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A procedure to identify a honey type

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Abstract

The aim of this work was to develop a new procedure to identify a honey type using the discriminant analysis. The physical-chemical parameters of quality of honeys were determined in 73 honey samples. Using only three parameters (total ash content, total acidity and dynamic viscosity) a nearly correct classification (98.67%) was achieved.

Keywords: Honey; Identification of honey; Discriminant analysis; Electrical conductivity; Total ash; Dynamic viscosity; Total acidity

1. Introduction

The growing interest on issues associated with the variety-related identification of products has been observed in the domain of nutrition science for several years already. In a number of scientific centres, various honey identification procedures have also been studied. A *pollen analysis* appeared to be the most frequently used method in such studies (Bambara, 1991; Bolchi Serini & Salvi, 1990; Feller-Demalsy & Parent, 1989; Gomez Ferreras, 1987; Ohe & Von Der Dustman, 1990; Szczęsna & Rybak-Chmielewska, 1993). In order to identify honeys by their varieties, a sensory analysis was applied (Kerlvliet, 1992; Serra Bonvehi & Gomez Pajuelo, 1988). For this purpose, many researchers attempted to analyse physical-chemical parameters of honeys, and among the parameters investigated, electric conductivity was found to be the most useful while identifying honeys. However, the electric conductivity measured allows for the separation of only some varieties of nectar honeys among all the multifloral nectar and honeydew honeys (Bańkowska-Penar & Pieczonka, 1987; Kubišova & Mastny, 1976; Popek, 1998). No anticipated results were obtained when trying to identify honeys on the basis of dyes (mainly flavonoids) contained in them (Bogdanov, 1989; Tisse, Dordonnat, & Guerere, 1994), or on the grounds of colour parameters measured using $L\ a^*\ b^*$ and XYZ systems (Ortiz Valbuena & Silva Losada, 1990). To achieve the same aim, other physical-chemical parameters were analysed, such as: content of aromatic acids and amino acids, general or active acidity, general ash content, carbohydrates amount, and, also, ratio between glucose and fructose amounts (Mateo & Bosch Reig, 1997; Pena Crecenta & Herrero Latorre, 1993; Persano Oddo, Sabatini, Piazza, & Accorti, 1995; Pirini, Conte, Lanfranco, Ornelia, & Lercker, 1992; Steeg & Montag, 1988). Nevertheless, with those methods, it is impossible to classify all honeys according to their individual types and varieties.

In addition, some other methods are suggested; they involve measurements of dozen or so parameters showing physical and chemical characteristics of honeys; measured values are statistically assessed with use of various analyses (analysis of variance, canonical analysis, principal component analysis, multivariate analysis, taxonomic analysis) for the purpose of choosing some of those features and making an optimal factor of a specific variety out of them (Krauze & Zalewski, 1991; Pena Crecenta & Herrero Latorre, 1993; Persano Oddo, Stefanini, Piazza, & Accorti, 1988; Salinas, Alvarez, Montero De Espinoza, & Lozano, 1994; Salinas, Monterro De Espinoza, Lozano, & Sanchez, 1994; Sanz, Perez, Herrera, Sanz, & Juan, 1995). Yet, with the methods suggested, the only objective achievable was that the honeys investigated were grouped into three or four groups which, unfortunately, did not strictly corresponded to the individual honey variety.

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In order to develop a procedure to identify a type and variety of honeys, based on the physical-chemical parameters of honey quality, two-phase calculations were conducted. The first phase involved a classic, single-factor variance analysis, while in the second phase, a method of discrimination analysis was used. Moreover, a model to use while identifying honeys was proposed.

2. Materials and methods

The experimental material in the present study consisted of 73 samples of honeys belonging to the variety types [nectar (rape, acacia, heather, linden, buckwheat, multifloral nectar from various plants) honeydew and nectar-honeydew]. The tested honey samples were produced in apiaries located throughout Poland, and each sample was from a different apiary.

The botanical origin and the purity of honey samples was controlled using a savouriness profiling method, it was a method developed by Cairnocros and Sjöström, and modified by Tilgner (1962). The method referred is a sort of compilation of the dilution indicator technique and the flavour profiling concept; the key principle of proceeding is to watch (monitor) the quality, intensity, and sequence of single notes. The notes are: flavour components and a flavour factor of an investigated product that is diluted in a neutral medium (redistilled water), and its dilution grade is gradually increased. The dilution processes ensues until they totally vanish. The method as indicated appears very practical in investigation procedures dealing with the identification of bee honey varieties. The profiling technique involving a dilution method allows of detecting masked flavour factors, and since several varying dilution grades are applied, it is easier to watch changes in the intensity and the occurrence sequences of notes which generate/present the complete flavour impression. The following assays were performed on the experimental material:

1. water content, using a refractometric method (AOAC, 1995; Codex Stanard, 1981);
2. total ash content by incinerating honey samples in a muffle furnace at a temperature of 550 °C (AOAC, 1995; Codex Stanard, 1981; Ładoński & Gospodarek, 1986);
3. reducing sugar content and total sugar content using the Lane-Eynon method (AOAC, 1995; Codex Standard, 1981);
4. sucrose content (Codex Stanard, 1981);
5. active acidity (pH) of aqueous 20% solutions using a CX-721 multi-function computer measuring instrument (AOAC, 1995);
6. total acidity of aqueous 20% solutions; acidic honey components were neutralized by a standard solution of sodium hydroxide (AOAC, 1995; Codex Stanard, 1981);
7. specific electrical conductivity of aqueous 20% solutions using a CX-721 multi-function computer (AOAC, 1995);
8. dynamic viscosity of aqueous 20% solutions using an Ubbelohde viscosimeter; a honey solution flow in a capillary of Ubbelohde's viscosimeter was measured (PN-87/C-8929/20, 1987);
9. colour parameters in the $L\ a^*\ b^*$ system using a CR-200 Minolta Chroma Meter (Gliszczyńska & Koziołowa, 1995).

A variance analysis was performed using an Anova packet of the Statistica softwear. The decision was made as to the significance of the differences between the group values (for the type or variety) of arithmetical means on the basis of the calculated value of the 'F' test (at the level of $\alpha=0.05$). Averages calculated were grouped in homogeneous groups with use of a Tukey test (Bożyk & Rudzki, 1977).

In the second phase, a linear discriminant analysis method (a discriminant function analysis method) was used (Jajuga, 1993; Pieczonka, 1998; Salinas, Alvarez, et al., 1994; Salinas, Monterro De Espinosa et al., 1994). This is one of the methods of a multi-dimensional statistical analysis; with this method it is possible to assess a set structure of experimental observations, i.e. to evaluate a position of individual elements of a set in a n-dimensional space, where "n" equals the number of measured parameters. The position of the set elements in space is designated by vectors of the parameters that describe them, whereas mutual relations occurring among the individual objects result from the indicated distance matrix.

A discriminant analysis packet of the Statistica softwear was used to perform calculations. The first step of the discriminant analysis was executed as a 'stepwise forward analysis'. For the purpose of this analysis a model was assumed in which the grouping variable was a type (variety) of honey, and the classifying variables were quality parameters obtained. The next steps involved some other quality parameters, and calculations were carried on until a classification matrix was obtained with vectors the parameters of which allowed for the incorporation of 100% of all the tested samples (objects) into that group (type and variety) from which they originated. This is presented in Table 2.

3. Results and discussion

The results achieved are shown in Tables 1–3.

Table 1
Results of the physical-chemical parameters (mean ± S.D.)

Group of honey	Acacia ($n=10$)	Linden ($n=10$)	Multifloral ($n=10$)	Buckwheat ($n=10$)	Heather ($n=8$)	Rape ($n=8$)	Honeydew ($n=10$)	Nectar-honeydew ($n=7$)
Electrical conductivity ($Scm^{-1}10^{-4}$)	2.192 ±0.493	5.495 ±0.337	6.835 ±0.402	3.570 ±0.351	6.091 ±0.153	3.557 ±0.292	9.972 ±0.60	9.3800 ±1.221
Total ash (%)	0.1005 ±0.0221	0.1535 ±0.0579	0.2884 ±0.0318	0.2441 ±0.0341	0.2191 ±0.0375	0.1415 ±0.0162	0.5609 ±0.0615	0.5875 ±0.1201
Water (%)	17.68 ±1.81	17.96 ±2.12	16.21 ±2.02	15.93 ±1.80	17.72 ±1.74	16.87 ±2.59	16.10 ±1.72	15.73 ±1.53
Acidity (^{0})	1.38 ±0.26	2.14 ±0.39	1.72 ±0.23	2.46 ±0.20	2.40 ±0.29	1.48 ±0.16	3.53 ±0.31	1.70 ±0.32
Total sugar (%)	84.02 ±3.02	80.30 ±3.43	75.10 ±1.58	74.58 ±2.62	72.88 ±1.09	81.06 ±1.96	73.19 ±2.99	71.80 ±2.43
Reducing sugar (%)	77.28 ±3.11	79.57 ±3.40	73.01 ±1.67	72.58 ±2.55	71.74 ±1.19	79.95 ±1.96	69.07 ±2.73	69.15 ±2.40
Sucrose (%)	6.13 ±0.93	0.71 ±0.28	2.19 ±0.74	1.47 ±0.28	0.99 ±0.27	0.99 ±0.47	3.89 ±0.33	2.49 ±0.32
Viscosity (mPa s)	1.755 ±0.021	1.624 ±0.035	1.565 ±0.030	1.616 ±0.017	1.681 ±0.052	1.635 ±0.016	1.586 ±0.042	1.525 ±0.053
L	25.13 ±0.47	26.81 ±0.75	29.17 ±0.98	22.30 ±1.59	25.46 ±3.45	38.26 ±1.73	23.70 ±3.01	25.71 ±2.88
*a**	−0.63 ±0.17	−0.73 ±0.45	−0.30 ±0.48	1.17 ±0.35	1.06 ±0.48	−1.02 ±0.38	0.17 ±0.30	0.26 ±0.05
*b**	3.48 ±2.17	5.88 ±2.66	6.54 ±1.46	2.89 ±0.96	6.57 ±1.39	8.59 ±1.00	4.25 ±0.86	5.05 ±0.87
pH	3.75 ±0.13	3.86 ±0.21	3.78 ±0.09	3.74 ±0.10	3.95 ±0.08	3.67 ±0.10	4.24 ±0.26	4.11 ±0.08

Table 2
Results of the discriminant function analysis method

Step	Measure	Honey (% of properly qualified samples)							
		Buckwheat	Honeydew	Nectar honeydew	Acacia	Heather	Multifloral	Rape	Linden
1	Electr. cond.	90	80	14	100	62	90	0	50
2	Electr. cond., Acidity	100	100	100	100	62	100	100	60
3	Electr. cond. Acidity Ash	100	100	100	100	75	100	100	80
4	Electr. cond. Acidity Ash Sucr.	100	100	100	100	75	100	100	80
5	Electr. cond. Acidity Ash Sucr. Sugar red.	100	100	100	100	100	100	100	100

It was stated in the paper that there are relationships among the physical-chemical quality parameters of honey types under investigation. It was stated that a special dependence exists among the specific electrical conductance, the total ash content, and the dynamic viscosity.

The high specific electrical conductance of honeydew and nectar-honeydew honeys results from the high content of total ash in them. Contrary to this fact, low levels of the specific conductance, as evidenced with regard to the acacia, buckwheat, and rape honeys, are caused by a low content of total ash in them. Moreover, the results presented in Table 1 prove that the total ash content in the investigated product first depends on the raw material, which a particular honey type is made of. It is so because the total ash content in the nectar honey varieties analysed is averagely 2.5 times lower than in nectar-honeydew and honeydew honeys.

Table 3
The results of the canonical analysis

Group of honey	Means of canonical variables	
	Root 1 [F1]	Root 2 [F2]
Rape	−8.91390	0.39739
Honeydew	6.43602	6.78307
Nectar-honeydew	12.40637	−4.30991
Acacia	−6.58457	−0.20796
Heather	0.33877	3.39515
Multifloral	4.47326	−3.74584
Buckwheat	−4.13347	−3.53060
Linden	−1.05951	−0.10136

As for the relationship between specific electrical conductance and dynamic viscosity, it was confirmed the following: a high specific electrical conductance of the nectar-honeydew and honeydew honeys is connected with a low level of their dynamic viscosity whereas a low level of the acacia and rape honey's parameter is to be referred to a higher value of their dynamic viscosity. During our investigations, we found that the dynamic viscosity showed comparable values for all investigated honey samples from various type and variety groups. We explained it on the base of the similar sugar content, a main component of honeys, in all of the honey samples analysed. In addition, a comparable degree of honey hydration was assumed to be a factor making the absolute viscosity very similar for all tested honey samples. Thus, the highest dynamic viscosity of the acacia honey recorded could be attributed to the relatively highest content of sucrose in the acacia honey. Whilst analysing the total acidity rates obtained, we can state what follows: the lowest acidity shown by the acacia and rape honeys is connected, if compared with the high active acidity (pH), with the fact that dissociated organic acids, contained in them, mainly determine the acidity of those honey varieties. Other honey components of an acidic character do not impact their acidity much. A similar tendency could be observed in a nectar-honeydew honey type. At the same time, with regard to sugars contained in each of the 73 investigated honey samples, an interesting inverse proportionality was stated between the sugar content and water content (humidity) in honeys. Sugars a dominating element of the bee honeys' dry mass ranging from 80% (Maurizio, 1966) to 99% (White, 1976), and this fact can be an explanation of this specific relation.

On the basis of the Variance Analysis's results, it can be inferred that the level of water is unvarying in all types and varieties of honey whereas the other parameters differentiate subgroups of honeys in the following manner:

- Electrical conductivity
  - group I - linden;
  - group II - rape, buckwheat;
  - group III - linden, heather, multifloral;
  - group IV - nectar-honeydew, honeydew
- Total ash
  - group I - rape, acacia, linden;
  - group II - buckwheat, heather, multifloral;
  - group III - nectar-honeydew, honeydew.
- Total acidity
  - group I - rape, acacia, multifloral, nectar-honeydew;
  - group II - buckwheat, heather, linden;
  - group III - honeydew.
- Sugar, reducing and total
  - group I - rape, acacia, linden;
  - group II - multifloral, buckwheat, heather, honeydew, nectar-honeydew.
- Sucrose
  - group I - rape, buckwheat, heather, linden;
  - group II - nectar-honeydew, multifloral;
  - group III - honeydew;
  - group IV - acacia.
- Viscosity
  - group I - nectar-honeydew; multifloral;
  - group II - acacia;
  - group III - linden, buckwheat, heather, rape honeydew.
- *L* (brightness)
  - group I - rape;
  - group II - linden, buckwheat, heather, multifloral, acacia, nectar-honeydew, honeydew.
- Parameter of colour *a**
  - group I - rape, acacia, linden;
  - group II - multifloral;
  - group III - buckwheat, heather, nectar-honeydew, honeydew.
- Parameter of colour *b**
  - group I - rape;
  - group II - linden, buckwheat, heather, multifloral, acacia, nectar-honeydew, honeydew.
- pH
  - group I - rape, buckwheat, acacia, multifloral;
  - group II - heather, linden;
  - group III - nectar-honeydew, honeydew.

The variance analysis is a fully adequate method to draw substantial important conclusions provided a given experiment consists of testing the impact of selected factors on the one characteristic (quality parameter). If, on the other hand, experimental data include several characteristics, it may happen that the final conclusion will be extremely difficult to reach. The variance analysis is a one-dimension method typical for the so-called "partial description." It does not enable any kind of comprehensive approach, nor any interpretation that embraces all the measured parameters together; it does not point out the best or optimal variant (subgroup) of the tested population, designated on the basis of several parameters measured. In this case, difficulties in the interpretation emerge from the fact that each successive quality parameter (among those measured) allows for the differentiation of two or three subgroups of honeys, including several varieties of each subgroups. (On the basis of the pH value, for example, three subgroups can be distinguished.) When some parameters (e.g. *L*) were measured, it was possible to distinguish one variety of honey. Thus, the variance analysis does not allow for the identification of types and varieties of honeys to a degree that could be called "comprehensive".

For this reason, a linear discriminant analysis method (discriminant function analysis method) was applied. The following fragments (presented in Table 2) of the classification matrix presented here indicate that electrical conductivity is a parameter that allows for the distinction of only one honey, the acacia honey, from the rest. The determination of electrical conductivity and acidity makes it possible to distinguish at that step all the varieties except for the heather honey (its three samples were classified together with the linden honey) and linden (three samples classified as heather, and one as rape). The incorporation of the results obtained from the determination of conductivity, acidity, ash content, and sucrose content into the calculations produced no essential changes in the classification outcome. It was only when the fifth characteristic was included, i.e. the content of reducing sugars, that it was possible to distinguish all the sub-groups.

Thus, it is obvious that the discriminant function analysis basing on the determination of the five honey's quality parameters: electrical conductivity, total acidity, ash content, sucrose, and reducing sugars will enable the identification of types and varieties of honeys.

In the second phase of the discriminant analysis, calculations were made in which a series of models was accepted and differentiated by quality parameters included in the analysis; here, those parameters were selected using some laboratory determination procedures since they were relatively easy and not too much time consuming. The best of those models proved to be the one in which conductivity, acidity, and viscosity were measured. The three parameters as pointed out made it possible to correctly classify almost all the samples (only one sample of a heather honey was assigned to the linden honey). The canonical analysis performed included a calculation of the average values of two canonical variables (Table 3) and the coefficients of those variables. The average values give a picture of the placement of the individual types and varieties in the system of two coordinates (canonical variables). The coefficients of the canonical variables form the following equations of the corresponding linear discriminant function:

$$F_1 = -28.658 + 3.254^{*}\text{cond.} - 4.723^{*}\text{acid} + 12.233^{*}\text{visc.}$$
$$F_2 = -70.989 + 0.378^{*}\text{cond.} - 4.725^{*}\text{acid.} + 36.128^{*}\text{visc.}$$

This model can be suggested for use to identify honeys. With it, three parameters (electrical conductivity, total acidity, and viscosity) can be determined, and two functions: F1 and F2 can be calculated; next, the calculated values can be compared with those presented in Table 3.

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