

## <u>Possibilities for the determination of diastase with QSI</u> <u>A comparison of the methods according to Schade, Phadebas and</u> Nitrophenol

The honey-own enzyme "Diastase" (scientifically it concerns a  $\alpha$ -Amylase) is a marker for the authenticity of honey and represents an important quality parameter. Accordingly, the diastase number (DN) is legally regulated according to the Schade scale (EU regulation 2001/110/EG, Codex Alimentarius). For honey with exception of baker's honey / industrial honey a minimum activity of 8 DN (Schade scale) is fixed. For honey types with low natural enzyme content such as lemon honey and a HMF content of maximum 15 mg/kg, the minimum activity of diastase must be at least 3 DN. Also in the worldwide honey trade, the required diastase activity is usually explicitly regulated in specifications. In 2017, QSI tested 13.981 samples for diastase. The average diastase number of all samples was 22,9 DN. 92.7% of all analysed samples were in the range of 8-50 DN.

The following methods are used to determine the diastase number:

Method	established	Standardization	Substrate	Selectivity
Schade-Method	1958	DIN10750 § 64 LFGB L 40.00-1	potato starch	low (α, β, γ-amylase)
Phadebas-Method	1975	none*	modified starch with dye	medium (mainly α-amylase)
Nitrophenol- Method	1998	planned by DIN	4,6-Ethyliden(G7)- 1[4-nitrophenyl(G1)]- 1,4-α-D- maltoheptaoside	high (only α-amylase)

<sup>\*</sup>Methodology specified by manufacturer or harmonized by IHC (IHC harmonized methods 2009)

For all three methods the activity is given as diastase number (DN) in Schade units or according to Schade scale and can therefore be used for an assessment of quality.

In the case of authentic honey, the activities obtained by all three methods should be quite comparable. However, the selectivity for  $\alpha$ -amylase is significantly higher in the more modern Phadebas method and especially in the Nitrophenol method than in the Schade method, which uses starch as substrate. Starch is not only degraded by  $\alpha$ -amylases, but also by  $\beta$ - and  $\gamma$ -amylases.  $\beta$ - and  $\gamma$ -Amylases are thus analytically measured in the Schade method, but not in the other two more specific methods.  $\beta$ - and  $\gamma$ -Amylases are contained also naturally in honey in small quantity. Therefore, in our experience, the Schade method finds slightly higher diastase activities than the other methods. All methods are conventional methods and harmonized over proficiency tests (ring trials). The results of the individual methods should therefore also be well comparable between different laboratories.

In the case of non-authentic honeys, honey-foreign amylases are to be interpreted as indirect detection of syrups made from starch. In our experience, amylases are also artificially added to meet the legal requirements for diastase activity, e.g. after ion exchange treatment (resin) or after the addition of sugar syrup which no longer has amylase activity. Such manipulated "honeys" show partly clear differences between the results of the three diastase determination methods, which can already indicate an adulteration. For this purpose we also offer an analysis package of all three diastase methods. However, limit values for "non-natural" deviations between the methods cannot be determined, since various different amylases can be added for a manipulation and different kinds of amylases might have different responses in the three diastase methods.

In addition, there are also different types of honey, which naturally show higher differences between the diastase methods.

To detect the manipulation of  $\alpha$ -amylases (diastase), we also offer the Famyp method (honey-foreign  $\alpha$ -amylases) and a direct measurement of the activity of  $\beta$ -,  $\gamma$ -amylases, which should be within a range of 0 to 5 units/kg (exceptions with higher natural  $\beta$ -,  $\gamma$ -amylase activity: certain varieties such as Pine honeys, Metcalfa, Quillaya, Avocado).

In summary, we offer our customers all three methods for diastase determination. Traditionally, we recommend the Schade method, as it is currently the only standardized method. On the other hand, the Nitrophenol method, which is currently being evaluated in the DIN (Germany), is more specific for honey's own  $\alpha$ -amylase (diastase). However, in some European countries, such as Poland, the Phadebas method is officially recognised as a reference method. Therefore, we will be happy to prepare a suitable offer for you. Please do not hesitate to contact us!

Yours sincerely
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