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## PHYSICO-CHEMICAL CHARACTERISTICS OF SELECTED HONEYS OF THE WEST POMERANIAN PROVINCE AND THE VALUE OF THEIR PRO-HEALTH

### CHARAKTERYSTYKA FIZYKOCHEMICZNA WYBRANYCH MIODÓW Z WOJEWÓDZTWA ZACHODNIOPOMORSKIEGO I ICH WARTOŚĆ PROZDROWOTNA

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**Streszczenie.** Ostatnio prowadzonych jest wiele badań dotyczących pozytywnego wpływu miodów na przebieg niektórych chorób. Podkreśla się jego szczególnie właściwości lecznicze w walce z chorobami układu krążenia. I chociaż większość autorów sugeruje pozytywny wpływ miodów w leczeniu tych schorzeń, to zwracamy uwagę na inne aspekty, często negatywne, związane ze spożyciem miodu dostępnego w sprzedaży. Przeprowadzono badania dotyczące zafałszowań 35 naturalnych miodów pszczelich pozyskanych z prywatnych pasiek i ze sprzedaży detalicznej. Miody zostały poddane próbie na obecność sacharozy (metodą Lane-Eynona), skrobi, syntetycznych barwników kwaśnych i zasadowych, wody (metodą refraktometryczną), kwasowość ogólną i aktywność przeciwutleniającą (metodą spektrofotometryczną). Spośród przebadanych miodów ponad 50% zawierało więcej niż 10% sacharozy; wykazano związek między zwiększoną zawartością sacharozy a występowaniem obniżonej kwasowości w miodach, co będzie wpływało niekorzystnie na właściwości przeciwbakteryjne produktu. Stwierdzono również obniżoną aktywność przeciwutleniającą miodów, mieszczącą się w zakresie od 10,32 do 80,56 procent inhibicji rodników DPPH, i stosunkowo wysoką zawartość wody w 17% miodów, co będzie negatywnie wpływało na stabilność mikrobiologiczną produktu pszczelego. Nie wykazano obecności skrobi i syntetycznych barwników w badanych próbkach miodu. Postuluje się zachowanie ostrożności w spożywaniu miodu o niesprawdzonej jakości przez osoby przewlekle chore.

**Key words:** honey, acidic and basic dyes, food adulteration, antioxidative activity, acidity, saccharose.  
**Słowa kluczowe:** miód, barwniki kwaśne i zasadowe, zafałszowania żywności, aktywność antyoksydacyjna, kwasowość, sacharoza.

## INTRODUCTION

In recent times the return to the use of natural products in prevention and treatment of diseases of affluence has been observed. Many preparations appeared at retail, which were produced using the advantages of apitherapy, the science which is an unconventional treatment method based on the use of bee's products. The products produced by bees have many therapeutic and cosmetic properties and the major argument for their introduction to

everyday use are their low cost and availability. The fact further supporting the use of natural bee's products is their wide applicability, from their use in food preparation and drinks sweetening, use as a component of isotonic drinks and cosmetics, to prevention and treatment of cardiovascular diseases and cancer (Majtan 2009). In one of the study the reduced effects of heart attack and arrhythmia were observed (Gharekhani *et al.* 2012; Najafi *et al.* 2012). For a honey to demonstrate its pro-healthy effect it has to be of good quality. Its properties, both organoleptic and physicochemical are strictly determined in Polish Standards. To test whether the honeys available in the West Pomeranian Voivodeship fulfill the criteria and are not adulterated, the available honey samples were analyzed with respect to the contents of water, saccharose and artificial acidic and basic dyes, as well as their antioxidative activity and acidity. Not all results of the performed analyses were positive in terms of honeys quality. Detected adulterations can not only lower the pro-healthy quality of honey but also contribute to higher risk of heart attack, sclerosis and arrhythmia.

The purpose of this study was to show whether the honeys available in our region can be approved as food products increasing human health. It is regarded that if the ingredients of a food products or its other properties have been changed and the buyer has not been informed then we deal with food adulteration (Gasparska 2009). Food adulteration also takes place when the substances are added to a food product which change its content and lower its nutritive value. One of the tested honey features was water content. Water content of honey can decide on its microbial stability. The disqualifying characteristic for a honey in Poland is the presence of artificial dyes and starch. However, there is no regulation with regards to saccharose content in honey, hence we expected to obtain results showing malpractice. There have been reports, that dishonest sellers add cheap sucrose syrup into honey to increase the retail amount of their product (Megharbi *et al.* 2009), so it was interesting to determine whether such activities are practiced in our region. After determining the surprising number of cases of honey adulteration with saccharose also honey acidity and antioxidative activity were tested, as the reduction of these parameters additionally had a negative effect on health quality of honeys.

## **MATERIALS AND METHODS**

The study comprised 35 (buckwheat, honeydew, multifloral, lime and acacia) samples of natural bee's honey, which was available at retail in West Pomeranian Voivodeship region in Poland. Among the tested honeys 15 were obtained from private apiaries and 20 from retail market, their origin is shown below (Fig. 1). Honeys were tested with respect to the content of saccharose, starch, synthetic acidic and basic dyes, water content and their acidity. All the analyses were performed according to the Polish Standard PN-88/A-77626. Additionally the antioxidative activity of honeys was measured using DPPH (2,2-diphenyl-1-picrylhydrazyl) (Molyneux 2004; Zych and Krzepiński 2010; Marinova and Batchvarov 2011).



Fig. 1. The origin of honey samples bought in Western Pomeranian province  
 Ryc. 1. Pochodzenie próbek miodu zakupionych w województwie zachodniopomorskim

### Measurement of reducing sugars content

The analysis was performed by Lane-Eynon's method based on the reduction of copper salt from Fehling solution by reducing carbohydrates present in honey in the presence of methylene blue as an indicator. In case of samples where honey solution did not discolor and the sample content remained blue it was assumed that the honey contained more than 5% of saccharose what proved its adulteration.

### Determination of the presence of starch

The analysis was performed in the presence of potassium iodide to test whether honey solution changed its color.

### Presence of synthetic dyes

The test was performed by using degreased yarn and 10% (m/m) solution of potassium bisulfate (acidic dyes) or 1% (m/m) solution of ammonia (basic dyes).

### Water content determination

The analysis was performed by using refractometer after bringing the honey to liquid form at the temp. 40°C. Using glass rod the honey was spread onto the whole surface of lower prism of refractometer and then covered with the top, opaque prism. The results were read with the precision of four decimal places and the index of refraction of 0.00023 per each degree of the temperature different from 20°C was taken into consideration. After each measurement the prisms were cleaned with water and ethanol. The final result was the arithmetical mean of the results of two measurements differing in less than 0.2%.

### Honeys acidity

The analysis was performed in triplicate, calculating the mean and standard deviation (SD). The calculations were based on the following formula considering the conversion to formic acid equaling 0.046.

$$X = (V_z \cdot C_z \cdot K \cdot V_o \cdot 100) / V_1 \cdot m$$

where:

$V_z$  – NaOH volume used during titration [ml],

$C_z$  – NaOH concentration = 0.1 mol/dm<sup>3</sup>,

$K$  – acid conversion unit,

$V_o$  – volume to which the test portion was supplemented – i.e. 50 ml,

$V_1$  – volume of the sample taken for titration – i.e. 50 ml,

$m$  – honey test sample [g].

The reference values for the acidity were from 0.05 to 0.3%. Honeys with higher acidity were discarded due to the fact they could undergo fermentation.

### Antioxidative activity of honey

The activity was measured with spectrophotometric method using synthetic radical DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma). Analyzed honeys of different semi-liquid state were homogenized and 10% water solutions were prepared for further analyses.

Absorbance of 10% solutions of tested honeys was measured using wavelength of  $\lambda = 518$  nm. 0.3 mM ethanol solution of DPPH was prepared and stored in dark at 5°C. Calibration of the spectrophotometer was performed with 96% ethanol. The absorbance of DPPH solution ( $A_0$ ) was measured by adding 1 cm<sup>3</sup> of 0.3mM DPPH ethanol solution to 3 cm<sup>3</sup> of 96% ethanol. For spectrophotometric analysis the tested sample was prepared by mixing 1 cm<sup>3</sup> of 0.3 mM ethanol solution of DPPH, 2.92 cm<sup>3</sup> of 96% ethanol and 0.08 cm<sup>3</sup> of 10% solution of tested sample. After 30 minutes from initiation of the reaction the absorbance was measured ( $A_{pr}$ ) and until the measurement the mixture with analyzed sample was stored in the dark.

Each measurement was taken three times. The results were presented as a mean value with standard deviation.

The ability of honey solution to prevent oxidative reactions, alternatively the % of inhibition, was calculated using the following formula (Molyneux 2004; Zych and Krzepitko 2010; Marinova and Batchvarov 2011):

$$\% inhibition = \frac{A_0 - A_{pr}}{A_0} \cdot 100$$

where:

$A_0$  – absorbance of DPPH radical solution,

$A_{pr}$  – absorbance of analyzed solution containing tested honey sample.

### Statistical analysis

The results were statistically analysed using the software package Statistica 8.0 (Statsoft, Tulsa, Oklahoma, USA). The arithmetical mean, standard deviation and the significance of differences with ANOVA were calculated. To assess the differences between the studied groups, for statistical data analysis, the Tukey test were used. The level of significance was  $p < 0.05$ .

## RESULTS

### Content of reducing sugars

Performed analyses determined the adulteration with saccharose in the majority of tested honey samples. Intensive blue color, giving evidence to adulteration with saccharose, remained in 19 tested samples which amounted to 54.3% (Fig. 2).

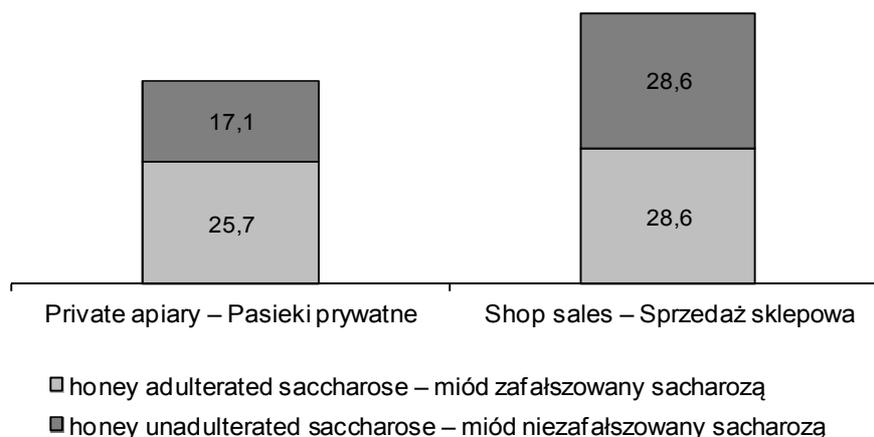


Fig. 2. Percentage of honeys adulterated with saccharose with reference to their origin  
Ryc. 2. Odsetek miodów zafałszowanych sacharozą w odniesieniu do ich pochodzenia

### Presence of starch and artificial dyes

Starch and synthetic dyes, both acidic and basic, were not detected in the tested samples of bee's honey.

### Water content

Water content in 34 honeys fell within the range of reference values and amounted to 14–20%. Higher water content was noted in only one sample – No. 8 (Table 1).

Table 1. Acidity, antioxidative properties [% of inhibition DPPH radicals] of honey and relation between refractive index and water content [% m/m]

Tabela 1. Kwasowość, właściwości antyoksydacyjne [% inhibicji rodników DPPH] miodu oraz zależności współczynnika załamania światła i zawartości wody [% m/m]

No. Lp.	Acidity $\pm$ SD Kwasowość $\pm$ SD [%]	Percent of inhibition of DPPH radicals $\pm$ SD Procent inhibicji rodników DPPH $\pm$ SD	Water content Zawartość wody [%]
1	0.13 $\pm$ 0.00048	32.44 $\pm$ 6.24	16.4
2	0.11 $\pm$ 0.00227	43.16 $\pm$ 6.64	16.5
3	0.10 $\pm$ 0.00396	30.88 $\pm$ 4.44	15.1
4	0.19 $\pm$ 0.01155	38.04 $\pm$ 8.64	15.6
5	0.09 $\pm$ 0.00099	27.12 $\pm$ 2.64	16.0
6	0.14 $\pm$ 0.00379	80.56 $\pm$ 2.96	17.7
7	0.27 $\pm$ 0.00078	61.28 $\pm$ 4.68	16.2
8	0.28 $\pm$ 0.01012	25.34 $\pm$ 3.32	20.1
9	0.11 $\pm$ 0.01044	70.84 $\pm$ 4.84	15.6
10	0.26 $\pm$ 0.00530	54.88 $\pm$ 4.84	16.8
11	0.15 $\pm$ 0.00429	34.40 $\pm$ 2.32	16.0
12	0.17 $\pm$ 0.00520	21.28 $\pm$ 3.80	14.7
13	0.07 $\pm$ 0.00083	75.20 $\pm$ 2.28	15.8
14	0.19 $\pm$ 0.01281	20.48 $\pm$ 3.76	15.1
15	0.07 $\pm$ 0.00063	38.41 $\pm$ 4.56	14.0
16	0.15 $\pm$ 0.01912	19.24 $\pm$ 4.64	14.5
17	0.17 $\pm$ 0.00773	33.40 $\pm$ 4.76	14.6
18	0.07 $\pm$ 0.00018	13.44 $\pm$ 2.56	16.3
19	0.40 $\pm$ 0.01515	15.06 $\pm$ 4.04	18.0
20	0.09 $\pm$ 0.00164	34.16 $\pm$ 3.52	14.9
21	0.20 $\pm$ 0.00213	28.12 $\pm$ 5.20	16.2
22	0.11 $\pm$ 0.00131	35.32 $\pm$ 5.24	18.3
23	0.24 $\pm$ 0.01305	29.28 $\pm$ 3.96	15.1
24	0.12 $\pm$ 0.00233	26.92 $\pm$ 2.36	16.1
25	0.12 $\pm$ 0.00790	16.00 $\pm$ 3.16	16.4
26	0.07 $\pm$ 0.00045	21.12 $\pm$ 4.72	16.3
27	0.12 $\pm$ 0.00411	32.48 $\pm$ 4.44	15.6
28	0.10 $\pm$ 0.00274	10.32 $\pm$ 3.32	14.9
30	0.14 $\pm$ 0.00384	55.64 $\pm$ 8.32	16.7
31	0.07 $\pm$ 0.00164	21.72 $\pm$ 6.36	16.0
32	0.12 $\pm$ 0.01406	57.92 $\pm$ 5.80	17.4
33	0.12 $\pm$ 0.01859	72.72 $\pm$ 2.04	17.2
34	0.08 $\pm$ 0.01269	26.52 $\pm$ 6.36	16.8
35	0.07 $\pm$ 0.00086	14.68 $\pm$ 3.68	16.8
36	0.14 $\pm$ 0.00034	28.64 $\pm$ 4.40	15.1
$\bar{x}$	0.14 $\pm$ 0.00597	38.41 $\pm$ 4.42	16.2

### Acidity of honey

The average acidity of all tested honeys amounted to 0.21% per formic acid. Honeys adulterated with saccharose had lower average acidity of 0.12%  $\pm$  0.006 than honeys with regular saccharose content – 0.24%  $\pm$  0.009 (Table 2). Statistically significant differences were found.

Table 2. Average acidity of honeys with respect to saccharose content  
Tabela 2. Kwasowość miódów z uwzględnieniem zawartości sacharozy

Division honeys Podział miódów	$\bar{x}$	$\pm$ SD
Honey adulterated saccharose Miody zafałszowane sacharozą	0.12*	0.06
Honey unadulterated saccharose Miody niezafałszowane sacharozą	0.24*	0.09

\*Significance difference at  $p < 0.05$  – Różnice istotne przy  $p < 0,05$ .

### Antioxidative properties of honeys

The average antioxidative value of honeys was determined to be  $38.41 \pm 4.42$  (Table 1). Antioxidative properties of buckwheat and honeydew honeys were statistically significantly higher than that of nectar honeys (linden, acacia, multiflorous) and amounted to, respectively 70.7 and 55.08 (Fig. 3). Antioxidative properties of honeys were not affected by the origin (private apiary or retail) and sucrose content.

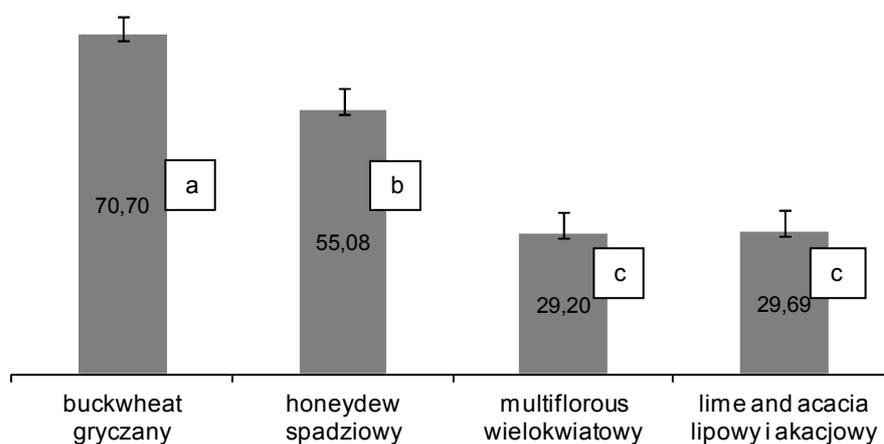


Fig. 3. Comparison average of antioxidative properties of honeys (SD); a, b, c – homogeneous groups in the Tukey test; significance difference at  $p < 0.05$

Rys. 3. Porównanie średnich właściwości antyoksydacyjnych miódów (SD); a, b, c – grupy jednorodnej wg testu Tukeya; istotność różnic przy  $p < 0,05$

## DISCUSSION

The first characteristic determining the quality of honey is its water content. It is important ingredient of bee's honeys and it can amount to 70% in nectars and ca. 30% in honeydew. During honey processing by bees the amount of water should be reduced several times, so in the final product its amount does not exceed 20% (Majewska 2009). Performed studies showed that 6 out of 35 honey samples contained more than 17% water. Honeys with higher water content can be utilized only in industrial processing, e.g. for the production of confectionery mass (Kowalski et al. 2011).

The increase in water content by 1% causes the five-fold increase in yeasts in the product (Chirife et al. 2006; Bogdanov et al. 2008). Due to that the excessive amount of water influences faster honey fermentation. Honey containing more than 19% water quickly undergoes fermentation, but the process can be as quick in honeys containing ca. 17% water (Bornus 1989). Polish standards allow the water content in honeys to reach 20%, the value which seems to be too tolerant. Taking into consideration the information given above it may be postulated that 17% of analyzed honeys will ferment in a short period of time and will be inadequate for consumption. The possibility to lower the water content of honey effectively in laboratory conditions gives certain scope for intervention at the time it is authorized for sale (Semkiw et al. 2008). But the consumption of honey in people with immunodeficiency can have a negative effect on their health.

Another feature significantly affecting its quality is the presence of saccharose in honey. Natural bee's honey is composed of 80% carbohydrates, including 90% of sugars, i.e. fructose and glucose. Their content ratio is from 1.0 : 1.0 to 1.3 : 1.0. Oligosaccharides, present in honeys in small amounts, including saccharose, maltose and isomaltose, have prebiotic properties, but their average content in nectar honeys should not exceed 10%, and the maximal content of saccharose in nectar honey should amount to 5% (Bornus 1989; Borawska et al. 2011). Honeys with higher content of saccharose are considered as adulterated. When performing analyzes it was determined that 19 out of 35 tested honeys were falsified with saccharose. Also the results obtained by other authors confirm that honeys often do not meet the standard requirements concerning saccharose content, due to direct addition of treacle into honey or to excessive feeding of bees before winter period. Also high saccharose content was proved to be the evidence of very early harvesting of honey, when the saccharose was not entirely transformed into glucose and fructose (Rizelio et al. 2012). This process occurs in bee's alimentary tract with the enzyme  $\beta$ -fructofuranosidase (invertase).

Elevated saccharose content in honeys negatively affects consumers' health. The superiority of glucose and fructose over saccharose are, among other factors that they are directly absorbed into the bloodstream, are better assimilated and are healthier for the heart, being better source of energy and exerting cardioprotective effect (Najafi et al. 2011). Additionally, the metabolism of saccharose demands the presence of vitamins thiamin, riboflavin and niacin, what can contribute to reduction of their amount in the organism and thus the occurrence of nervous system and heart diseases. Moreover, saccharose acidifies the organism being the energy source for unfavorable bacteria colonizing alimentary tract. Excessive amount of saccharose in a diet contributes to obesity, sclerosis, high blood pressure, short-sightedness, gastric hyperacidity and intestinal diseases. It weakens the efficiency of heart, liver and especially kidneys, increases the susceptibility to depression and also anxiety and hyperactivity in children. By stimulating excessive activity of pancreas and adrenal cortex it leads to diabetes and hypoglycemia. It also increases the concentration of triglycerides and C-reactive protein linked to the occurrence of heart attack (Abdulrhman et al. 2012). The latest recommendations of the American Heart Association suggest limitation in saccharose consumption to 25 grams for women and ca. 40 grams for men. Therefore the consumption of honey should be taken with caution and control. For a more complete range of knowledge about the quality of honey and in particular saccharose content would be further expanding the future study to incorporate of quantitative HPLC methods.

The quality of honey is also influenced by the presence of artificial acidic and basic dyes. Their addition to natural bee's honeys in Poland is strictly prohibited and honeys containing those substances are not allowed at retail (DzU 2010, No. 232). In this study it was noted that the color of honeys was only determined by the presence of natural colorants. They include carotenoids, such as chlorophyll, xanthophyll, anthocyanin and flavonoids. Honey color is also affected by the presence of colloid substances formed from proteins, water and beeswax particles. Honey darkening is a natural process and is influenced by melanoidins, the substances formed during the reactions of sugars with vitamin C and amino acids present in honey. Honey color is also determined by crystallization process when the product becomes much lighter than before the process (Majewska and Trzaneek 2009). The color of honey is often linked to higher antioxidative properties due to total polyphenols content. It is known from the literature that the highest antioxidative properties are displayed by buckwheat, honeydew and heather honeys, which have the widest applicability in cardiovascular diseases (Wilczyńska 2013). Current research confirmed this knowledge, however the average antioxidative values of honey amounting to 38.41% were lower than those reported in other sources. The cause of lower antioxidative properties could be improper storage conditions of honey, referring to e.g. temperature not exceeding 18°C.

The results of starch detection in honeys were negative. Starch is a polysaccharide composed of high number of glucose molecules. In plants starch is formed in a process of sugars condensation and serves as their storage material (Bornus 1989). In honey the enzymatic degradation of starch is carried out by the enzyme  $\alpha$ -amylase and enzymatic activity of honey is determined by the ability to hydrolyze starch by this enzyme (Lempka 1970). Partial starch hydrolysis leads to formation of maltodextrin which efficiently slows the process of honey crystallization.

The presence of starch and starch dextrans formed during enzymatic starch hydrolysis is unacceptable in mature bee's honeys and disqualifies the honey for sale. Naturally present honey dextrans, called maltodextrins, are significantly different from starch dextrans. Maltodextrins do not change their color to blue in the presence of iodine as in the case of starch dextrans. The tested honey samples did not change color, thus it was concluded they were not adulterated with starch and starch dextrans. The presence of starch dextrans in honeys has a negative influence of living processes of bees (Londzin 2004).

The last analyzed characteristic of honeys was their acidity. The most abundant acid in honey is gluconic acid, but except that also other acids are present: malic, lactic, citric, tartaric, oxalic, succinic, butyric and formic acid. The amount of organic acids in honey is relatively low – 0.05–0.3% per formic acid. Excessive acidity is observed in fermented honeys, what is mostly the result of the growth of various microorganisms on the surface.

Unripe honeys and those produced by feeding the bees with sugar display lower acidity and thus possess worse antibacterial properties. Hydrogen peroxide is a major antibacterial component in most honeys, but its effectiveness after the consumption is highly limited. It is formed by glucose oxidase and its effect is strengthened by the presence of vitamin C and metal ions (iron, chromium, cobalt, magnesium), especially copper. The most antibacterial honey due to the presence of methylglyoxal is manuka honey obtained from tea tree (Mavric et al. 2008; Alnaimat et al. 2012).

## CONCLUSIONS

1. Considering the content of starch and synthetic acidic and basic dyes it should be stated that honeys available at retail in West Pomeranian Voivodeship do not pose health threat.
2. 17% of bee's honeys due to relatively high water content may undergo fast fermentation process accompanied by yeasts growth.
3. Honey intended for the feeding of the chronically ill should be monitored particularly in terms of water content and saccharose.

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**Abstract.** Recently there have been many studies on positive effect of honeys on various diseases, especially in treatment of cardiovascular diseases. Even though the majority of results suggest positive influence in the treatment of those diseases the authors of those research wanted to indicate other aspects, often negative, connected to the consumption of honey available at retail. The performed study relates to the adulteration of 35 natural honeys obtained from private apiaries and at retail. The honeys were subjected to the quality tests PN-88/A-77626 on the presence of saccharose (Lane-Eynon's method), starch, artificial acidic and basic dyes, water (refractometric method), total acidity and antioxidative activity (spectrophotometric method). Among the tested honeys more than 50% contained more than 10% of saccharose, and the relation between higher saccharose content and the presence of lower acidity in honey was determined, what will have unfavorable influence on antibacterial properties. Also lowered antioxidative activity of honeys was observed – within the range of 10.32–80.56 percent of inhibition of DPPH radicals and relatively high water content in 17% honeys, what will negatively affect the microbial stability of the product. The presence of starch and synthetic dyes in honey samples were not determined. It is postulated to maintain caution in nutrition honey with no approved quality of the chronically ill.

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