.8 mg/l and 241.4 mg/l of gallic acid equivalents (Figure 1), and was similar to those of commercial meads and white wine published by Wintersteen *et al.* (2005).

The meads from Slovakia were produced using batch fermentation of acacia honey, cherry floral honey or honeydew forest apian honey, while the meads from South Africa were produced by continuous fermentation of wild natural plants of Eastern Cape apian honey using immobilised yeast. With the exception of two Slovak mead samples (Slovak Pale and Slovak Dark), all the samples tested were flavoured. Therefore, it was a very interesting observation that, with the exception of one unidentified compound present in one of the South African meads, all main volatile aroma compounds were very similar in their abundance in all the samples from both of the countries.

The parameters dependent on the mead style, such as the residual sugar and alcohol contents, differed, while the polyphenols and volatile component contents were very similar. The total sulphur dioxide content was higher in the African meads, because the Slovak ones were preserved using sorbate. However, the sulphur dioxide content in the African meads did not exceed the European limits of 200 mg/l. The protein content, which depends on the technological process of honey pretreatment, was lower in all of the African meads.

The results of this study indicate that there are no significant differences exist between the parameters of the Slovak and South African meads such as polyphenols. The residual sugar content was significantly higher in all of the Slovak meads, because the consumers in Slovakia typically prefer sweet mead reminding them of honey, and drink it often served hot, only on special occasions such as at Christmas markets. In South Africa, mead is drunk with dinner instead of wine.

Scientific studies on mead are very rare. As reported by Mendes-Ferreira *et al.* (2010), there were only three reports investigating some aroma compounds of meads (Wintersteen *et al.* 2005; Sroka & Tuszynski 2007; Vidrih & Hribar 2007) before 2010. In these studies, it was shown that the higher alcohols and esters analysed varied considerably depending upon the honey floral source (Wintersteen *et al.* 2005; Vidrih & Hribar 2007), and heat treatment of honey-must (Wintersteen *et al.* 2005). Global analysis of aroma profiles revealed quantitative differences between different meads confirming the contribution of both yeast metabolism and honey-must composition to the diversity of "bouquet". Therefore, a comparison of the parameters of meads, including volatile profiles, produced on two different continents and using different fermentation technologies is of great importance.

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