CONTENT OF CARBOHYDRATES AND SPECIFIC ROTATION ANGLE OF HONEY

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Abstract

The main carbohydrates of honey are fructose, glucose, sucrose and maltose. Invertase hydrolyzes sucrose about fructose and glucose. Therefore low content of sucrose and high content of glucose and fructose in honey are parameters for characterization of honey quality. Several sorts of honey contain heightened content of maltose. Therefore it is possible to use this criteria for identification several honey sorts. Each carbohydrate has a specific angle of rotation of polarized light (specific rotation). It depends on relations and content of carbohydrates in honey. The aim of the present research was to establish the relationship between honey sorts and content of carbohydrates as well as specific rotation and possibilities of using these criteria (content of carbohydrates, specific rotation, and activity of invertase) for characterization of honey quality. Following parameters were determined with different physico – chemical methods: specific rotation – by method of polarimetry, content of sugars with high pressure liquid chromatography and activity of invertase – spectrophotometrically. The following results of honey analysis were obtained: the activity of invertase 4–30 (invertase number); content of carbohydrates (sucrose 0.5–3.0%, glucose 30–38%, fructose 35–42%, and maltose 1–6%); specific rotation $[\alpha]_D^{20}$ at -16° to -5°. The obtained results indicated that content of carbohydrates partially dependent on honey sorts. Content of sucrose depends from invertase activity in honey. Invertase is good parameter for honey characterization. Specific angle of rotation of polarized light is not available for identification of honey sorts.

Key words: honey, sugars, invertase, polarized light, identification.

Introduction

Honey is a complex natural product, containing more than 400 different substances, e.g. various carbohydrates, organic acids, proteins, amino acids, enzymes, aroma substances, mineral substances, pigments, waxes, etc (Belitz *et al.*, 2005).

The main carbohydrates of honey are fructose, glucose, sucrose and maltose. Invertase hydrolyzes sucrose about fructose and glucose, but enzymes amylases hydrolyzed starch about carbohydrate maltose, glucose and dextrose (Кашковский, Кузнецова, 2003).

The name of mix from sugars glucose and fructose is invert sugars. The content of glucose and fructose in honey average is 31.3% and 38%. In her turn content of maltose in honey is to 9%, but content of sucrose to 8%, in several honey sorts yet more. Proportions of glucose and fructose partially determine the crystallizations speed of honey. Fructose determines the hygroscopic features of honey, but glucose – the speed of honey crystallization. Honey with partially crystallization, the top liquid layer, basically contains fructose (Farmer, 2003; Belitz et al., 2005; Kasenburger, 2006).

The highest content of sucrose and lowest content of invert sugars (glucose and fructose) characterize the bad maturing of honey or else about bee feeding with sucrose (Шабаршов, 2002).

EU and Latvia are adopted the following standards of quality control for honey sugars: invert sugars in flower honey – no less than 60%, in honeydew honey – no less than 45%; sucrose – no more than 5%, in some exceptions – 10% (Council Directive 2001/110/EC, 2002; LR MK noteikumi Nr.522, 2003).

Several sorts of honey contain heightened content of maltose. Therefore it is possible to use this criteria for identification several honey sorts (Чепурной, 2002).

Each carbohydrate has a specific angle of rotation of polarized light (specific rotation). It is depending on relations and content of carbohydrates in honey. As now, that specific rotation of carbohydrate fructose is -92.4°, specific rotation of glucose +52.7°, specific rotation of sucrose +66.5°, but specific rotation of maltose 130.4^o (Чепурной, 2002).

One of the characterizations criteria of honey quality is activity of enzymes. Important enzyme in honey is invertase. Invertase is more sensitive to heat than amylases and loses activity during storage faster compared to amylases. That is why in few countries (Italy,

Switzerland) invertase is used as additional criteria to characterize honey quality. As a freshness indicator invertase is also used in honey standards of the beekeepers association in Germany, Belgium and Spain (Bogdanov *et al.,* 1999).
In conformity to EU recommendations it was proposed that fresh and unheated honey should have an invertase number (IN) higher than 10, but for honey with low enzymatic activity IN higher than 4 is recommended (Bogdanov, 1997).
The aim of the present research was to establish the relationship between honey sorts and content of carbohydrates as well as specific rotation and possibilities of using these criteria (content of carbohydrates, specific rotation, and activity of invertase) for characterization of honey quality. Research the relationship between invertase activity and content of sucrose in honey. Following parameters were determined with different physico – chemical methods.

Materials and Methods (Bogdanov, 2002)
In our work was analysed various honey sorts (various flowers, wild flowers, lime blossom flowers, dropwort flowers, phacelia flowers, sweet clower flowers, heather flowers, meadow flowers and buckwheat flowers) from different regions of Latvia.
Content of carbohydrates (glucose, fructose, sucrose, and maltose) in honey was determined by method of high pressure liquid chromatography (HPLC).
Principle. After filtration of the solutions, the sugar content is determined by HPLC with RI – detection. Peaks are identified on the basis of their retention times.
Calculation. Quantitation is performed according to the external standard method on peak areas or peak heights.
Parameters for method:
column: Altima Amino 100A 5 u,
flow rate: 1.3 ml min^{-1},
mobile phase: acetonitrile/water (70:30 v/v),
column and detector temperature: 30 °C,
sample volume: 10 µl.
Specific rotation was determined by method of polarimetry.
Principle. The angular rotation of a clear, filtered aqueous solution is measured by means of a polarimeter. The value is related to the carbohydrate composition.
Calculation. The specific optical rotation, $[\alpha]_D^{20}$ is the angle of rotation polarized light at the wavelenght of the sodium D line (λ=589.3 nm) at 20 °C of an aqueous solution of 1 dm depth and containing 1g ml^{-1} of the substance.

$$[\alpha]_D^{20} = \frac{\alpha \cdot 100}{l \cdot m}, \qquad (1)$$

where: α – angular rotation found,
l – length in decimetres of the polarimeter tube,
m – grams of dry matter taken.

The activity of invertase in honey samples was determined by method of spectrophotometry.
Principle. p-Nitrophenyl-α-D-glucopyranoside (pNPG) is used as substrate for the determination of the invertase number in honey. pNPG is split into glucose and p-nitrophenol by invertase. By adjusting the pH value to 9.5 the enzymatic reaction is stopped and at the same time nitrophenol is transformed into nitrophenolate anion, witch corresponds to the amount of converted substrate and is determined spectrophotometrically at 400 nm.
Calculation. Invertase activity is expressed in invertase units (IU kg^{-1}) or in invertase number (IN), where 1 IN=7.344732 IU kg^{-1}. One IU is defined as the number of micromoles of substrate destroyed per minute and expressed per kilogram of honey. One IN is defined as the number of gram of sucrose hydrolysed per hour and expressed per 100 grams of honey.

$$IN = 21.64 \times A_{400}, \quad (2)$$

where: A – the value of absorbtion,

21.64 – slope of the linear regression of IN (y axis) on ΔA_{400} (x axis).

Results and Discussions

Various honey samples from different regions of Latvia (gathered in 2007) were investigated. Analyses were done in laboratories of the Chemistry department of Latvian University of Agriculture.

Results of investigations of different kinds of honey are shown in Table1.

Table 1

Content of carbohydrate in honey and specific rotation

Kinds of honey	Regions of gathering	Content of carbohydrate in honey, %				Specific rotation, $[\alpha]_D^{20}$
		Fructose	Glucose	Sucrose	Maltose	
Various flowers	Ludza	38.64	33.13	2.32	1.04	-12
Various flowers	Jekabpils	37.10	37.94	1.92	3.92	-9
Various flowers	Cesis	40.17	36.13	2.28	3.02	-14
Wild flowers	Madona	36.40	32.67	2.31	1.26	-5
Wild flowers	Cesis	41.74	33.09	2.12	0.73	-16
Lime blossom	Riga	37.72	35.21	1.98	1.95	-7
Lime blossom	Talsi	38.04	36.31	2.17	2.11	-8
Dropwort flowers	Valka	40.50	38.17	2.14	0.87	-16
Heather flowers	Limbazi	37.97	33.20	2.40	4.29	-10
Meadow flowers	Riga	39.12	37.03	2.32	1.75	-14
Buckwheat flowers	Saldus	37.96	38.48	1.72	3.12	-16
Phacelia flowers	Jelgava	41.52	38.51	2.31	6.00	-14
Sweet clower	Riga	37.30	37.77	2.69	4.99	-8

From results of "specific rotation" it is possible to ascertain, that specific rotation of honey is not depending from a honey kinds. It means that the parameter of specific rotation cannot be used for identification of honey kinds.

It is necessary to note, that at all samples of honey, and a parameter of specific rotation is a negative size. Polarized light of all analyzed honey samples turn on left.

From results of content of carbohydrates in honey it is possible to ascertain, that the content of carbohydrates are not full depending from honey kinds. At analyzed honey samples the attitude fructose/glucose is in an interval 0.98–1.26.

It is known, that the crystallization of some honey kinds are faster, and the crystallization of some honey kinds are more slowly. Speed of crystallization in honey is defined with a proportion and the content of carbohydrates in honey.

It is known, that carbohydrate glucose promotes the crystallization of honey, and however carbohydrate fructose breaks crystallization of honey.

Crystallization of such kind as heather blossom honey is slowly. About the analysis of ours research can see, that the content of glucose in heather blossom honey one of the smaller.

It is necessary to note, what even in boundaries of one kinds of honey, honey can have a different speed of the crystallization.

The content of reducing sugars is differentiated in quality standards of flowers and honeydew honey. Standards of EU, *Codex Alimentarius* and Latvia require the following: in flower's honey it has to be $\geq$60 g 100 g^{-1}, in honeydew honey it has to be $\geq$45 g 100 g^{-1}. The same content ($\geq$45 g 100 g^{-1}) of these sugars has to be in blends of both flower and honeydew

honey (Codex Alimentarius Standart 12–1981, Rev. 2, 2001; Council Directive 2001/110/EC, 2002; LR MK noteikumi Nr. 522, 2003).

In both standards the norm of sucrose is required to be no more then 5%. In some sorts of honey the sucrose content can be higher. So in honey from lucerne and citric plant sucrose content can achieve 10%, and in honey from lavender even 15%.

The data of our analyses give evidence that content of reducing sugars and sucrose in explored honey's samples complies with requirements of quality standards of EU and LR for honey, as well as they correspond to data given in the literature (Belitz et al., 2005).

If to compare the content of carbohydrate maltose in honey between different kinds of honey, it is necessary to note, that despite of some kinds of honey with the raised content of carbohydrate maltose in honey (sweet clover and honey of phacelia), at other kinds of honey the maintenance of carbohydrate maltose approximately similar.

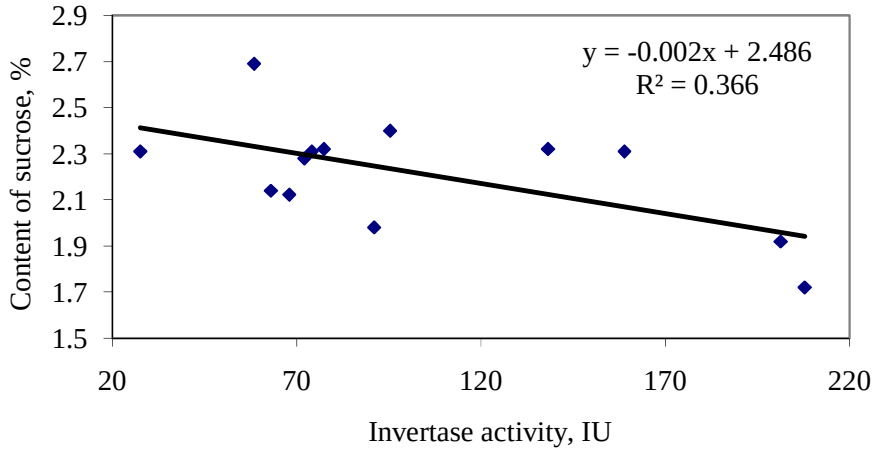

Figure 1. Influence of invertase activity in honey on the content of sucrose in honey

However it is possible to approve, that the content of carbohydrate maltose in honey partially depends on a kinds of honey.

It is know, that enzyme invertase hydrolyses sucrose about glucose and fructose. In our work also was determined activity enzyme invertase in honey samples. The correlation between activity enzyme invertase and the content of sucrose in honey was determined. Our observations are disclosed in Figure 1.

From the results of regression analysis (significance level 0.05) ascertained, that there is the some dependence between the content of sucrose in honey and invertase activity. As can see from Figure 1, the more the content of sucrose in honey, then less is activity invertase in honey. If to compare activity of invertase in honey between different kinds of honey, it is necessary to note, that lowest activity of invertase in honey are at such kinds of honey as honey from phacelia, meadowsweet honey and sweet clover honey. At other samples, activity of invertase in honey corresponds with criteria of "Interantional Honey Commission" discussions. Despite of rather low activity of invertase at some samples of honey, it is impossible to approve, that in these samples the lowered quality. About quality of honey it is possible to judge, estimating other parameters, which characterise quality of honey.

Conclusions

1. Specific rotation of light cannot be used for identification of honey kinds.
2. The content of maltose in honey partially depends on a kind of honey.
3. Content of invertsugars (glucose and fructose) and sucrose in honey correspond with quality criteria of EU and LR.
4. Content of sucrose in honey partially depends on a activity of enzyme invertase in honey.

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