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Linear Regression Model of the Ash Mass Fraction and Electrical Conductivity for Slovenian Honey

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Summary

Mass fraction of ash is a quality criterion for determining the botanical origin of honey. At present, this parameter is generally being replaced by the measurement of electrical conductivity (*κ*). The value *κ* depends on the ash and acid content of honey; the higher their content, the higher the resulting conductivity. A linear regression model for the relationship between ash and electrical conductivity has been established for Slovenian honey by analysing 290 samples of Slovenian honey (including acacia, lime, chestnut, spruce, fir, multifloral and mixed forest honeydew honey). The obtained model differs from the one proposed by the International Honey Commission (IHC) in the slope, but not in the section part of the relation formula. Therefore, the Slovenian model is recommended when calculating the ash mass fraction from the results of electrical conductivity in samples of Slovenian honey.

Key words: honey, ash, electrical conductivity, linear regression model

Introduction

In the present research, which is part of an extensive study of the physicochemical and sensory characteristics of Slovenian unifloral and mixed honeys, the behaviour of five unifloral and two mixed types of honey is examined with respect to the electrical conductivity and ash mass fraction.

Electrical conductivity and ash mass fraction are measures of the mineral content of honey (*1–4*). The ash mass fraction is a quality criterion for determining the botanical origin of honey (*2,4–6*); brighter honeys usually containing fewer elements than darker ones. At present, the determination of ash mass fraction is generally being replaced by the measurement of electrical conductivity (*κ*) (*2–4,7*). The total ash mass fraction could be retained as a quality factor during a transition period until conductivity is accepted as a worldwide standard (*7*).

Electrical conductivity depends on the ash and acid content of honey, the higher their content, the higher the resulting conductivity (*2*). The relation between *κ* and mineral content has already been demonstrated by many researchers, who have determined that the above-mentioned parameters are in a linear relationship, which can be expressed with various equations (*4,8–12*). A higher mineral content results in a higher *κ* value. Minerals are introduced into honey primarily with the pollen. The mineral and ash mass fraction therefore depend on the predominant pollen present in honey (*2*); they are, as such, appropriate parameters for honey type determination (*4,8–12*).

The pollen in honey derives from both nectar and nectarless plants. When calculating the percentage of pollen present during pollen analysis, the pollen of nectarless plants is excluded. In this way only pollen from nectar plants determines the type of honey in pollen ana-

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lysis. But the ash mass fraction and κ value depend on the whole pollen spectrum of the honey. In spite of this, mineral and ash mass fraction are honey type dependent (*10*).

A linear relationship exists between electrical conductivity on the one side and soluble and insoluble ash (*11*), sulphated ash (*9*) and total ash mass fraction (*4,8, 9,12*) on the other side. Italian model (*4,8*) that describes a relationship between electrical conductivity and total ash mass fraction is: κ=1.74 w(ash)+0.14; where κ is the electrical conductivity in mS/cm, and w(ash) is the total ash mass fraction in g per 100 g of honey. This model is a general one; it covers a range of κ from 0.09 to 2.11 mS/cm and, according to Vorwohl, it has been proposed for use around the whole Europe by International Honey Commission (IHC) (*1*). This model should replace an older, slower and more difficult method for determination of total ash mass fraction by ashing. The main problem, however, is that this model is based on Italian honey types and has not been tested in many other honey-producing countries in Europe. The other weakness of this model is that it cannot be used for samples with κ equal to or lower than 0.14 mS/cm. Nevertheless, in Italy and other parts of southern Europe there are many honey types, such as rosemary and citrus, which possess very low κ, usually below 0.14 mS/cm.

Spanish model for determining electrical conductivity, based on Basque honey (*9*), is: κ= 1.205 w(ash)+0.092. This model was constructed by using data for κ measured in honey solution prepared according to the procedure proposed by Sancho *et al.* (*13*), where 10 g of honey is weighed per 75 mL of distilled water. This procedure does not need water content information before electrical conductivity determination. Therefore, this model is not directly comparable to the Italian model, but it has to be recalculated first. Spanish researchers who set up this model performed recalculation and found out that both models are similar. Sancho's procedure is very simple, but unified procedures in honey analysis should be used around Europe to enable researchers to compare the results without any recalculations needed because of different honey solution preparations. Therefore, in our opinion, the IHC procedures should be followed as much as possible.

Another example from the literature is the Irish model. Downey *et al.* (*12*) determined a linear regression of the total ash mass fraction and electrical conductivity to be κ=0.771 w(ash)+0.1084 for Irish honey. This model is accurate only for low κ values (0.1–0.5 mS/cm) and therefore is not comparable to the Italian or Spanish one. Europe or countries of the European Union are very diverse in climate and pedology conditions; thus honeys produced in different parts are variable. It is therefore unreasonable to expect honey from Europe to be homogeneous and that a model established for honey from a distinct part of Europe would be accurate for the whole of Europe. Consequently, the question arises, which model should be used for Slovenian honey?

There are 7 main types of honey in Slovenia: acacia (*Robinia pseudoacacia*) and multifloral (mixed meadow plants and fruit trees) of nectar origin, lime (*Tilia* spp.) and chestnut (*Castanea sativa*) of nectar and honeydew origin, forest honeydew honey (mixed forest trees), fir (*Abies alba*) and spruce honey (*Picea abies*) of honeydew origin. Among these honey types, great differences exist, both in κ and ash mass fraction as well as in the sensory characteristics and pollen spectrum.

Materials and Methods

All samples (290) included in this research were produced in the years 2004 and 2005 and were gathered directly from Slovenian beekeepers. The sampling was carried out by beekeepers immediately after harvesting, and in accordance with written instructions. Honey samples were stored at room temperature in plastic containers. In this way no mineral or dust contamination could occur. Collected samples were produced in different regions of Slovenia. Samples were of different botanical origin, mostly of mixed botanical origin (185 samples), with no specific sensory characteristics of a particular honey type. Other samples (105) included 15 samples each of the seven main Slovenian honey types (acacia, multifloral, lime, chestnut, forest, spruce and fir).

Sample preparation

Ash mass fraction and electrical conductivity were determined in 290 samples of Slovenian honey according to the AOAC method (*14*) and the harmonised methods of the International Honey Commission (*7*). Before the analyses, all crystallised samples were homogenised and liquefied at 40 °C in order to enable water content determination.

Methods

Content of water

The refractive method is based on the principle that the refractive index of honey increases with the solids content. Water content of honey is determined from the refractive index of the honey by reference to a standard table. The method is recommended in harmonised methods (*7*).

Electrical conductivity (Method A)

This method is valid for the determination of electrical conductivity (κ) of honey in the range of 0.1–3 mS/cm. Electrical conductivity is measured using a 20 % (by mass per volume) solution in water, where the 20 % refers to the honey dry matter. This method is recommended in the harmonised methods (*7*).

Electrical conductivity (Method B)

A modified method was applied for the determination of electrical conductivity (κ) in honey using a 20 % (by mass) honey solution in water, where the 20 % refers to the honey dry matter. This method is less time and glassware consuming. We used it to assess the differences between the two methods.

Ash mass fraction

Ash mass fraction of honey is the residue obtained by a defined procedure and is expressed as a percentage by mass. The honey is ashed at 600 ºC and the residue is weighed (*14,15*).

Sensory analysis

Sensory analysis of all samples was used for selecting the samples with characteristic properties of each type of Slovenian honey. Sensory characteristics described by Golob *et al.* (*16*) for each type of Slovenian honey were used in this analysis. Sensory analysis includes appearance, smell, taste, and aroma assessment. Through sensory analysis, 15 most representative samples of each type were selected from the 290 samples. All samples were sensory assessed by at least three members of the sensory panel.

Statistical analysis

All analyses were carried out in triplicate. For calculating the relationship between ash mass fraction and κ, only average values of each sample were used. The following statistical parameters were calculated: average value, standard deviation and coefficient of variability. After the correlation analysis, analysis of variance (ANOVA) and Duncan's test were performed. Correlations were obtained by Pearson's correlation coefficient in bivariate linear correlations. Differences between means at the 95 % ($p\leq0.05$) confidence level were considered significant.

Results and Discussion

Fifteen samples of 7 types of honey (in total 105 samples) were analysed for water content, ash mass fraction and κ. The average values and standard deviations (SD) are presented in Table 1. Using ANOVA and Duncan's test, we determined that the average values for ash mass fraction and κ were statistically significantly different among some types of Slovenian honey at $p\leq0.05$. Acacia and chestnut honey differed from all other types, whereas no difference existed between the multifloral and lime. There was also no difference among forest, spruce and fir honey, but these types differed from all the others.

Water content completely depends on the maturity of honey; it was therefore not meaningful for comparison among the different types of honey.

The average mass fraction of ash in our acacia honey was lower than in Croatian honey (*17*). On the other hand, the average mass fraction of ash in Slovenian multifloral honey was higher than in Croatian multifloral honey. The range of ash mass fraction in our samples, from 0.04 to 1.04 g per 100 g, was wider than previously reported for Spanish thyme honey (*18*), multifloral honey (*5,19*) and chestnut honey (*5*). The average ash mass fraction in their samples was higher than we measured for nectar honey types, whereas the average κ was lower for thyme honey than for our floral and lime honey and higher than κ for acacia honey. The average mass fraction of ash in Slovenian multifloral honey was comparable to the ash mass fraction determined for Indian multifloral honey (*20*).

Our main prediction about linear regression model for κ and ash mass fraction was that different models were valid only for specific types of honey. However, as can be seen in the last two columns of Table 1, this anticipation was incorrect, since no statistically significant differences between models for different types of honey had been found. The linear regression model of ash mass fraction and κ is therefore independent of honey type. From the available literature we understand that this hypothesis has not previously been tested.

All Slovenian honey types can be described with one general model, which is presented in Fig. 1. Yet, this model is imperfect due to the lack of data for κ in the ranges from 0.8 to 1.0 mS/cm and from 0.25 to 0.5 mS/cm, as well as above 1.5 mS/cm.

Table 1. Average values and SD for water content, ash mass fraction and κ_B

Type of honey	Number of samples	Basic statistics	w(water)/%	w(ash)/%	κ_B/(mS/cm)	Linear regression between κ and w(ash)	
						Model	R^2
Acacia	15	Average±SD	16.2±1.0	$(0.06\pm0.01)^a$	$(0.20\pm0.03)^a$	κ=2.09 w(ash)+0.08	0.81
		Range	14.5–15.5	0.04–0.09	0.16–0.26		
Multifloral	15	Average±SD	16.0±0.8	$(0.21\pm0.05)^b$	$(0.58\pm0.12)^b$	κ=2.19 w(ash)+0.12	0.86
		Range	15.2–17.8	0.11–0.27	0.33–0.78		
Lime	15	Average±SD	16.7±1.0	$(0.25\pm0.04)^b$	$(0.73\pm0.07)^b$	κ=1.95 w(ash)+0.23	0.82
		Range	15.2–18.9	0.18–0.30	0.55–0.86		
Chestnut	15	Average±SD	16.2±1.1	$(0.70\pm0.13)^d$	$(1.62\pm0.28)^d$	κ=2.07 w(ash)+0.15	0.92
		Range	14.6–18.0	0.55–1.04	1.20–2.25		
Forest	15	Average±SD	14.9±0.6	$(0.53\pm0.06)^c$	$(1.28\pm0.13)^c$	κ=1.91 w(ash) +0.26	0.89
		Range	14.1–15.8	0.44–0.63	1.06–1.47		
Spruce	15	Average±SD	15.8±1.1	$(0.57\pm0.09)^c$	$(1.31\pm0.19)^c$	κ=1.93 w(ash)+0.22	0.88
		Range	14.3–18.4	0.41–0.71	0.96–1.47		
Fir	15	Average±SD	15.3±0.8	$(0.55\pm0.08)^c$	$(1.29\pm0.15)^c$	κ=1.99 w(ash)+0.21	0.98
		Range	13.8–16.6	0.38–0.65	1.07–1.63		

Data with different superscript in the same column are statistically significantly different at $p\leq0.05$

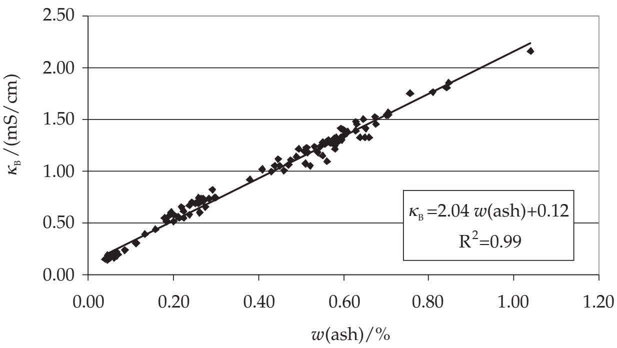

Fig 1. Linear regression of κ_B and w(ash) in 105 samples (7 types and 15 samples of each type)

Consequently, we decided to expand this research and include more honey samples. The number of samples thus rose from 105 to 290. These extra samples were of mixed botanical origin and could not be classified as just a single type of honey. As such, these samples were only used for linear regression model construction. The model presented in Fig. 2 was generated from the data for κ and ash mass fraction of all 290 samples. Here, the κ varies from 0.16 to 2.25 mS/cm and the ash mass fraction varies from 0.04 to 1.04 g per 100 g of honey. The determined linear regression model is therefore valid only in this range.

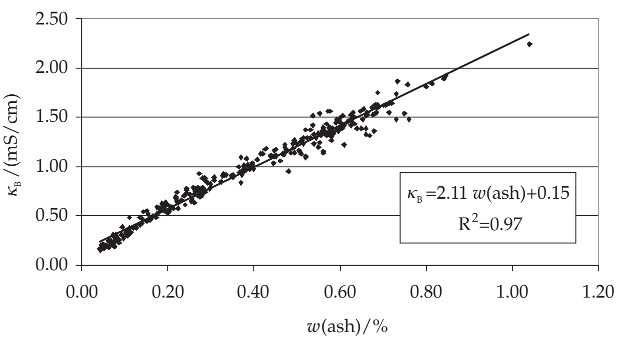

Fig 2. Linear relationship between the κ_B and w(ash) in Slovenian honey (290 samples)

When comparing our model with the one proposed by IHC, it becomes obvious that the method A for κ determination of electrical conductivity should be used because it is recommended in the harmonised methods and was used when constructing the Italian model. However, more time and glassware are needed for this method. In order to save time, method B was used. Sancho *et al.* (*13*) also used some simplifications for the κ determination but they also needed to use proper corrections when comparing their results to the results of analyses performed according to IHC (*7*). Our modified method for the determination of electrical conductivity is faster than others and less glassware is needed. We attempted to assess differences between the two methods. Preliminary results of measurements on 31 honey samples revealed that significant differences exist between these two methods. Method B gave results that were on average 3 % lower than the results of method A. A statistically significant relationship (R^2=0.999) exists between these two methods. We prefer the method B due to savings in time and glassware use, but only if used with proper correction. Our correction and relationship between the two methods is presented in Fig. 3, where κ_B is on the abscissa while κ_A is on the ordinate.

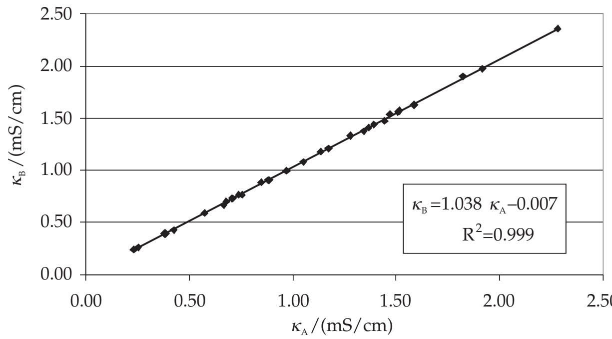

Fig 3. Linear relationship between two methods for κ determination: κ_B and κ_A

All our results were recalculated using the above-mentioned relationship between electrical conductivity measured by the two methods and a new model was constructed from the new data. This model is presented in Fig. 4.

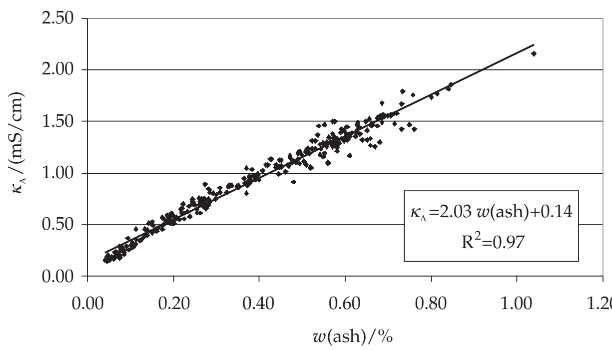

Fig 4. Linear relationship between the κ_A and w(ash) in Slovenian honey

The final regression model (y=ax+b), as presented in Fig. 4, between the mass fraction of ash and κ_A or κ_B (κ=2.03 w(ash)+0.14) differs from the one proposed by the International Honey Commission (IHC): κ=1.74 w(ash)+0.14, where κ is the electrical conductivity in mS/cm and ash is the ash mass fraction in g per 100 g (*7*). The slope differs greatly, but the section part of the relation formula does not. The relation was gathered by analysing 290 samples of different pure types of Slovenian honey and their blends.

As an illustration: if a sample of Slovenian honey has κ=1.0 mS/cm, the ash mass fraction is 0.42 g per 100 g as calculated by the Slovenian model, or 0.49 g per 100 g when using the model proposed by the IHC. The difference of up to 17 % in ash mass fraction is obtained when the different models are compared. Observed disparity is an outcome of a different type and a different geographical origin of honey used in the research of Piazza *et al.* (*4*) compared to our present work.

Measuring the κ_A value is prescribed to take place in 20 % (by mass per volume) water solution of honey, and is therefore always calibrated using the dry mass of honey. But the mass fraction of ash is determined in the natural honey, not in its dry matter content. It would thus be wise to make a comparison between κ and the mass fraction of ash in the dry matter of honey. The model of linear regression for κ and ash mass fraction in dry matter (κ=2.41 w(ash)+0.14) is presented in Fig. 5.

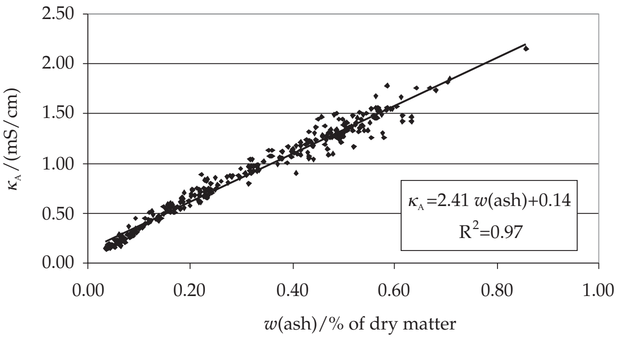

Fig 5. Linear relationship between the κ_A and w(ash) of dry matter for Slovenian honey

This model has steeper slope and the same section part as the model without correction for water content, which was presented in Fig. 4, and is even less comparable to the IHC model. The use of this model should be further discussed with other researchers in this field, especially since Sancho *et al.* (*13*) claimed that the effect of moisture content on κ determination is very small and therefore can be neglected.

Conclusions

When comparing our model with the proposed one, it can be concluded that Slovenian honey obviously has higher κ values at the same ash mass fraction, which might be explained by its higher acid content. Therefore, when calculating the ash mass fraction from κ in the samples of Slovenian honey, it is recommended that the Slovenian formula be used for this relationship. The Slovenian model is valid in a range of κ from 0.16 to 2.25 mS/cm. Honey samples with κ lower than 0.16 mS/cm are very rare in Slovenia, so this is not really a weak point of this model.

A faster and less complicated method B for κ_B determination can only be used if a proper correction factor is employed for recalculating the correct value. In Fig. 4, the linear regression model of the κ_B and ash mass fraction in Slovenian honey are presented. The latter model should be used for ash mass fraction calculation in our laboratory, but we use the modified method for κ determination. However, all simplifications of the honey solution preparation may represent difficulties when comparing the results among different laboratories that use different procedures. Therefore, a uniform standard in honey analysis provided by International Honey Commission should be used in as many laboratories as possible around Europe. The exposed problem of the importance of measuring the ash mass fraction in dry matter when establishing models of relationship between κ and ash mass fraction should be discussed with leading European researchers in the field of honey researches.

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