

# A comparison of the Schade and Phadebas methods for determining diastase activity in honey

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## Abstract

The aim of this work was to assess methods for determining diastase activity in honey. Eight samples of honey from the retail network and 48 blends of honey with syrups (Apifood and Apivital) were tested using the Schade method and the Phadebas enzyme method. One of the eight samples of honey failed to meet the requirements of the Decree No. 76/2003 Coll. Diastase activity values of 11.13 units according to Schade, though 7.61 Schade units according to the Phadebas method, were measured in this sample. The two methods produced different diastase activity values, with the values according to Phadebas being on average 12.48% lower. A statistically significant difference ( $p < 0.05$ ) was found between the methods. The cause of this difference in the results produced by the methods used was probably enzyme substrate specificity – the specific properties of the starch used as a substrate. The Schade method can be recommended for testing a small number of samples, while the Phadebas method is more suitable for serial analyses.

*Bee product, diastase activity, enzymes, honey, Phadebas method, Schade method*

## Introduction

Honey contains a number of enzymes, some of which come from plants (catalase, acid phosphatase) and some of which get into the honey from the bees' pharyngeal glands (diastase, invertase and glucose oxidase) (Čížková et al. 2010).

Diastase is an enzyme that breaks down starch into simpler saccharides. The activity of the enzyme diastase is an important legislative parameter for honey, and according to the Decree No. 76/2003 Coll. the diastase activity may not be less than or equal to 8 Schade units or, for certain types of honey, less than or equal to 3 Schade units.

Diastase activity ( $\alpha$ -,  $\beta$ -,  $\gamma$ -amylase) is, as is the case for other enzymes, reduced by excessive heating of the honey (Přidal and Vorlová 2002; Tosi et al. 2004; Tosi et al. 2008; Samborska and Czelejewska 2014). The reduction of diastase activity is directly proportional to the temperature and period of heating. Diastase activity and the content of hydroxymethylfurfural are quality control parameters whose measurement can be used to confirm the heating of honey (Tosi et al. 2008). The values of these parameters are used together for an approximate determination of the intensity of heating to which the honey has been subjected (Ramirez Cervantes et al. 2000). A reduction to the diastase activity also occurs during long-term or unsuitable storage (Babacan and Rand 2007) or the addition of foreign sugar to honey. If honey is adulterated by the addition of a saccharose solution, hydrolysed starch or corn syrup with a high fructose content (HFCS), the dilution of the honey leads to a reduction to the diastase activity. Adulteration may be masked by the addition of foreign bacterial amylases such as "baker's yeast amylase" (Voldřich et al. 2009).

Diastase activity can be determined according to the Harmonised Methods of the European Honey Commission (Bogdanov et al. 1997) by the use of two methods – the Schade method and the Phadebas enzyme method.

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An evaluation of these two methods for the determination of the diastase activity of honey has been performed in this work.

### Materials and Methods

Floral honeys ( $n = 4$ ) and honeydew honeys ( $n = 4$ ) purchased from retail chains on the Czech market according to availability in April 2016 were tested. The declared countries of origin of these honeys were the Czech Republic, a blend of honeys from the EC and outside the EC, a blend of honeys from the EC, and a blend of honeys from outside the EC. The honeys were stored in glass or plastic retail packaging at room temperature ( $23 \pm 2$  °C).

Blends of these honeys with the syrups Apifood ( $n = 24$ ) and Apivital ( $n = 24$ ) were also tested. Blends were produced in concentrations of 10, 15 and 20% to a resultant volume of 100 g for the purpose of extending concentration series of diastase activity which are reduced by these syrups in a directly proportional relationship. Syrups were purchased in retail chains on the Czech market according to availability in April 2016 and stored in plastic packaging under the same conditions as the honeys. The honeys and blends of honeys with syrups were analysed within two months of purchase.

Determination of diastase activity according to Schade and the Phadebas enzyme method (a modification of this method according to the instructions from the manufacturer Megazyme was used) was performed in accordance with the Harmonised Methods of the European Honey Commission (Bogdanov et al. 1997; Megazyme 2007) using a Spekol 11 instrument. Statistical calculations were performed with the use of the program Microsoft Office Excel 2003, and a paired Student's T-test was used to compare the two methods.

### Results and Discussion

The spectrophotometric methods according to Schade and Phadebas (enzyme method) can be used to determine diastase activity. The units of diastase activity (Goethe units) are defined as the amount of enzyme that can convert 0.01 g of starch in 1 hour at 40 °C under test conditions. The results are converted into Goethe units (or Schade units) per gram of honey (Bogdanov et al. 1997).

The principle of the Schade method lies in the fact that a standard starch solution, which is capable of forming a colouration of a certain intensity with iodine, is hydrolysed by an enzyme from a sample of honey under standard conditions. The fall in the intensity of the blue colouring is measured at intervals. Changes in absorbance in relation to time or a regressive equation are used to determine time  $t_x$  required to attain a specific absorbance of 0.235 (the German DIN standard uses a factor of 0.301). Diastase activity is calculated as 300 divided by  $t_x$  (Bogdanov et al. 1997).

The method using Amylasyme tablets, which are similar to Phadebas tablets, is based on the principle that hydrolysis of the substrate accompanied by the release of soluble colour products occurs in the presence of diastase ( $\alpha$ -amylase). After terminating the reaction with the addition of Trizma base solution, the sample is filtered and measured on a spectrophotometer at 590 nm. The absorbance of the measured filtrate is directly proportional to the diastase activity in the sample (Megazyme 2007).

An overview of the values measured by the two methods is given in Table 1. A diastase activity above 8 Schade units was measured in all the samples of honey purchased on the retail market in the Czech Republic by the Schade method which is in accordance with the Decree No. 76/2003 Coll. as amended. One sample did not, however, satisfy the demands of this decree when measured by the Phadebas method. Values of diastase activity of 11.03 Schade units according to the Schade method, though just 7.61 Schade units according to the Phadebas method, were measured in this sample.

The two methods produced different values of diastase activity. The results produced by the Phadebas method were 12.48% lower on average. A statistically significant difference between the two methods was found by a Student's T-test ( $p < 0.05$ ). An assessment of the methods for determining diastase activity in honey is given in Table 2.

Čížková et al. (2010) also studied the determination of diastase activity in honey, and determined the diastase activity according to Schade and with a commercial Phadebas

Table 1. Values for the diastase activity in honey and blends of honey with syrups (Schade units)

| Method             | Schade | Phadebas |
|--------------------|--------|----------|
| Average            | 15.15  | 13.26    |
| Standard deviation | 3.84   | 5.01     |
| Median             | 14.98  | 12.91    |
| Minimum            | 9.70   | 5.84     |
| Maximum            | 23.11  | 23.31    |

Table 2. An assessment of methods for determining diastase activity

| Method                         | Schade    | Phadebas       |
|--------------------------------|-----------|----------------|
| Time demands of method         | demanding | fast           |
| Instrumentation                | the same  | the same       |
| Calibration solutions required | required  | no calibration |
| Financial cost                 | low       | high           |

set in a group of 15 samples. They also recorded different values for the diastase activity using the two methods, with the diastase activity according to Phadebas being on average 35% lower, although the results obtained by the two methods correlated with each other relatively well. We hold the view, like Čížková et al. (2010), that the cause of the difference in the results produced by the two methods was probably the substrate specificity of the enzyme – the specific properties of the starch that was used as a substrate. While the starch is precisely defined in the commercial set and should always have the same properties, the specification for the Schade method is merely general and workplaces performing tests must verify the method with a view to the specific starch used.

The Schade method can be recommended for testing a small number of samples. The overall cost is lower, though this method is five times as time-consuming. The Phadebas method is better suited to serial analyses, though it is necessary to count on costs eight times higher per analysis.

## Conclusions

Determination of the diastase activity in honey was performed by two spectrophotometric methods. One of the eight samples of honey failed to satisfy the demands of the Decree No. 76/2003 Coll. A value of 11.13 Schade units of diastase activity was measured in this sample by the Schade method, though just 7.61 Schade units by the Phadebas method.

The two methods produced different values of diastase activity, with the results according to the Phadebas method being on average 12.48% lower. A statistically significant difference was found between the two methods ( $p < 0.05$ ). The cause of the difference between the results produced by the methods used was probably the substrate specificity of the enzyme – the specific properties of the starch used as a substrate.

The Schade method can be recommended for testing a small number of samples. It is more time-consuming, though total costs are lower. The Phadebas method is better suited to serial analyses.

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