Diastase testing – Different methods Schade, Phadebas® and Nitrophenol

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The natural honey enzyme "Diastase" (scientifically it concerns an α-Amylase) introduced by the bee into honey is an important quality parameter and further a potential marker for honey authenticity. Its activity decreases when honey is heated or stored over a long time-period.
Accordingly, the diastase number (DN, descriptive for the enzyme activity) is legally regulated (EU regulation 2001/110/EC, Codex Alimentarius, USP/FCC Honey identity standard 2021).
For honey - with exception of baker's honey - a minimum activity of 8 DN (Schade scale) is fixed. For honey types with low natural enzyme content such as lemon honey and an HMF content of max. 15 mg/kg, the activity of diastase must be at least 3 DN (all three standards stated above).
Also in the worldwide honey trade, the required diastase activity is usually explicitly defined in trade specifications. However, the method used for the determination of the diastase activity is not described in the EU honey directive or Codex Alimentarius, just the scale according to Schade is fixed.
In the 2021 USP/FCC honey identity standard, the Schade method is depicted. In the German DIN method 10750 meanwhile both the Schade method and since March 2022 also the Nitrophenol method are depicted, but not the Phadebas® method.

Table 1: Methods used to determine the diastase activity

Method	established	Standardization	Substrate	Selectivity	Expanded MU **
Schade-Method	1958	DIN 10750-1 German § 64 LFGB L 40.00-1	Potato starch	low (α, β, γ-amylase)	12%
Phadebas®-Method	1975	none*	Cross-linked starch polymer carrying a blue dye	high (only α-amylase)	24%
Nitrophenol-Method	1998	DIN 10750-2	4,6-Ethyliden(G7) -1[4-nitrophenyl (G1)] -1,4-α-D-maltoheptaoside	high (only α-amylase)	8%

*Methodology specified by manufacturer / also described by International Honey commission in IHC harmonized methods 2009; Phadebas results should match with Schade method results
** MU = expanded measurement uncertainty / max. variation, calculated based on RSD by 4 different German honey labs

For all three methods the activity is given as "Diastase Number" (DN) in according to "Schade scale" and can therefore be used for an assessment of quality in accordance with the standards and legislation worldwide.
For authentic honey the activities obtained by all three methods should be rather comparable. However, the selectivity of the Schade method for α-amylase is lower as compared to the Phadebas® and the Nitrophenol method, which both use specific substrates that detect only α-amylases.
Starch as substrate is not only degraded by α-amylases, but also by β- and γ-Amylases. β- and γ-Amylases are thus analytically included in the Schade method, but not in the other two more specific methods. Natural honey also shows a certain β- and γ-Amylases activity. Therefore, in our experience, the Schade method often finds slightly higher diastase activities than the other two methods. All methods are conventional methods and harmonized over proficiency tests (ring trials). The results of the three individual methods should therefore also be well comparable between different laboratories considering the expanded analytical measurement uncertainty and homogeneous samples. However, in some cases differences are observed between the three methods in one lab or between different labs, that cannot be explained with the analytical measurement uncertainty. The question is: what is the root cause for these significant differences?
In the following different aspects are highlighted giving more insight into this topic.

In 2021 in total 12,126 honey samples were tested at QSI for diastase (all three methods). The natural range of the diastase activity is broad and depends on the kind of honey and how long and at which temperature it was stored.

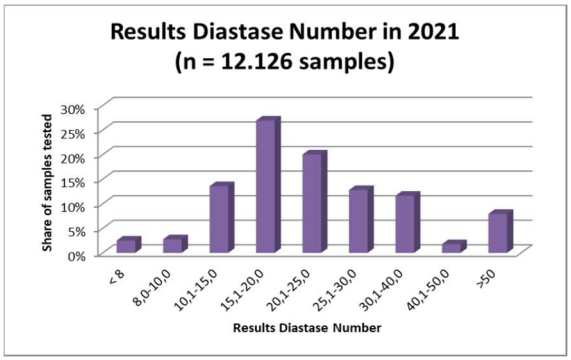

Figure 1: Overview of diastase activities observed in customer samples at QSI in 2021

Honey-foreign amylases (diastase, saccharase) might be introduced into honey via bee feeding during the nectar flow (not allowed) or intentional addition of foreign sugar syrups made from starch (fraud) or unintentionally by the beekeeper via feeding supplements (produced by yeasts, brewer´s yeast) or via other artificial manipulation (fraud). For some honey varieties beta-gamma-amylases might be introduced via honeydew insects (known exceptions of unifloral honeys see below).
In our experience, besides the natural origin (bee) diastase is also artificially added to meet the legal requirements for its activity, to cover ion exchange treatment (resin), heat/storage damage or after dilution with sugar syrups, which do not have any diastase activity. Such manipulated "honeys" can possibly show differences between the results of the three diastase determination methods, which might indicate the presence of foreign sugars or adulteration.
However, limit values for "non-natural" deviations between the three methods cannot be stated. Various amylases can be possibly added for a manipulation and different kinds of amylases might have different responses in the three diastase methods or amylases can be introduced via honeydew insects or inadequate feeding. Thus, also authentic honeys in certain cases show significant differences in the results. This is known from many ring trials where QSI participated with all three different diastase methods.
To detect the manipulation of α-amylases (diastase), we also offer the Famyp method (honey-foreign α-amylases) as well as the determination of thermostable α-amylases (diastase activity should be < 1 DN after strong heat treatment). In addition, we offer the specific determination of the activity of β-, γ-amylases, which should be less than 5 units/kg (exceptions with higher natural β-, γ-amylase activity: certain varieties such as Pine honeys, Metcalfa, Quillaya, Avocado).

Traditionally, QSI recommends the Schade method for diastase (also included in the common commercial trade analysis package), which is standardized, robust and does not rely on kits or tablets from third parties. On the other hand, the Nitrophenol and the Phadebas® method are more specific for α-amylase (diastase) only. In some European countries, such as Poland, the Phadebas® method is officially recognised as reference method.
We further recommend to testing bee feed for enzyme activities to exclude feeding as root cause for unexpected honey results.
QSI offers all three diastase methods (also in packages) and you as customer can select the most suitable method for your quality control. We will gladly provide you with an attractive quote!

Please contact us if you have any questions related to this or any other topic!

Your QSI - Team