

## APPLICABILITY OF PHYSICO-CHEMICAL PARAMETERS OF HONEY FOR IDENTIFICATION OF THE BOTANICAL ORIGIN\*

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### ABSTRACT

**Background.** Efforts are being made to apply physicochemical parameters analysis in the identification of varietal honeys. With many variables describing a given population, it is feasible to differentiate between basing on principal component analysis (PCA). The aim of this study was to investigate selected physicochemical quality characteristics of nectar honey, with particular emphasis paid to carbohydrate composition, and to determine its applicability in identifying the variety of floral honey.

**Material and methods.** The experimental materials were samples of commercial honey available at retail in Krakow in 2005-2007 period. The following analyses were performed: water content by the refractometric method, sugars content according to Luff-Schoorl, content of sugars using HPLC, electrical conductivity, specific rotation, and acidity of honey.

**Results.** Application of HPLC allowed the precise qualitative identification of sugars, which was impossible to be determined by Luff-Schoorl method. The obtained results were analysed using principal component analysis (PCA).

**Conclusions.** Based on the obtained results and performing the statistical analysis, it was found that the relationship between specific rotation and the total acidity can be used to distinguish buckwheat honeys from other analysed varieties. Moreover, it was demonstrated that the relationship between the specific rotation and maltose content can be used to distinguish between acacia honeys vs. buckwheat and lime honeys.

**Key words:** honey, chromatography, principal component analysis

### INTRODUCTION

Honey is called the product made by honeybees from the nectar of plants or honeydew. Depending on the origin, there are distinguished three types of honey: nectar, honeydew and nectar-honeydew [Wojtacki 1988]. Both nectar and honeydew are derived from the juice contained in the conductive tissues of higher plants, but the nectar is directly derived from the plant, while honeydew is collected by bees as secondary material, resulting from the activities of insects

[Bańkowska-Pennar 1983]. Nectar is a sweet liquid, secreted by small glandular type organ, called nectary, which is present in the majority of angiosperms. It consists mainly of water (30 to over 90%) and sugars (up to approximately 70%, the most abundant are glucose, fructose and sucrose). In the smaller quantities, there can also occur nitrogen compounds, organic acids (mainly malic, citric, tartaric, oxalic and succinic acids), pigments (carotenoids, xanthophylls),

\*This work was financially supported as scientific project N312441237.

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enzymes, essential oils, some vitamins and minerals (i.e. potassium, sodium, calcium) [Bańkowska-Pennar 1983, Hołderna-Kędzia and Kędzia 1994]. The largest group of chemical compounds in honey are carbohydrates. The main carbohydrates of the most honeys are: glucose, fructose and sucrose. They are often accompanied by complex sugars [Bańkowska-Pennar 1983, Popek 2001]. Glucose and fructose compose about 70-80% of all the sugars contained in nectar honeys and 55-65% in honeydew honeys [Chmielewska-Rybak 1987]. Their quantitative relationship varies depending on the origin of honey [Przewodnik... 1993]. Except monosaccharides honeys also contain certain amounts of disaccharide, but their content ranges from 3.29-18.60% and oligosaccharides from 0.13-10.00% [Wojtacki 1988]. The most widespread disaccharide in the plants world is sucrose. In the nectar honeys its content usually does not exceed 3% [Chmielewska-Rybak 1987]. It is assumed, that mature nectar honeys should not contain more than 5% of sucrose [PN-88A-77626 1988]. Its content is higher in unripened honey [Rybak-Chmielewska 2007 a]. The content of sucrose in nectar or honeydew is much higher, but the enzyme  $\beta$ -fruktofuranosidase (invertase) added by bees breaks it into simple sugars: glucose and fructose. A much higher content of sucrose is in the product that bees produce after feeding them a sugar syrup [Przewodnik... 1993]. In a similar quantity, as sucrose, another reducing disaccharide maltose, it is present in the honey, which content, according to some authors may be higher than sucrose, and ranges from 2.8 to 7.5% [Przewodnik... 1993]. The distinctive taste of honey comes not only from the presence of the sugars, but also organic acids. Indeed, they greatly enrich and differentiate the taste bouquet of honey, depending on its variety, and determine honey's maturity [Wojtacki 1988]. The acidity of honey varies from about 2-4 degrees of acidity and depends on its variety and type. The highest acidity was observed in buckwheat honey, whereas acacia and rape the lowest [Hołderna-Kędzia and Kędzia 1994]. In honey formic, malic, tartaric, succinic, acetic, lactic, citric, oxalic, gluconic, malonic, valeric acids were identified, but the main acid found in honey is gluconic, which constitutes 70-90% of total acids [Gertig and Przysławski 2007, Hołderna-Kędzia and Kędzia 1996]. honey acidity depends mainly on the type of material, maturity level,

and season, in which it was produced [Wojtacki 1988]. While ripening of honey its acidity increases as well. The excessive acidity is characteristic for fermented honeys, and usually is the result of the various microorganisms development on the their surface [Hołderna-Kędzia and Kędzia 1996].

The so-called acacia honey is produced during the flowering of black locust (*Robinia pseudoacacia* L.), also known as "false acacia", "white acacia" or "white linden tree" This plant is an excellent feeding ground for the bees [Hołderna-Kędzia 2001 a]. Pure acacia honey is characterised by light cream colour passing into a clear, and therefore is considered as the brightest of all honeys. After crystallization it takes colour from white to straw [Gala 1994].

Linden honey is produced by bees from the flowers of the small-leaved Lime (*Tilia mordata* Mill.), and large-leaved Lime *Tilia platyphyllos* (*Tilia platyphyllos* Scop.) that are commonly grown in Poland [Hołderna-Kędzia 2001 c]. It is distinguished not only by a strong aroma resembling a linden flower fragrance, but also sweet and clear, quite spicy, slightly bitter taste [Wojtacki 1988]. Lime honey colour in a liquid state ranges from greenish-yellow to pale amber, while its texture and colour resembles castor oil. After crystallization it takes the form of fine-grained, changing its colour from white yellow to golden yellow. Pure lime honey has quite a sharp taste, and a very distinctive and strong flavour resembling the smell of mint [Wojtacki 1988].

Buckwheat honey comes from buckwheat (*Fagopyrum sagittatum* Gilib.), which is a good melliferous plant. Bees collect nectar from small blooming buckwheat flowers during the summer [Hołderna-Kędzia 2001 b]. The strained buckwheat honey colour ranges from dark to brown. Stored for longer periods in conditions enabling the access of light, it changes colour from dark brown to almost black, and therefore is considered to be the darkest of all honeys. During crystallization it forms thick, hard crystals deposited in the liquid honey [Przewodnik... 1993]. After crystallization lumps adopt coarse, non-uniform structure with a slightly lighter color [Wojtacki 1988]. It has a very intense and pleasant aroma of buckwheat flowers. The taste is spicy and sweet, slightly burning [Hołderna-Kędzia 2001 b]. As well as acacia honey it contains a lot of fructose, and therefore it is difficult to

crystallize, and remains in the liquid state up to two months [Przewodnik... 1993].

Efforts are being made to apply physico-chemical parameters analysis in the identification of varietal honeys [Bańkowska-Pennar 1983, Hołderna-Kędzia 2001 c]. The most characteristic parameter for identifying honeys was electrical conductivity. Mainly it allows to distinguish nectar honey, and some varieties of multi-floral honey from honeydew [Popek 2001]. Other physicochemical parameters including analysis of amino acids, aromatic acids, water content, total acidity and sugar content, total ash, acidity and the ratio of the active concentrations of glucose to fructose were used to distinguish among honey varieties. This, however, did not bring the expected results in identifying the all varieties of honeys [Popek 2001].

With many variables describing given population, it is feasible to differentiate between basing on principal component analysis (PCA). This analysis is one factor analysis method. Using the PCA method a new set of factors called principal components could be obtained from a variety of factors. The most of variation is usually explained by the two initial factors. The principal component analysis can also be used to reduce the size of the statistical data set [Beretta et al. 2005, Karoui et al. 2007, Sanz et al. 1995].

The aim of this study was to investigate selected physical and chemical quality characteristics of floral honey available in the retail trade in Krakow, with particular emphasis paid to carbohydrate composition. Also an attempt was undertaken to apply principal component analysis (PCA) to identify the varieties of honeys.

## MATERIAL AND METHODS

The research material consisted of commercial honeys purchased in retail stores in Krakow. These samples came from the season 2005-2007.

They were coded according to the following scheme:

XX.A.B.YY

where:

- XX – is a honey descriptor (NA – acacia nectar, NL – lime nectar, NG – buckwheat nectar),
- A – indicate on the honey producer,
- B – is the number of the package,
- YY – the last digits of the honey production year.

## METHODOLOGY

**Determination of water content by the refractometric method.** The samples of honey were closed tightly in test tubes and heated in a water bath at 50°C ( $\pm 0.2$ ) until all the crystals dissolved. Next the samples were cooled to room temperature and mixed. The refractive index was measured by means of Abbe refractometer [RL3 1811/83 PZO Warsaw]. According to results obtained the water content in honey was established as well [PN-88A-77626 1988].

**Determination of sugars.** Determination of sugars was performed by chromatographic method according to the International Honey Commission (IHC) recommendations [Bogdanov 2002] with Lupano [1997] modification. Chromatographic separation was performed on an amino column [100-5 Eurospher Knauer NH2] with RI detection. As a mobile phase the acetonitrile/water 87/13 (v/v) was used. The flow rate was set on 1.3 ml/min. Samples were prepared at a concentration of 5 g honey per 100 mL, and as a solvent a solution of methanol/water 1/3 (v/v) was used. Before injection samples were filtered on the 45  $\mu$ m filter. Loop volume was 10  $\mu$ L.

An analysis of sugars before and after inversion by Luff-Schoorl method was performed. The methods principle is the reduction of copper (II) ions by carbonyl group of sugar. The amount of reduced copper was calculated on the basis of the difference in the volume of thiosulfate used in the blank and test [PN-90/A-79120.06 1990].

**Determination of conductivity.** This analysis was performed according to the International Honey Commission (IHC) recommendations [Bogdanov 2002]. The electrical conductivity of a solution of 20 g dry matter of honey in 100 ml distilled water was measured using conductometer CPC-551 (Elmetron Poland). The electrical conductivity of honey was expressed in  $\text{mS}\cdot\text{cm}^{-1}$  and was measured at 20°C.

**Determination of specific rotation.** Identification was performed according to the IHC recommendations [Bogdanov 2002]. 12 g of honey was accurately weighed, and dissolved in distilled water in a volumetric flask (100 ml). Than 10 ml of Carrez I solution was added and sample was stirred for 30 seconds. Next 10 ml of Carrez II solution was added, stirred again for 30 seconds and filled up to the mark. The next day the

content of flask was filtered through a fluted filter into a dry beaker. The clean polarimeter tube (with a length of 2 dm) was filled with solution, placed in a polarimeter, and rotation angle ( $\alpha$ ) was read.

The calculation of results based on Biot equation:

$$[\alpha]_D^{20} = \frac{\alpha \cdot 100}{l \cdot p}$$

where:

$\alpha$  – rotation value, °,

$l$  – length of the polarimeter tube, dm,

$p$  – honey sample weight, g, on dry basis.

**Determination of honey acidity.** This analysis was performed according to the (IHC) recommendations [Bogdanov 2002]. 10 g of sample was dissolved in 75 ml of CO<sub>2</sub>-free distilled water in 250 ml beaker. The solution was stirred with a magnetic stirrer, and pH meter electrode was immersed in it, and pH value was read. Next the sample was titrated with 0.05 M NaOH at a rate of 5.0 ml/min. Titration was completed at pH = 8.5. Then 10 ml of 0.05 M NaOH was immediately added and whole sample was titrated with 0.05 M HCl to pH 8.30. The result is given in mmol/kg:

$$\text{free acidity} = (\text{cm}^3 \text{ of } 0.05 \text{ M NaOH from a burette} \\ - \text{cm}^3 \text{ of blank}) \times 50 / \text{g of sample}$$

$$\text{lactone acidity} = (10.00 - 0.05 \text{ cm}^3 \text{ M HCl} \\ \text{from a burette}) \times 50 / \text{g of sample}$$

$$\text{the total acidity} = \text{free} + \text{lactone acidity.}$$

**Statistical calculations.** Statistical analysis was performed using STATISTICA 6.0 (StatSoft, Tulsa, USA). The ANOVA was done and, the significance of differences between means was shown using the Tukey test. Principal components analysis (PCA) was conducted as well. The analysis was performed on four samples of acacia honey, four samples of linden and three samples buckwheat honey. All results are expressed as the average of at least three repetitions.

## RESULTS AND DISCUSSION

By analyzing the water content in the investigated honeys, it was found, that the results fit in to mandatory requirements [PN-88A-77626 1988]. The lowest water content was observed in acacia nectar honey (NA.1.1.06), and highest in buckwheat honey

(NG.1.1.06, NG.1.2.06). The most negative value of specific rotation was observed in case of buckwheat nectar honey (NG.2.1.05), which was also characterised by the highest content of reducing sugars by Luff-Schoorl's method. Chromatographic analysis revealed, that this honey was characterized by the highest content of glucose among all analysed samples. The highest specific rotation was observed in case of linden nectar honeys (NL.1.1.07, NL.1.2.07; Table 1 and 2).

Analysing the mean values of conductivity in the investigated samples, it was discovered that the highest values of this parameter were observed in lime nectar honeys (NL.1.1.07, NL.1.2.07), whereas the lowest in acacia honey (NA.2.1.06, NA.2.2.06; Table 2). Obtained results are consistent with PN-88/77626 requirements, which specifies that the electrical conductivity should not be less than  $2.0 \cdot 10^{-4} \text{ S} \cdot \text{cm}^{-1}$ .

The highest pH value showed acacia honey (NA.1.1.06, NA.1.2.06). In other honeys, this parameter reached lower values, while the buckwheat honeys were characterised by the highest total acidity. This is consistent with the quality requirements of the standard PN-88/77626 as well.

Carbohydrate profile was determined by high performance liquid chromatography (HPLC). The greatest amount of glucose was noted in buckwheat and lime honeys (Table 1, 2), and acacia honeys were characterised by lower content one. Among sugars, acacia honeys contained the highest amounts of fructose – more than 50% (Table 1 and 2); in other honeys this value was lower. Another sugar found in honey is sucrose. Its content was at a fairly low level (Table 1 and 2). Buckwheat honeys had contained the lowest amounts of this sugar. The low sucrose content may be due to a long storage period of honey and its proper ripening [Rybak-Chmielewska 2007 b].

On the other sugars may be mentioned disaccharide maltose. The most abundant this sugar was present in acacia honeys, and in the smallest quantities occurred in buckwheat honeys (Table 1 and 2).

On the base of obtained physicochemical studies, the principal component analysis (PCA) was performed. It is a statistical method that allows concluding about objects described by many variables. This method is used to study the relationship between the two sets of multidimensional variables.

**Table 1.** Results of honey physicochemical analysis

Honey code	The content of reducing sugars %	The content of reducing sugars after inversion %	Glucose content %	Fructose content %	Saccharose content %	Maltose content %	Specific rotation °
NA.1.1.06	86.69 ±0.86 bc	92.01 ±1.42 ab	36.22 ±0.24	53.60 ±1.06 i	1.01 ±0.08 abf	4.24 ±0.41 a	-19.925 ±0.624 b
NA.1.2.06	91.04 ±0.41 a	93.18 ±1.35 a	34.04 ±0.24	51.48 ±0.41 gh	0.56 ±0.47 cde	4.02 ±0.24 ab	-20.219 ±0.706 b
NA.2.1.06	90.32 ±0.36 a	92.71 ±0.04 a	32.39 ±0.25 b	51.21 ±0.17 fg	1.00 ±0.23 abf	4.15 ±0.36 a	-22.463 ±0.385 a
NA.2.2.06	88.70 ±0.75 de	90.80 ±3.38 abc	32.96 ±0.06 b	52.60 ±0.32 hi	0.79 ±0.04 abde	4.15 ±0.16 a	-23.297 ±0.517 a
NL.1.1.07	86.72 ±0.82 bc	87.88 ±0.00 c	44.20 ±0.02 d	48.56 ±0.11 bc	0.65 ±0.16a cde	3.86 ±0.16 ab	-11.738 ±0.519 e
NL.1.2.07	85.61 ±0.74 b	90.12 ±2.75 abc	43.46 ±0.80 cd	47.88 ±0.80 ab	0.89 ±0.04 abef	3.79 ±0.06 ab	-11.276 ±0.426 e
NL.3.1.07	91.28 ±0.61 a	91.68 ±1.26 ab	46.24 ±0.16 a	49.19 ±0.15 ce	1.28 ±0.01 f	3.52 ±0.30 bd	-16.320 ±0.195 c
NL.3.2.07	90.10 ±0.78 ae	90.31 ±1.47 abc	46.76 ±1.14 a	50.17 ±0.94 ef	1.05 ±0.03 bf	3.02 ±0.13 cd	-16.538 ±0.372 c
NG.1.1.06	88.44 ±0.37 d	88.79 ±0.07 bc	40.39 ±0.17	46.17 ±0.20 d	0.67 ±0.13 abcde	2.92 ±0.41 cd	-13.327 ±0.513 d
NG.1.2.06	87.64 ±0.76 cd	90.79 ±1.79 abc	42.44 ±0.37 c	47.00 ±0.38 ad	0.30 ±0.20 c	2.78 ±0.41 c	-13.453 ±0.116 d
NG.2.1.05	90.41 a	92.50 a	47.02 a	48.03 abc	0.42 ±0.05 cd	1.71 ±0.14	-26.515 ±1.718

Samples coding – see material and methods section.

Mean values in the columns and denoted by the same letter are not statistically significant different at  $p < 0.05$ .

**Table 2.** Results of honey physicochemical analysis

Honey code	Free acidity mval/kg	Lactone acidity mval/kg	Total acidity mval/kg	pH	Water content %	Conductivity S/cm
NA.1.1.06	12.75 ±0.42	5.93 ±0.00 a	18.69 ±0.42 a	4.88 ±0.36	15.80 ±0.00	2.57 ±0.002
NA.1.2.06	15.17 ±0.86	2.73 ±0.44 b	17.90 ±0.42 a	4.33 ±0.41	17.62 ±0.00	2.73 ±0.001 a
NA.2.1.06	19.25 ±1.73 b	2.40 ±0.84 b	21.66 ±0.88 b	3.53 ±0.08 a	16.95 ±0.03 a	2.07 ±0.000
NA.2.2.06	20.46 ±0.02 b	1.80 ±0.01 b	21.66 ±0.88 b	3.72 ±0.18 ab	16.95 ±0.03 a	2.02 ±0.003
NL.1.1.07	32.49 ±0.04 a	6.31 ±0.41 a	38.81 ±0.38 c	3.89 ±0.00 ab	16.94 ±0.03 a	5.33 ±0.001
NL.1.2.07	32.73 ±0.39 a	5.71 ±0.43 ad	38.41 ±0.01 c	3.92 ±0.01 b	16.94 ±0.03 a	5.20 ±0.002
NL.3.1.07	31.97 ±0.79 a	4.82 ±0.01 cd	36.79 ±0.78	3.63 ±0.01 ab	17.35 ±0.03 b	2.97 ±0.001 b
NL.3.2.07	30.08 ±0.83	4.51 ±0.43 c	34.59 ±0.40	3.67 ±0.02 ab	17.02 ±0.00	2.98 ±0.001 b
NG.1.1.06	62.61 ±0.88	7.90 ±0.00	70.51 ±0.89	3.63 ±0.03 ab	17.75 ±0.03 c	4.88 ±0.002
NG.1.2.06	53.77 ±0.37	3.95 ±0.42c	57.72 ±0.80	3.85 ±0.04 ab	17.75 ±0.03 c	4.82 ±0.002
NG.2.1.05	46.19 ±0.54	6.64 ±0.83 a	52.83 ±0.30	3.59 ±0.05 ab	17.35 ±0.03 b	2.74 ±0.002 a

Samples coding – see Material and methods section.

Mean values in the columns and denoted by the same letter are not statistically significant different at  $p < 0.05$ .

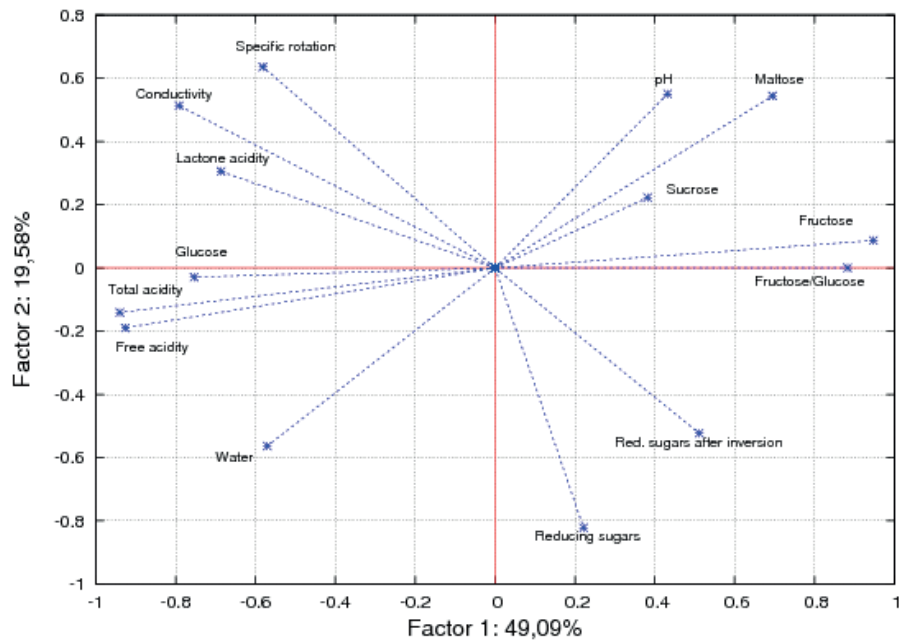


Fig. 1. Projection of variables on the factor plane x

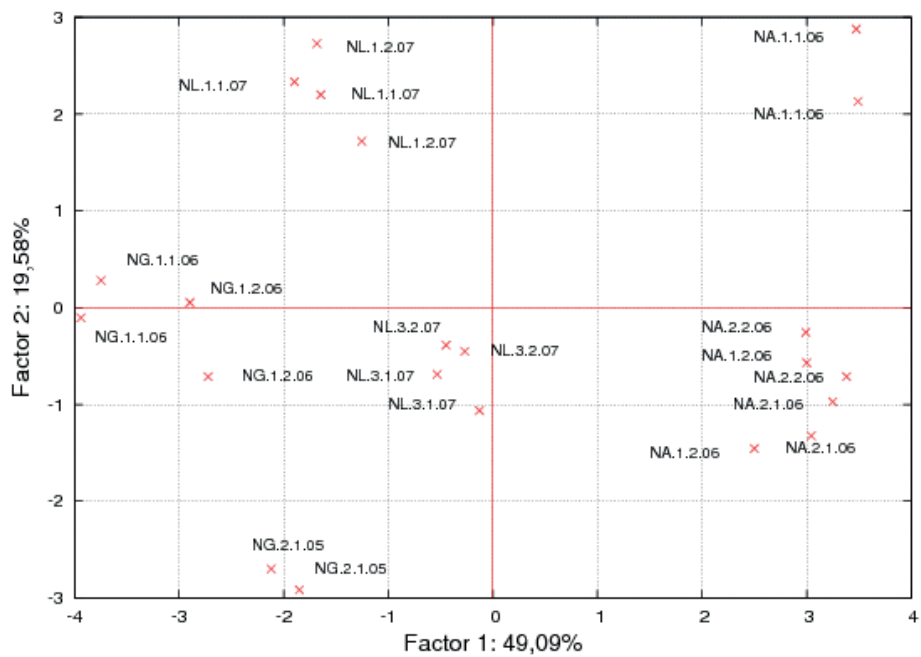


Fig. 2. The projection of cases on the factor plane x. Samples coding see material and methods section

The first two components explained 68.67% of the variation in the sample. Figure 1 shows the projection of variables onto a “x” factor plane. Included variables are: free, lactones and total acidity, pH, conductivity, specific rotation, content of reducing sugars and reducing sugars after inversion, content of glucose, fructose, sucrose maltose, water, and fructose/glucose ratio.

Parameters such as free and total acidity, the contents of glucose, fructose, and the of fructose to glucose ratio were the least related to the factor (2) because they were located closer to “x” axis, but in turn they are strongly linked to the factor (1).

Figure 2 shows the projections of cases on “x” factor plane, and they can be separated from the general population. Basing on this analysis, in the case of this research, e acacia honey could be separated (they form a compact group), which unfortunately failed to buckwheat and lime honeys. In case of greater number of analysis on broader material it could be expected that also these two groups of honey can be separated.

As a result of correlating the specific rotation (which was the most strongly associated with factor 2)

with total acidity (which was most strongly associated with factor 1, but reached negative values) the following graph, presented in Figure 3, was obtained. It was concluded, that line given by equation  $f(x) = 61.4765 + 1.3671 \cdot x$  separates buckwheat honeys from others.

Also correlation between specific rotation and maltose content was found (Fig. 4), and straight line given by equation  $f(x) = -19.789 + 0.5952 \cdot x$  separates acacia honeys from the remaining ones.

## CONCLUSIONS

Based on the obtained results and performing the statistical analysis, it was found that the relationship between specific rotation and the total acidity can be used to distinguish buckwheat honeys from other analyzed varieties. Moreover it was demonstrated that the relationship between the specific rotation and maltose content can be used to distinguish between acacia honeys vs. buckwheat and lime honeys.

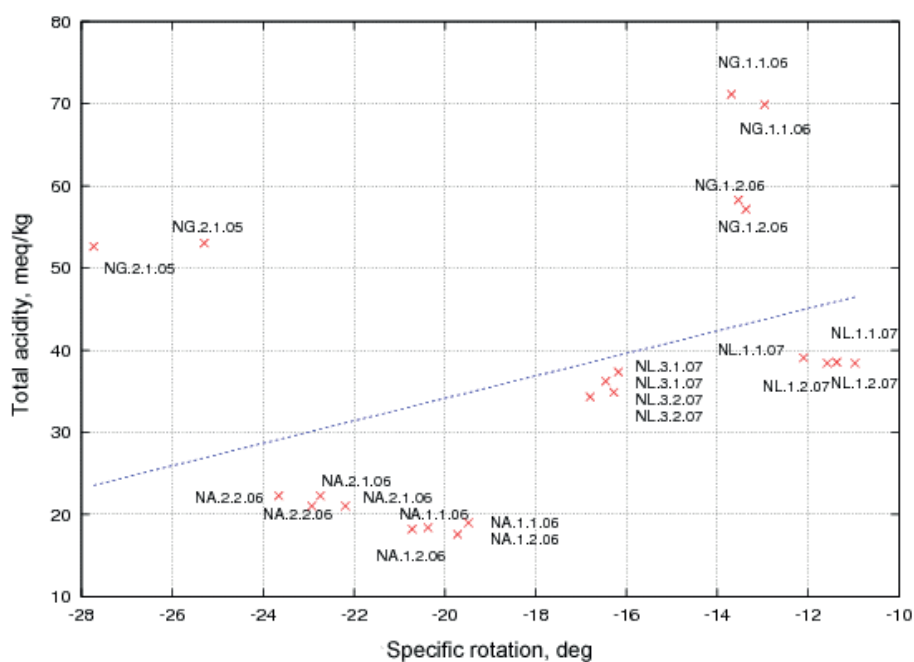


Fig. 3. Correlation of specific rotation from the total acidity  $f(x) = 61.4765 + 1.3671 \cdot x$

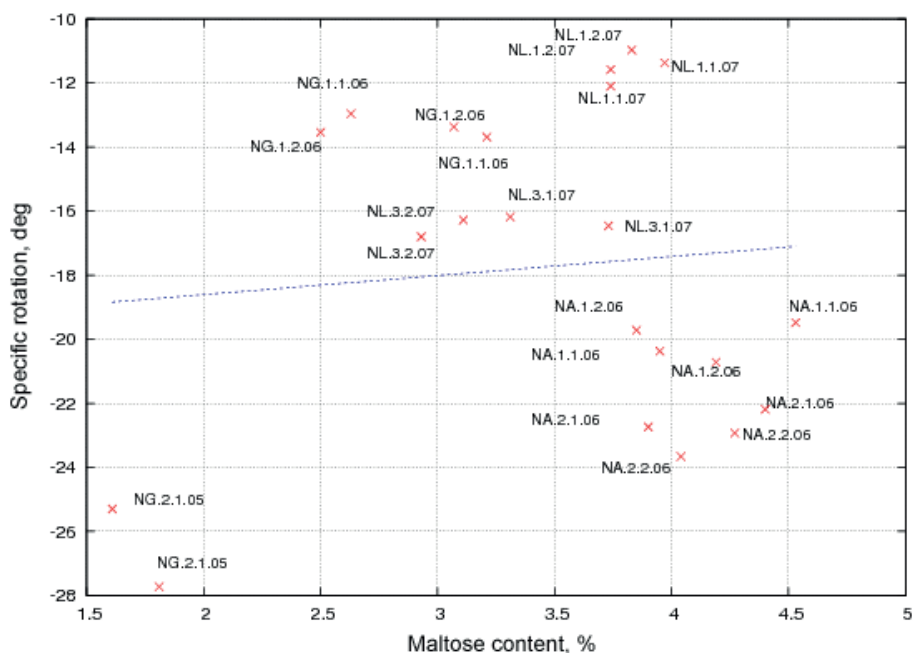


Fig. 4. Correlation of maltose from the specific rotation  $f(x) = -19.798 + 0.5952 \cdot x$

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## MOŻLIWOŚĆ ZASTOSOWANIA WYNIKÓW ANALIZ PARAMETRÓW FIZYKOCHEMICZNYCH MIODÓW DO IDENTYFIKACJI ODMIANOWEJ

### STRESZCZENIE

**Wstęp.** Czynione są starania, aby zastosować wyniki analiz fizykochemicznych analizy do identyfikacji miodów odmianowych. Jednym ze sposobów, umożliwiających wykorzystanie wielu zmiennych opisujących daną populację, jest rozróżnienie na podstawie analizy głównych składowych (PCA). Celem pracy było zbadanie wybranych fizykochemicznych cech jakościowych miodów nektarowych, ze szczególnym uwzględnieniem składu węglowodanowego, oraz określenie ich przydatności w identyfikacji odmianowej miodów nektarowych. Materiałem doświadczalnym były próbki miodów, dostępne w handlu detalicznym na terenie Krakowa, z lat 2005, 2007 z pasiek z terenów południowej Polski.

**Materiał i metody.** W analizowanych produktach oznaczono: zawartość wody metodą refraktometryczną, zawartość cukrów metodą Luffa-Schoorla, zawartość cukrów metodą chromatograficzną, przewodność elektryczną, skręcalność właściwą oraz kwasowość miodu. Badanie za pomocą chromatografu Knauer pozwoliło na dokładną identyfikację jakościową cukrów, niemożliwą do przeprowadzenia w metodzie Luffa-Schoorla. Uzyskane wyniki opracowano statystycznie, wykorzystując analizę głównych składowych (PCA).

**Wnioski.** W wyniku powiązania skręcalności właściwej z kwasowością całkowitą i skręcalności właściwej z zawartością maltozy oddzielono miody gryczane i akacjowe od pozostałych odmian.

**Słowa kluczowe:** miód, chromatografia, analiza głównych składowych

Received – Przyjęto: 26.07.2012

Accepted for print – Zaakceptowano do druku: 19.11.2012

For citation – Do cytowania

Kowalski S., Łukasiewicz M., Berski W., 2013. Applicability of physico-chemical parameters of honey for identification of the botanical origin. *Acta Sci. Pol., Technol. Aliment.* 12(1), 51-59.