The Criteria of Honey Quality and Its Changes during Storage and Thermal Treatment
Medus kvalitātes kritēriji un to izmaiņas medus uzglabāšanas un termiskās apstrādes laikā

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Abstract. Honey is a very complicated natural product. The quality of honey may be characterized by various chemical and physical parameters. The content of reducing sugars (fructose, glucose, maltose), saccharose, moisture and amino acid proline, characterize the naturalness and maturity of honey. The content of hydroxymethylfurfural (HMF) and activity of enzymes invertase and glucose oxidase indicate the unaffectedness of honey as well as honey's storing and treatment conditions. The acidity and pH indicate the maturity of honey and characterize stability and changes of the honey quality during storage. The oxidation-reduction potential (redox potential) is an indicator of activity of various biochemical and other oxidation-reduction reactions in honey. Samples of flower honey from different regions of Latvia were investigated. The following parameters were obtained: content of reducing sugars (73.7–82.8%), content of saccharose (0.8–3.5%), moisture (15.8–20.2%), content of proline (211–1843 mg·kg^{-1}), content of HMF (7.1–15.0 mg·kg^{-1}), activity of enzyme invertase (41.4–171.1 IU), activity of enzyme glucose oxidase (32–125 mg·kg^{-1}), total acidity (11–37 mval·kg^{-1}), pH 3.95–4.62, and oxidation-reduction potential (70–183 mV). Investigated honey samples showed that honey was of high quality, natural, and mature. The content of HMF increased, but activity of enzymes invertase and glucose oxidase decreased during heat treatment as well as during storage of honey.
Key words: honey, quality criteria, storage, thermal treatment.

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.

In conformity with EU Standards, quality of honey may be characterized by various chemical and physicochemical parameters (Kvalitātes, klasifikācijas …, 2003). For testing of thermal treatment of honey, hydroxymethylfurfural (HMF) content in honey is usually determined.

In small amounts HMF can be found in natural, i.e. thermally non-treated honey too. As the result of thermal treatment or lengthy storage of honey, the content of HMF in honey increases (Nollendorfa, 2002; Чепурной, 2000; Коврига, 2000).

HMF in honey is formed from carbohydrates, mainly from fructose, which is thermally more labile than saccharose and glucose. Fructose disintegrates at approximately 60 ˚C (Машенков, 2001; Belitz, Grosch, 1992).

In different countries there are different standards limits set for maximum permissible HMF content in honey, for example, in Latvia (as in all EU) – 40 mg·kg^{-1} (Kvalitātes, klasifikācijas …, 2003; Lees, 1998), in Russia – 25 mg·kg^{-1} (Угринович, Фарамазян, 2002).

As alternative criterion for assessment of honey quality during storage and thermal treatment, the activity of enzyme invertase may by used. Discussions about determination of invertase activity and content of amino acid proline in honey took place in International Honey Commission (Bogdanov et al., 1997).

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 criterion to characterize honey quality. As a freshness indicator invertase is also used in honey standards of the beekeepers associations in Germany, Belgium and Spain (Persano et al., 1999; Bogdanov et al., 1997).

Invertase activity is expressed in invertase units (IU · kg^{-1}) or in invertase number (IN), where 1 IN = 7.344732 IU · kg^{-1}. One invertase unit (IU · kg^{-1}) is defined as the number of micromoles of substrate destroyed per minute and expressed per kilogram of honey. One invertase number (IN) is defined as the number of gram of saccharose hydrolysed per hour and expressed per 100 grams of honey.

In conformity to EU recommendations it was proposed that fresh and unheated honey should have an invertase number (IN) higher than 10 (for honey with low enzymatic activity IN higher than 4 is recommended) (Bogdanov et al., 1997).

One of the criteria of honey quality is content of carbohydrates (sugars). Sugars in honey are up to 95% of dry matter and these are mainly monosaccharides. For control of honey quality, the content of reducing sugars and saccharose is determined.

The main sugars of honey are glucose and fructose. Saccharose in honey can be found in small amounts, since in the process of honey maturing it is inverted into glucose and fructose. Fructose determines the hygroscopic features of honey, glucose – the speed of honey crystallisation.

EU has 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%; saccharose – no more than 5%, in some exceptions – 10% (Kvalitātes, klasifikācijas..., 2003).

Honey contains 11–21 free amino acids, mostly proline. The content of proline in honey is a criterion of honey ripeness, unaffectedness and, in some cases, also of sugar adulteration. A minimum value for genuine honey of 180 mg · kg^{-1} is accepted by International Honey Commission (Bogdanov et al., 1997).

One of the main characteristics of the quality of honey is its water content (moisture), which depends on the grade of honey maturity. In immature honey, water content is higher than in mature honey. EU standards define the following standards for maximum water content in honey: heather honey and baker's honey – no more than 23%, honey of other origin – no more than 20% (Kvalitātes, klasifikācijas..., 2003).

Honey having a higher water content is readily susceptible to fermentation by osmophilic yeasts. Yeast fermentation is negligible if water content is less than 17.1%, while between 17.1–20.0%, fermentation depends on the count of osmophilic yeast buds (Belitz, Grosch, 1992).

Mature honey has stable pH and total acidity, therefore these parameters are important characteristics of the quality of honey. EU standards prescribe the following total acidity standard – no more than 40 mequivalents of acid in 1000 g of honey.

Several substances found in honey participate in oxidation-reduction processes. For example, glucose oxidase enzyme oxidates glucose (100%) and mannose (9%). As a result of enzymatic oxidation of glucose, gluconic acid and hydrogen peroxide are formed (Schlammer, 1996; Pomeranz et al., 1994; Belitz, Grosch, 1992).

The oxidation-reduction potential of honey partially indicates the characteristics of honey and physicochemical processes in it during storage and treatment. According to oxidation-reduction potential, honey can be divided into different kinds. Every such kind of honey has its characteristic oxidation-reduction potential (Чепурной, 2002; Заикина, 1999).

The aim of the present research was to analyze honey quality and determine the dependence of different parameters of honey (activity of enzymes glucose oxidase and invertase, content of HMF) on the way of honey treatment.

Materials and Methods

Samples of different types of honey from different regions of Latvia were investigated: honey of various flowers, wild flowers, meadow flowers, heather flowers, lime-blossom, rape flowers, buckwheat flowers, and camomile flowers.

The following parameters were determined:

- content of reducing sugars and saccharose – by method of photometry (Коренман, 1989);
- content of HMF – by method of photometry (Bestimmung …, 1992);
- content of proline – by method of photometry (Bogdanov, 2002);
- activity of invertase – by method of photometry (Bogdanov, 2002);
- moisture of honey – by method of refractometry (Bogdanov, 2002);
- activity of glucose oxidase – by express method (Glucose-oxidase ..., 2004);
- oxidation-reduction potential and pH of honey – by method of potentiometry;
- total acidity – by method of alkalimetry (Bogdanov, 2002).

Determination of reducing sugars and saccharose in honey (after an appropriate treatment) was based on the absorbency (λ = 440 nm) after the reaction of reducing sugars with potassium ferricyanide.

Determination of HMF in honey was based on the absorbency (λ = 550 nm) after the reaction of honey solutions with solutions of barbituracids and p-toluidine.

Determination of proline in honey was based on its reaction with ninhydrin. Proline and ninhydrin form a coloured complex. After adding 2-propanol, the absorbency (λ = 500–520 nm) of sample solution and reference solution was determined.

p-Nitrophenyl-α-D-glucopyranoside (pNPG) is used as a substrate for determination of invertase activity in honey. pNPG is split into glucose and p-nitrophenol by invertase. By adjusting (with reaction-terminating solution of tris-(hydroxymethyl) aminomethane in water) the pH value to 9.5, enzymatic reaction is stopped and, at the same time, nitrophenol is transformed into nitrophenolate anion that corresponds to the amount of converted substrate and was determined photometrically (λ = 400 nm).

For determination of oxidation-reduction potential, pure honey samples as well as honey solutions in water (proportion 1:4) were used. During the measurement process, the water oxidation-reduction potential was taken into account too. The oxidation-reduction potential of solutions was determined after one hour.

For glucose oxidase activity determination, an express method was developed. If honey is mixed with a

4 times greater amount of water at 20 ˚C, glucose oxidase will generate hydrogen peroxide. After 1 hour, the maximum amount of hydrogen peroxide is observed.

For investigation of relationship between the activity of glucose oxidase and the HMF content as well as their dependence on heating temperature, honey solutions were used thus ensuring homogeneous environment for the measurement sample and greater stability of measurement and to allow to analyze the graphical interconnection of the above parameters.

Results and Discussion

Results of investigations of different kinds of honey are shown in Tables 1–3. Data in Table 1 indicate high quality of honey, its naturalness, and maturity, in Table 2 – unaffectedness and freshness, and in Table 3 – illustrate decrease in invertase activity during storage.

Investigating the activity of glucose oxidase, honey was hated at different temperatures during 24 hours. The following correlation was found (Fig. 1): with the increase of temperature, activity of glucose oxidase decreases, therefore the measurement of glucose oxidase can be used as one of the parameters of honey quality. Thus, determination of glucose oxidase activity could successfully replace such thermal treatment and storage criteria of honey as determination of amylase value.

In order to facilitate better comparison of different characteristics of honey, depending on the heating temperature, also HMF content in honey was determined: honey was heated at different temperatures for 24 hours (Fig. 2). Figure 2 leads to conclusion that HMF content in honey starts to increase considerably over 50 ˚C. Comparison of other parameters of honey quality shows that the activity of glucose oxidase is more sensitive to changes in the heating temperature of honey. Compared to changes in HMF content in honey, changes in glucose oxidase activity are more rapid and start at a lower honey heating temperature (Figures 1 and 2).

Table 1

The content of reducing sugars, saccharose and moisture, total acidity and pH of different kinds of honey (average values)

Sample of honey	Reducing sugars, %	Saccharose, %	Total acidity, mval kg^{-1}	pH	Moisture, %
Various flowers	82.8	3.5	12	4.20	17.0
Various flowers	75.9	1.5	21	3.95	20.2
Wild flowers	74.3	0.8	34	4.09	15.8
Wild flowers	82.3	2.3	35	4.04	17.4
Lime blossom	79.2	1.2	14	4.62	17.5
Rape flowers	79.0	3.0	11	4.16	18.2
Buckwheat flowers	73.7	2.1	37	3.97	19.0
Camomile flowers	80.3	0.8	34	4.10	17.4
Meadow flowers	73.8	2.2	25	4.12	16.2
Heather flowers	80.5	0.9	22	4.09	16.6

Table 2

The content of HMF and proline, oxidation-reduction potential and activity of glucose oxidase of different honey samples (average values)

Sample of honey	HMF, mg kg^{-1}	Proline, mg kg^{-1}	Oxidation-reduction potential, E_H, mV	Activity of glucose oxidase, μg kg^{-1}
Various flowers	10.8	330	80	35
Various flowers	9.6	518	70	60
Wild flowers	8.5	974	183	125
Wild flowers	7.1	1654	75	125
Lime blossom	12.5	211	140	32
Rape flowers	7.1	239	125	60
Buckwheat flowers	15.0	870	145	75
Camomile flowers	10.2	687	142	100
Meadow flowers	7.9	1843	90	35
Heather flowers	9.4	1268	72	75

Table 3

Changes in invertase activity in honey during storage at 18±2 ˚C (average values)

Sample of honey	Invertase activity in 2003		Invertase activity after 12 months		Invertase activity after 20 months	
	$IU \cdot kg^{-1}$	IN	$IU \cdot kg^{-1}$	IN	$IU \cdot kg^{-1}$	IN
Various flowers	171.1	23.3	144.0	19.6	119.5	16.3
Various flowers	88.4	12.0	70.3	9.6	55.5	7.6
Wild flowers	140.0	19.1	115.1	15.7	94.3	12.8
Wild flowers	96.4	13.1	76.2	10.3	58.7	8.0
Lime blossom	41.4	5.6	32.6	4.4	23.5	3.2
Rape flowers	167.2	22.8	136.1	18.5	108.7	14.8
Buckwheat flowers	71.0	9.7	57.3	7.8	45.0	6.1
Camomile flowers	168.2	22.9	139.1	18.9	108.1	14.7
Meadow flowers	91.3	12.4	73.2	10.0	58.6	8.0
Heather flowers	60.4	8.2	48.4	6.6	37.4	5.1

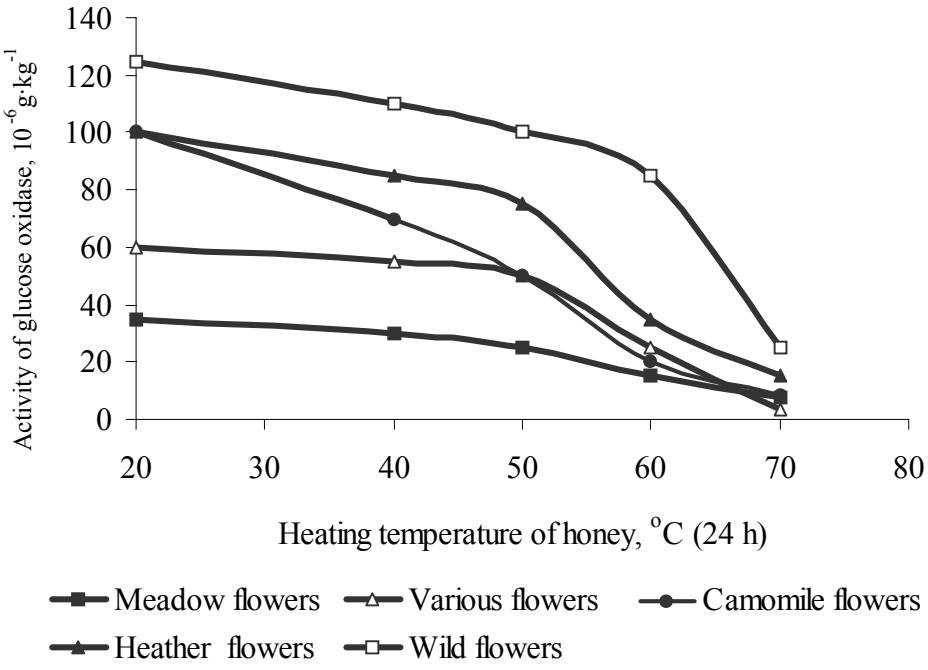

Fig. 1. The activity of glucose oxidase in honey and its dependence on the heating temperature.

Using the obtained data, the relationship between glucose oxidase activity and HMF content was analyzed (Fig. 3). Figure 3 shows that the activity of glucose oxidase decreases when HMF content is increasing. The research was performed using analysis data of samples of different kinds of honey. The obtained correlation exists at 95% probability. The results of this research confirm the above mentioned fact that measurements of glucose oxidase activity in honey can be used as indicator of thermal treatment of honey.

Figure 4 demonstrates dependence of invertase activity in honey upon the heating temperature. The activity of invertase decreases significantly already in an hour at the temperature of 50-70 ˚C.

Analysis of Figures 1, 2 and 4 suggests that rapid changes in honey quality take place at the temperature of 50-60 ˚C.

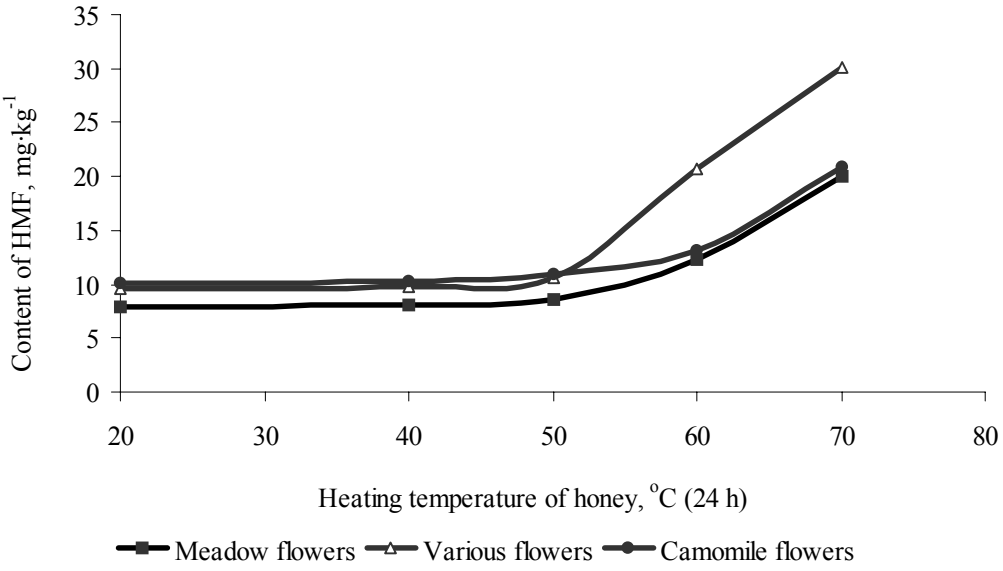

Fig. 2. The content of HMF in honey and its dependence on the heating temperature.

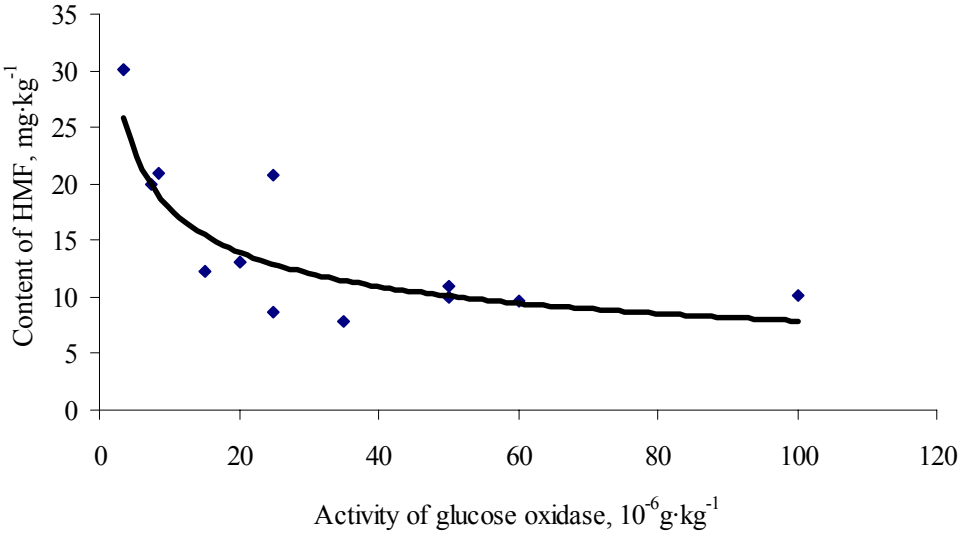

Fig. 3. The relationship between HMF content and activity of glucose oxidase in honey.

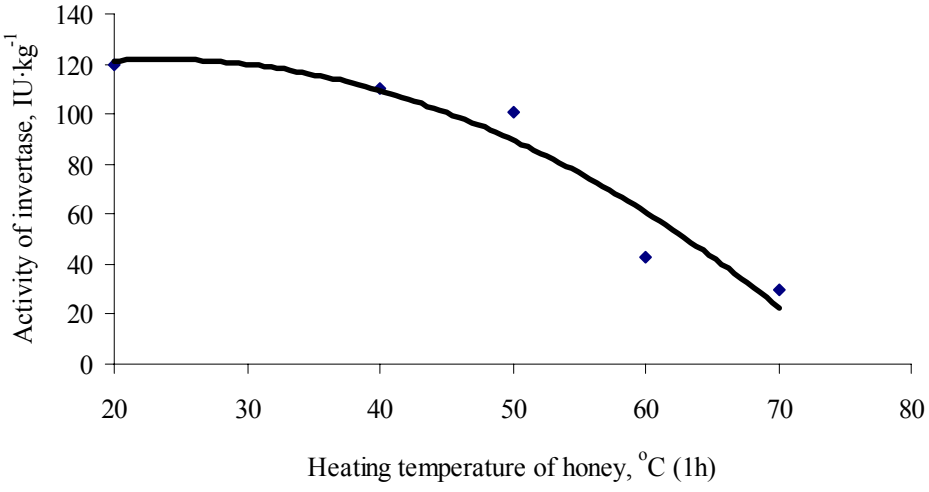

Fig. 4. The activity of invertase in honey and its dependence on the heating temperature.

Conclusions

1. The obtained results of honey analyses indicate the high quality of honey produced in Latvia.
2. The quality of honey corresponds to EU Standards and recommendations.
3. The measurement of glucose oxidase and invertase activities can be an alternative indicator determining the possibility of honey heating, in addition to determination of HMF content and amylase value in honey.
4. The content of proline can be used as additional criterion to characterize honey quality.
5. The activity of invertase in honey during 20 months of storage at 18 ± 2 ˚C decreases 1.4–1.8 times.
6. The dependence of analyzed parameters (HMF content, activity of glucose oxidase, activity of invertase) on the heating temperature of honey shows rapid changes in honey quality at the temperature of 50–60 ˚C.

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Anotācija

Darbā pētīta Latvijā iegūtā dažādu šķirņu medus kvalitāte. Medus kvalitātes novērtēšanā izmantoti gan ES pastāvošie un ar tiem saskaņotie Latvijas Republikas standarti, gan arī Starptautiskajā medus komisijā plaši diskutētie alternatīvie medus kvalitātes kritēriji. Medus kvalitātes raksturošanai noteikti sekojoši parametri: reducējošo cukuru, saharozes, mitruma, aminoskābes prolīna un hidroksimetilfurfurola (HMF) saturs, fermentu invertāzes un glikozes oksidāzes aktivitātes, medus oksidēšanās–reducēšanās potenciāls, medus kopējais skābums un pH. Pētītas invertāzes, glikozes oksidāzes un HMF izmaiņas un savstarpējās sakarības medus karsēšanas rezultātā, kā arī invertāzes aktivitātes izmaiņas medus uzglabāšanas laikā.