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# INVERTASE AND DIASTASE ACTIVITY IN HONEYS OF CZECH PROVENIENCE

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**Abstract**

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Apart from a number of other components, honey contains also enzymes which are responsible for converting nectar and honeydew to honey. One of the most important enzymes is invertase. Its sensitivity towards temperature is very high. In some European countries invertase activity determination is used as a parameter related to the freshness of honey, to its warming or storing conditions. The aim of this study was to determine invertase activity in various types of 54 honey samples of Czech provenience. Thirty-seven fresh samples coming directly from beekeepers were analysed and seventeen samples from the market, all gained in the year of 2000. At fresh honeys we evaluated the results for various honey types: blossom (n = 13), compound (n = 15) and honeydew (n = 9). The results of fresh honeys show that invertase activity varies at individual honey types and ranges from 0.8 to 25.9 IN (15.77 ± 6.3). Blossom honeys show a statistically *($P < 0.05$)* significant variability (12.12 ± 5.75 IN) from compound honeys (17.46 ± 6.46 IN) and honeydew honeys (18.22 ± 4.67 IN). Concerning invertase activity, honeydew honeys did not greatly differ from the compound ones. At the same time, the diastase activity was determined at all honeys as well as the ratio of both enzymes. In the invertase/diastase ratio, statistically significantly different *($P < 0.05$)* were only compound honeys (0.59 ± 0.18) from the type of honeydew honeys (0.82 ± 0.27). At market samples the invertase activity values (from 0.6 to 7.4 IN) were much lower *($P < 0.01$)*. From the results of correlation analysis of the particular parameters it follows that the invertase activity is a much more sensitive indicator of heating, conditions and storing time of honey than is diastase or HMF. Due to its variability for individual honeys, it is difficult to determine limit values of invertase activity for determining honey freshness.

honey, invertase, diastase, quality evaluation

Apart from a number of other components, honey contains also enzymes which are responsible for converting nectar and honeydew to honey. In honey there are α- glucosidase (invertase), α- and β- amylase (diastase), glucose oxidase, catalase and acid phosphatase. The enzyme activity in honey has been widely studied for many years (Rinaudo et al., 1973; Bogdanov et al., 1987, 1999; Krauze and Zalewski, 1991; Oddo et al., 1999; Bonvehi et al., 2000; Sanchez at al., 2001).

One of the most important honey enzymes is α- glucosidase (sacharase, invertase), glycoprotein originating from hypopharyngeal glands of bees. In the process of honey ripening it converts sucrose to saccharide - glucose and fructose which are physiologically usable for the bee. Invertase hydrolyses both sucrose and maltose (Belitz and Grosch, 1992). This enzyme also has the transglucosidase effects. The amount of excreted invertase depends on many aspects such as the age, physiological stage, food, condition of a bee colony, temperature and intensity or type of honey flow (Rinaude et al., 1973; Crane, 1990; Lipp, 1994; Oddo et al., 1999).

The enzyme sensitivity towards temperature is very high but the speed of enzyme destruction at the temperature of below 15 °C is very small. At 20 °C the invertase decrease rises by 1.5 to 1.7 % per month. At higher temperature the loss of invertase activity in dependence on temperature may be even higher. Seven to eight invertase isoenzymes are known (for example Acacia honeys).

In some European countries invertase activity determination is used as a parameter related to the freshness of honey, to its warming or storing conditions. The European Honey Commission proposes the invertase activity determination as a perspective criterion of the quality of honey (Bogdanov et al., 1997, 1999). It presents a more sensitive parameter than the diastase activity determination and it is easier to determine (Crane, 1990; Sanchez et al., 2001; Oddo et al., 1999). It is also a more sensitive parameter than the determination of hydroxymethylfurural (HMF). For this purposes, the HMF has been used by Kubiš et Ingr (1998) in honeys of the Czech provenience.

The aim of this study was to determine invertase activity in various types of honey of Czech provenience; it means in samples according to different honey groups, on the one hand, and to honey from beekeepers and commercial, on the other hand. Discussion on the role of invertase in the evaluation of the quality of honey with regard to other quality parameters was subsidiary aim of the present study.

## MATERIAL AND METHODS

### Samples

As material we chose both samples bought at the market in the year of 2000 – 17 of them (at the start of their minimal triennial durability), and samples coming directly from beekeepers in the same year – 37 samples (from honey flow in 2000). The honeys were grouped on the basis of pollen analysis. For the samples coming from the market, Czech provenience was verified by pollen analysis.

The market samples were packaged in jars of 400 - 500 g with the bottling date between May to September 2000. Samples coming directly from beekeepers were taken between May to August 2000 packaged in jars of 500 g. All samples were gained by extracting and straining. The samples were stored in original packages in the dark at the temperature of the laboratory (22 ± 2 °C) until analysed. All analyses were carried out within one month from obtaining the sample.

### *Methods*

#### *Determination of invertase activity*

Invertase activity was determined using the Siegenthaler method. As a substrate is used p-Nitrophenyl-alfa-D-glucopyranosid (pNPG) which is decomposed by invertase from honey to glucose and p-nitrophenol. By modifying pH to 9.5 the enzymatic reaction is stopped and at the same time nitrophenol is transformed to nitrophenal anion which is equivalent to the transformed substrate and is determined spectrophotometrically at 400 nm (UV/VIS Spectometer Lambda 11, Perkin Elmer, USA). The honey invertase activity was calculated from the measured absorbency by multiplying by the factor of 158.94 and calculated to a kilo of honey (U/kg). Then the value was expressed as invertase number (IN). The IN indicates the amount of sucrose per gram hydrolysed in 1 h by the enzymes contained in 100g of honey under test conditions (Bogdanov et al., 1997). Each sample was analysed in 2 parallel determinations.

#### *Determination of diastase activity*

Determination of diastase activity was done in order to compare the activity of both enzymes. It was evaluated spectrophotometrically using the Shade method (UV/VIS Spectometer Lambda 11, Perkin Elmer, USA).The diastase activity is calculated as diastase number (DN). DN expresses units of diastase activity (Gothe unit). One unit is defined as the amount of enzyme that will convert 0.01 g of starch to the prescribed end-point in 1 h at 40°C under the conditions of test (Bogdanov et al., 1997).

#### *Determination of electrical conductivity*

Electrical conductivity of honey was determined for a honey solution containing 20% ofdry substance of honey in 100 ml of distilled water (Bogdanov et al., 1997). Measuring was carried out with a thermostat conductive cell and a conductometer LF 315 (fy WTW GmbH, Germany). Each sample was analysed in three parallel determinations

#### *Determination of hydroxymethylfurfural (HMF)*

Determination of hydroxymethylfurfural (HMF) was done using the Winkler method where the solution of the tested honey when reacting with p-toluidin and barbituric acid and in the presence of hydroxymethylfurfural (HMF) gives a wine-red compound (Bogdanov et al., 1997). The absorption was measured at 550 nm on a one-ray Lambda 11 (fy Perkin Elmer, USA). Concentration of HMF was determined with the help of a calibrated line using the method of linear regression. Each sample was analysed in three parallel determinations.

#### *Determination of the botanical origin - microscopical analysis*

The honey origin was verified by qualitative and quantitative microscopic pollen analysis (melissopalynology). The honey samples were

divided according to their origin into several types and subtypes (blossom origin) as follows:

a) blossom honey –
   1. monofloral,
   2. multifloral and
   3. multifloral with predominance of some plant(s);
b) honeydew honey and
c) compound honey (blend of honeydew and blossom honey).

The honeys belonging to a1) group originate mainly from nectar of only one plant species and proportions of the other nectars are fractional. All monofloral honeys had both physical attributes and result of their microscopic analysis typical of the given monofloral origin (e.g. *Robinia* honeys did not crystallise etc.). The honeys of a1) group are not so-called experimentally monofloral - i.e. gained from technically isolated growths as it was carried out e.g. by Demianowicz (1961). The mixture of nectars from different plant species is typical of honey samples belonging to a2) group. The honeys of a3) group originate also from the mixture of different nectars but one, two or three sources of nectar at maximum are obviously predominant. These honeys, in contrast to a1) group, did not have both physical attributes and result of their microscopic analysis typical of any monofloral honeys for any of found predominant sources of nectar. The methodology was consistent with the international methodology including their supplements and adaptations proposed by (Louveaux, Maurizio & Vorwohl, 1970, 1978).

The results were elaborated by calculating basic statistic characters, correlation analysis and using of the T-test.

## RESULTS

In Tab. I there are basic statistic parameteres of both enzymes and their proportion in the honeys from beekeepers (fresh) and honeys from the market (commercial). The invertase activity of fresh honeys ranged widely from 0.8 to 25.9 IN. On the other hand, at commercial honeys the range was from 0.6 to 7.4 IN.

In Tab. II we can see the results of the determination of invertase activity, diastase and their proportion in the individual types (blossom, compound and honeydew) of fresh honeys. The commercial honeys could not be strictly classified in the most cases because they have been during processing mixed with more honey sorts with aim to produce a honey with better taste features. The lowest activity was found at blossom honeys (from 0.8 to 20.4 IN). A wide range of activities was found at compound honeys (from 4.0 to 25.9 IN). At honeydew honeys the enzyme activity ranging from 10.8 to 24.6 IN was found.

I: *Invertase, diastase, IN/DN ratio and HMF in fresh and commercial honeys*

| | Fresh honey | | | |
|---|---|---|---|---|
| | Invertase activity [IN] | Diastase activity [DN] | IN/DN ratio | HMF [mg . kg$^{-1}$] |
| Mean | 15.8 | 24.3 | 0.67 | 3.89 |
| Standard deviation | 6.3 | 9.3 | 0.23 | 3.25 |
| Standard error | 1.0 | 1.5 | 0.04 | 0.53 |
| Coefficient of variation [%] | 39.9 | 38.3 | 34.3 | 83.50 |
| Minimum value | 0.8 | 11.2 | 0.05 | 0.00 |
| Maximum value | 25.9 | 45.4 | 1.44 | 15.40 |
| | Commercial honey | | | |
| Mean | 3.1 | 13.6 | 0.22 | 17.34 |
| Standard deviation | 2.1 | 2.2 | 0.14 | 12.32 |
| Standard error | 0.5 | 0.5 | 0.03 | 13.18 |
| Coefficient of variation [%] | 67.9 | 15.9 | 61.63 | 71.06 |
| Minimum value | 0.6 | 10.9 | 0.05 | 1.60 |
| Maximum value | 7.4 | 17.8 | 0.53 | 49.30 |

II: *Invertase, diastase, IN/DN ratio and HMF in fresh honeys group*

| | Fresh honey | | | |
|---|---|---|---|---|
| | Invertase activity [IN] | Diastase activity [DN] | IN/DN ratio | HMF [mg . kg$^{-1}$] |
| | Floral honey (n = 13) | | | |
| Mean | 12.1 | 18.2 | 0.65 | 3.11 |
| Standard deviation | 5.8 | 5.9 | 0.22 | 4.08 |
| Standard error | 1.6 | 1.6 | 0.06 | 1.13 |
| Coefficient of variation [%] | 47.5 | 32.5 | 33.40 | 131.20 |
| Minimum value | 0.8 | 11.2 | 0.05 | 0.00 |
| Maximum value | 20.4 | 30.3 | 0.91 | 15.40 |
| | Compound honey (n = 15) | | | |
| Mean | 17.5 | 29.4 | 0.59 | 4.55 |
| Standard deviation | 6.5 | 7.4 | 0.18 | 2.51 |
| Standard error | 1.7 | 1.9 | 0.05 | 0.65 |
| Coefficient of variation [%] | 37.0 | 25.3 | 30.41 | 55.04 |
| Minimum value | 4.0 | 15.9 | 0.20 | 1.40 |
| Maximum value | 25.9 | 40.3 | 0.85 | 10.30 |
| | Honeydew honey (n = 9) | | | |
| Mean | 18.2 | 24.6 | 0.82 | 3.92 |
| Standard deviation | 4.7 | 11.5 | 0.27 | 3.13 |
| Standard error | 1.6 | 3.8 | 0.09 | 1.04 |
| Coefficient of variation [%] | 25.7 | 46.6 | 32.69 | 79.73 |
| Minimum value | 10.8 | 13.6 | 0.54 | 0.00 |
| Maximum value | 24.6 | 45.4 | 1.44 | 11.30 |

Diastase activity (Tab. I and II) for fresh honey samples ranged from 11.2 to 45.4 DN, for commercial samples from 10. 9 to 17.8 DN. When comparing the various types of fresh honeys (Tab. II) the lowest values were found at blossom honeys (from 11.24 to 30.3 DN), at compound honeys they ranged from 15.9 to 40.3 DN. Honeydew honeys showed the highest values of enzyme (from 13.6 to 45.4 DN).

The relation of both enzymes expressed by the invertase/diastase ratio is clear from Tab I and II. The invertase/diastase ratio for fresh honeys ranged from 0.05 to 1.44. At commercial honeys a much smaller range was found (from 0.05 to 0.53). The invertase/diastase ratio (mean ± SD) for fresh honeys (0.67 ± 0.23) statistically considerably differed ($P < 0.01$) from the values of commercial honeys (0.22 ± 0.14). Among fresh honeys the type of compound honeys was statistically ($P < 0.05$) very different (0.59 ± 0.18) from that of honeydew honeys (0.82 ± 0.27).

In Tab. III we can see the gained correlation ratios of enzymatic activities of both enzymes, diastase/invertase ratio, conductivity and HMF of fresh honeys. The relation of both enzymes is expressed by the correlation r = 0.7492, $P < 0.01$). Regression analysis is figured in Fig. 1. Statistically significant are the correlations between invertase activity and conductivity (r = 0.4968, $P < 0.01$) and invertase with IN/DN ratio (r = 0.4563, $P < 0.01$).

In Tab. IV we can see correlation coefficients of enzymatic activities of both enzymes, their ratios, conductivity and HMF for commercial honeys. The correlation coefficient of invertase activity and diastase was lower – amounting to r = 0.5732, $P < 0.05$. Regression analysis for this relation is figured in Fig. 2. Between invertase activity and the ration of IN/DN (r = 0.9419) were found statistically significant dependency ($P < 0.01$). No statistically significant dependency between HMF and the activity of both enzymes at fresh or commercial samples was proved (Tab. III and IV).

III: *Correlations among individual parameters of fresh honeys*

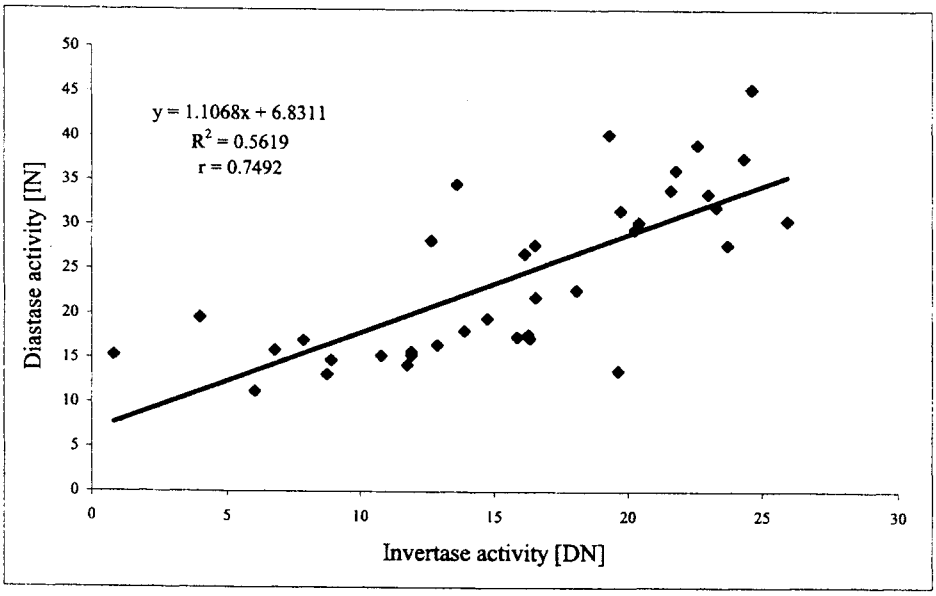
| Parameter | Conductivity | HMF | Invertase [IN] | Diastase [DN] | IN/DN ratio |
|---|---|---|---|---|---|
| Conductivity | 1 | | | | |
| HMF | 0.2273 | 1 | | | |
| Invertase [IN] | 0.4968** | -0.2087 | 1 | | |
| Diastase [DN] | 0.3647* | 0.0033 | 0.7492** | 1 | |
| IN/DN ratio | 0.2797 | -0.4192** | 0.4563** | -0.2088 | 1 |

(statistical significance * P< 0.05;** P< 0.01)

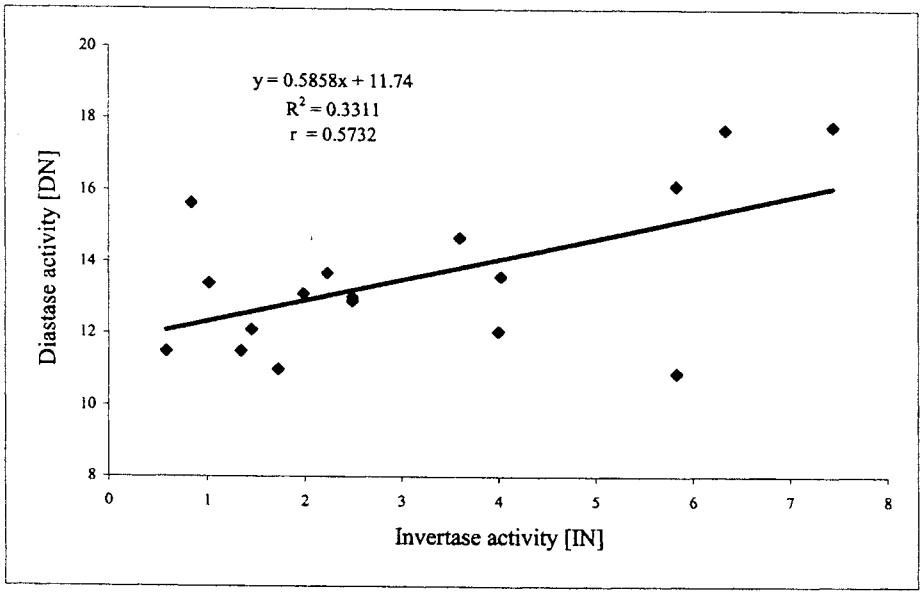
IV: *Correlations among individual parameters of commercial honeys*

| Parameter | Conductivity | HMF | Invertase [IN] | Diastase [DN] | IN/DN ratio |
|---|---|---|---|---|---|
| Conductivity | 1 | | | | |
| HMF | -0.0309 | 1 | | | |
| Invertase [IN] | 0.0280 | -0.4150 | 1 | | |
| Diastase [DN] | 0.1502 | -0.2738 | 0.5732* | 1 | |
| IN/DN ratio | -0.0633 | -0.4179 | 0.9419** | 0.2954 | 1 |

(statistical significance * P< 0.05;** P< 0.01)




1: *Regression and correlation between invertase and diastase in fresh honeys*




2: *Regression and correlation between invertase and diastase in commercial honeys*

## DISCUSSION

The division of the honeys into three types (blossom, compound and honeydew) complies with the legislation of the Czech Republic. At nectar honeys, the lowest value of IN (0.8) was found for the sunflower honey, represented by only one sample. At this unifloral honey Oddo et al. (1999) found higher values (from 9.0 to 16.3 IN). At the only acacia honey, the value we found was higher (8.8 IN) than that given by Oddo et al. (1999) - from 0.4 to 7.7 IN. At unifloral honeys *Brassica* (n = 7) values from 8.9 to 20.4 IN were found and they significantly differed from other samples of this group.

On the whole, blossom honeys, whose invertase activity was 12.12 ± 5.75, statistically considerably varied *($P < 0.05$)* in invertase activity from compound and honeydew honeys. This may be due to the fact that blossom honeys are usually honeys of early spring and the lower contents of the enzyme is caused by lower concentration of nectar with a higher participation of saccharide and a reduced activity of bee colonies during their growing (Oddo et al., 1999).

At compound honeys a great variability in invertase activity was found (17.46 ± 6.46 IN). These values are difficult to compare with other authors as this type of honey is not included in most national or international legislations. With regard to other honey types, the only statistically significant differences were those concerning blossom honeys *($P < 0.05$)*.

As far as the invertase content is concerned, the honeydew honey type statistically *($P < 0.05$)* differed from blossom honeys (18.22 ± 4.67 IN) but not from compound honeys. The same values for honeydew honeys were also found by Oddo et al. (1999). The higher values of invertase in honeydew honeys can be attributed to the fact that for honeydew honeys invertase comes from honeydew (at compound honeys according to the proportion of honeydew). To the honeydew it gets by means of salivary glands and the gut of plant-sucking insects (Crane, 1990).

The invertase/diastase ratio at fresh honeys (0.67 ± 0.23) is statistically *($P < 0.01$)* very different from the values of commercial honeys (0.22 ± 0.14). Within the evaluated types of fresh honeys, the group of compound honeys is statistically *($P < 0.05$)* most different (0.59 ± 0.18) from the group of honeydew honeys (0.82 ± 0.27). This parameter was studied at fresh and commercial honeys by Oddo et al. (1999) and Kiermeier and Köberlein (1954). Our results at fresh honeys resemble those found by Oddo et al. (1999). At fresh honeys for this parameter he found a wide range of values (0.1 – 2) and thus the matter of the suitability of this parameter for the detection of honey freshness is rather disputable. Our results at commercial honeys (0.05 – 0.53) are similar to those found by Kiermeier and Köberlein (1954) in their essay which gives the range of 0.2 – 0.5 for commercial honeys. In contrast with our results, at fresh honeys they give values higher than 0.5.

The relation of invertase to other given parameters is expressed by correlation coefficients in Tab. III and IV.

The correlation coefficients found by us (r = 0.7492) between both enzymes agrees with Krauze and Krauze (1991), who found a correlation coefficients of 0.7380. On the other hand, Oddo et al. (1999) found a coefficient higher (r = 0.835), as well as Huidobroet et al. (1995) (r = 0.878). At commercial samples a lower correlation coefficients between both enzymes was found: r = 0.5732. This phenomenon can be explained by more temperature susceptibility of invertase than of diastase because the major part of commercial honey is during processing decrystallised by heating. Diastase activity of commercial honeys is approximately only twice lower than in fresh honey in contrast to invertase activity, which is six-times lower (Tab. I). This phenomenon is also caused by more common stability as it has been found by White el. al. (1964). When comparing the activity of both enzymes at commercial samples and fresh ones, it may be said that at commercial samples the values of both enzymes are statistically significantly lower *(P < 0.01)*. These differences are more pronounced at invertase activity (15.77 ± 6.30 IN at fresh honeys, compared with 3.12 ± 2.12 IN at commercial honeys).

From the comparison of correlation coefficients of invertase activity and diastase with other parameters at fresh and commercial honeys, we can state that at the commercial ones, a higher correlation between the ratio of both enzymes and invertase activity was found. In contrast with the essay by Bogdanov et al. (1987), not even at commercial samples did we find a significant correlation with HMF. From the correlation relations of the activity of both enzymes and HMF it is, however, obvious that invertase is more sensitive to heat and that is probably why it correlates with HMF more than diastase. This comes clear when comparing fresh samples from the beekeepers (potentially less heated) with the commercial ones where heating is a basic technique.

All these gained results indicate that regardless great type variations of invertase activity, it is a much more sensitive indicator of heating, conditions and storing time of honey than is diastase or HMF. Oddo et al. (1999), Sanchez et al. (2001) came to the same conclusion.


## SUMMARY

Invertase activity was determined from 54 samples. We analysed 37 fresh samples coming directly from beekeepers and 17 samples from the market. At fresh honeys we evaluated the results for various honey types: blossom (n = 13), blend (n = 15) and honeydew (n = 9).

The results of fresh honeys show that invertase activity varies at individual honey types and ranges from 0,8 to 25,9 IN (15,77 ± 6,3). Blossom honeys show a statistically *(P < 0,05)* significant variability (12,12 ± 5,75 IN) from compound honeys (17,46 ± 6,46 IN) and honeydew honeys (18,22 ± 4,67 IN). Concerning invertase activity, honeydew honeys did not greatly differ from the compound ones. Concerning the enzyme activity, the blossom honeys, or the type most represented – the rape-seed honey (n = 7), did not differ from other samples of this type. At the same time, the diastase activity was determined at all honeys as well as the ratio of both enzymes.

In the invertase/diastase ratio, statistically significantly different *(P < 0,05)* were only compound honeys (0,59 ± 0,18) from the type of honeydew honeys (0,82 ± 0,27).

At market samples the invertase activity values (from 0,6 to 7,4 IN) were much lower *(P< 0,01)*. The results show that the invertase/diastase ratio is not a suitable criterion for honey freshness.

From the results of correlation analysis of the particular parameters it follows that the invertase activity is a more sensitive indicator of heating, conditions and storing time of honey than is diastase or HMF.

Due to its variability for individual honeys, it is difficult to determine limit values of invertase activity for determining honey freshness, which is, moreover, dependent also on condition of honey bee colony in all probability.

SOUHRN

Aktivita invertasy a diastasy v českých medech

U 54 vzorků medů byla stanovena aktivita invertasy. Bylo analyzováno 37 vzorků čerstvých medů přímo od včelařů a 17 vzorků medů z tržní sítě. U čerstvých vzorků bylo provedeno vyhodnocení výsledků pro jednotlivé skupiny medů: květové (n = 13), smíšené (n = 15), medovicové (n = 9).

Výsledky u čerstvých medů ukazují, že aktivita invertasy je různá u jednotlivých skupin medů a pohybuje se od 0,8 do 25,9 IN (15,77 ± 6,3). Statisticky významně *(P< 0,05)* se svou aktivitou lišily medy květové (12,12 ± 5,75 IN) od medů smíšených (17,46 ± 6,46 IN) a medovicových (18,22 ± 4,67 IN). Medy medovicové se aktivitou invertasy nelišily signifikantně od medu smíšených. Ve skupině medů květových se nejvíce zastoupená skupina medů řepkových (n = 7) nelišila enzymovou aktivitou od ostatních vzorků této skupiny.

Současně byla u všech medů stanovena aktivita diastasy a stanoven poměr obou enzymů. Poměrem invertasa/diastasa se statisticky významně *(P < 0,05)* lišily pouze medy smíšené (0,59 ± 0,18) od medů medovicových (0,82 ± 0,27).

U vzorků z tržní sítě byly hodnoty aktivity invertasy (od 0,6 do 7,4 IN) signifikantně nižší *(P < 0,01)* ve srovnání s medy přímo od včelařů. Z korelačních vztahů aktivity obou enzymů, invertasa/diastasa a HMF je zřejmé, že aktivita invertasy je citlivějším parametrem zahřátí a skladování medu než aktivita diastasy a obsah HMF. Stanovení hraničních hodnot aktivity invertasy pro stanovení čerstvosti medu je však obtížné, vzhledem k její variabilitě u jednotlivých medů nejspíš v závislosti na kondici včelstev.

med, invertasa, diastasa, hodnocení jakosti

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