Invertase activity in honey1

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Apidologie 30 (1999) 57–65

Original article

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(Received 13 March 1998; accepted 23 September 1998)

Abstract – The invertase activity was determined for 499 honeys (27 multifloral and 472 unifloral from *Arbutus*, *Carduus*, *Castanea*, *Citrus*, *Erica*, *Eucalyptus*, *Hedysarum*, *Helianthus*, *Rhododendron*, *Robinia*, *Rosmarinus*, *Taraxacum*, *Thymus*, *Tilia*, fir honeydew and honeydew produced by *Metcalfa pruinosa*), in order to determine its variability and establish the range characteristic for each honey type. The results show that invertase activity varies considerably in the different honey types (from less than 0.5 to more than 30 IN). *Robinia*, *Arbutus*, *Citrus*, *Erica* and *Rosmarinus* have the lowest values (usually less than 10) and the two honeydew honeys the highest (more than 18). The diastase content of the samples was also measured, to compare the content of the two enzymes, and a certain correlation was observed ($r = 0.835$, $P < 0.001$). The IN/DN ratio ranges from less than 0.1 to more than 2. The possible role of invertase and IN/DN ratio in honey quality evaluation is discussed.

unifloral honey / invertase / diastase / enzyme / honey freshness

1. INTRODUCTION

Honey contains small amounts of different enzymes, the most important of which are diastase (α-amylase), invertase (α-glucosydase), glucose oxidase, catalase and acid phosphatase. In particular, invertase is the enzyme responsible for converting sucrose to fructose and glucose which are the main sugars in honey [29].

The origin of invertase in honey is commonly attributed to the bee [23, 29, 30]. The nectar collected is mixed with secretions from the salivary and hypopharyngeal

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[1] The research was partly carried out in the frame of the project AMA (Ape, Miele, Ambiente) financed by the Italian Ministero per le Politiche Agricole.

glands of foraging bees. In the hive, when the nectar is passed from bee to bee before being stored in the cells, more secretions are added, enabling nectar to ripen into honey [19]. This process – and consequently the amount of added enzymes – depends on various factors such as age, diet and physiological stage of the bees, strength of the colony, temperature, abundance of nectar flow, etc. [4, 5, 10, 12, 13, 26].

Enzymes as honey components have been the object of much research over the years: the primary interest was as a possible means of distinguishing between natural and artificial honeys [29], but diastase and invertase are largely used in Europe as a measure of honey freshness, because their activity decreases in old or heated honeys [6–9, 11, 22, 24, 32].

In previous research [20], the variability of diastase content in honeys of different botanical origin was studied. The aim of the present research is to determine whether the same variability occurs for invertase activity, and establish the range of activity for each honey type. In addition, the possible role of invertase in honey quality evaluation is discussed.

2. MATERIALS AND METHODS

Invertase activity was determined for 499 honeys: 27 of them were multifloral and 472 were unifloral, from the following sources: *Arbutus, Carduus, Castanea, Citrus, Erica, Eucalyptus, Hedysarum, Helianthus, Rhododendron, Robinia, Rosmarinus, Taraxacum, Thymus, Tilia,* fir honeydew and honeydew produced by *Metcalfa pruinosa* (Say). The botanical origin of the samples was established by their melissopalynological, organoleptic and physico-chemical characteristics [21]. The multifloral samples were from various regions, different phytogeographical areas and altitudes. The honeys, produced in 1995–1997, were kept at –20 °C until they were analysed. Their freshness was verified through HMF determination (samples with more than 10 mg/kg were rejected).

Invertase was determined using the Siegenthaler method [25], harmonised by the European Honey Commission [3]. The enzyme activity is evaluated photometrically, by measuring the decomposition of the substrate p-nitrophenyl α-D glucopyranoside into the product p-nitrophenol (which has a maximum absorbance at 400 nm). The results were expressed as invertase number (IN). The IN indicates the amount of sucrose per gram hydrolysed in 1 h by the enzymes contained in 100 g of honey under test conditions.

The diastase activity of the same samples was also measured, in order to compare the content of the two enzymes. It was evaluated by the Phadebas method [2, 3], and results were expressed in Schade units (or diastase number, DN). One unit corresponds to the enzyme activity of 1 g honey that can hydrolyse 0.01 g of starch in 1 h at 40 °C.

3. RESULTS

3.1. Invertase

The results of invertase determinations, reported in *table I*, show that this enzyme varies considerably in the different honey types studied (from less than 0.5 to more than 30 IN). *Robinia, Arbutus, Citrus, Erica* and *Rosmarinus* have the lowest values (mostly less than 10 IN). Honeys from *Hedysarum, Taraxacum, Rhododendron, Carduus, Tilia* and *Helianthus* show low to medium values (from 5 to 20), with considerable overlapping between one type and the next. *Thymus, Eucalyptus* and *Castanea* honeys have medium to high values (between 14 and 30 IN), the two honeydew honeys have the highest values (more than 18) and the multifloral samples range from 7 to 28 IN.

3.2. Diastase

The results of the diastase analysis (*table I*) confirm the ranges already found on a different sampling in previous research [20, 21]: *Arbutus* honey shows the lowest values (DN from 0 to 8); honeys from *Robinia, Erica, Taraxacum, Citrus, Rhododendron,* are characterised by low values, almost

Table I. Invertase, diastase and IN/DN ratio in Italian unifloral honeys.

Honey type (n)	Invertase content (IN)		Diastase content (DN)		IN/DN ratio	
	x ± s.d.	Min.–Max.	x ± s.d.	Min.–Max.	x ± s.d.	Min.–Max.
Robinia (40)	3.6 ± 2.2	0.4–7.7	8.3 ± 2.3	3.9–14.9	0.42 ± 0.23	0.07–0.89
Arbutus (16)	4.0 ± 2.8	0.5–8.9	4.3 ± 2.2	0.0–8.4	1.12 ± 0.51	0.40–2.03
Citrus (54)	5.3 ± 2.3	1.0–9.5	9.3 ± 2.7	4.4–15.8	0.57 ± 0.21	0.10–0.97
Erica (15)	6.1 ± 3.4	1.6–10.7	8.3 ± 4.0	3.9–15.4	0.54 ± 0.22	0.24–0.95
Rosmarinus (27)	8.3 ± 2.1	3.9–11.8	8.8 ± 2.1	5.0–13.6	0 99 ± 0.14	0.73–1.32
Hedysarum (20)	9.0 ± 3.0	5.0–14.0	20.0 ± 2.5	14.3–23.6	0.44 ± 0.13	0.27–0.67
Taraxacum (14)	10.3 ± 2.9	5.4–14.5	9.1 ± 3.0	4.8–13.6	1.03 ± 0.25	0.58–1.35
Rhododendron (25)	11.1 ± 2.7	7.3–17.7	12.2 ± 2.1	8.2–15.8	0.91 ± 0.17	0.68–1.32
Carduus (35)	12.6 ± 2.8	6.5–16.4	20.0 ± 5.1	12.0–32.4	0.64 ± 0.13	0.37–0.90
Tilia (33)	12.8 ± 3.8	6.7–19.8	17.7 ± 3.9	12.0–27.2	0.73 ± 0.17	0.46–1.12
Helianthus (22)	13.0 ± 2.4	9.0–16.3	16.5 ± 3.3	12.0–23.0	0.81 ± 0.19	0.40–1.23
Multifloral (27)	15.4 ± 4.5	7.5–28.4	22.0 ± 6.0	9.2–37.1	0.72 ± 0.17	0.33–1.04
Thymus (26)	18.4 ± 3.5	13.8–26.8	32.8 ± 6.2	21.0–50.0	0.59 ± 0.11	0.38–0.92
Eucalyptus (47)	21.0 ± 3.9	13.5–28.3	24.0 ± 5.6	14.1–35.9	0.90 ± 0.22	0.61–1.34
Castanea (40)	21.6 ± 4.0	14.7–29.2	24.9 ± 4.7	14.3–34.9	0.88 ± 0.14	0.64–1.17
Metcalfa honeydew (39)	23.4 ± 2.4	18.5–28.1	32.9 ± 6.8	21.1–49.3	0.73 ± 0.13	0.53–1.04
Abies honeydew (19)	23.9 ± 3.0	17.9–30.6	21.7 ± 6.9	15.0–39.9	1.13 ± 0.21	0.77–1.42

always less than 15; *Helianthus, Tilia, Hedysarum, Abies* honeydew, *Eucalyptus* and *Castanea* honeys range mostly from 15 to 35; *Thymus* and honeydew from *Metcalfa* show the highest values (20–50). Two new types, *Rosmarinus* and *Carduus*, not described so far, are characterised by low (8.8 ± 2.1) and medium-high values (20.0 ± 5.1), respectively.

3.3. Invertase/Diastase correlation

By comparing the invertase and diastase activity of the 499 samples (*figure 1*), a certain correlation is observed between the two enzymes ($r = 0.835$, $P < 0.001$) and, even if they do not follow exactly the same trend, honeys with a low invertase content generally also have a low diastase content and vice versa (*figure 2*). A very similar correlation coefficient ($r = 0.878$) was found by Huidobro et al. [14]; slightly lower ($r = 0.738$) the one reported by Krauze and Krauze [17].

The results of invertase/diastase ratio (*table I*) show quite a wide variability, both between and within different honey types, from less than 0.1 to more than 2.

4. DISCUSSION

4.1. Variability in invertase content

Our results on invertase content in different honey types, are more or less similar to those found by Dustmann et al. [8], Krauze and Zalewski [18] and Huidobro et al. [14]. Notably different values are reported by Karabournioti and Drimjias [15] for *Thymus* and for *Abies* honeydew: 35 ± 9 and 39.7 ± 8.7 U/kg[1], respectively (corresponding to 4.8 ± 1.2 and 5.4 ± 1.2 IN), while their results for *Citrus* are in the same range as ours (23.2 ± 8.8 U/kg, corresponding to 3.2 ± 1.2 IN).

The variability in enzyme activity found in the different honey types is probably due to a series of factors, such as: nectar collection period (and consequently the physiological stage of the colony); abundance of nectar flow and its sugar content (a high flow of concentrated nectar leads to lower enzyme content); age of the bees (when the honey bee becomes a forager its glands produce more digestive enzymes); pollen consumption, etc. [4, 5, 10, 12, 13, 26].

Arbutus honey, which is produced in late autumn, has virtually no enzymes at all, probably because in overwintering bees the gland activity is considerably reduced. *Erica, Citrus* and *Rosmarinus* honeys are collected in early spring when the colony feeds brood and young bees, whose glands produce less enzymes, are predominant. The low enzyme content in *Robinia* honey is probably due to the high nectar flow that does not allow bees to adequately process it. All honeys with a high enzyme content are produced in summer when brood rearing is less intensive and foragers are predominant; in particular, honeydew honeys, which have the highest invertase content, derive from a raw material (honeydew) already rich in enzymes, especially invertase, coming from the secretions of the salivary glands and the gut of plant-sucking insects [19].

4.2. Evaluation of honey freshness

Honey freshness is generally evaluated by determining the value of parameters that increase or decrease with overheating and/or ageing. The most commonly used are hydroxymethylfurfurale (HMF), diastase and invertase. The first is produced by the degradation of fructose: it is virtually absent in fresh honey and increases more or less quickly, according to storage conditions and certain chemical properties of the honey,

[1] 1 U/kg = the enzyme activity that can transform 1 μM substrate in 1 min under optimal conditions. It corresponds to 7.345 IN.

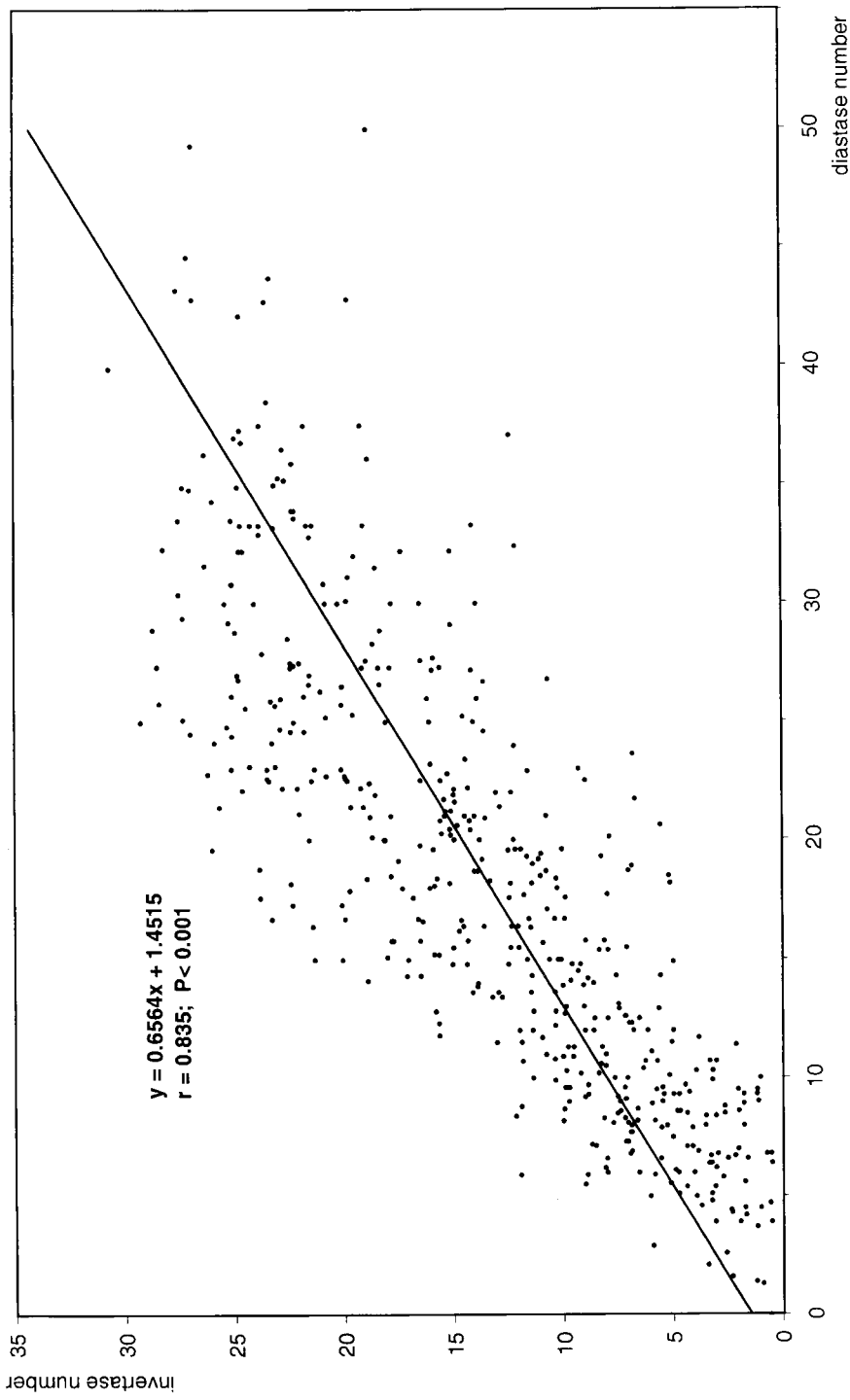

Figure 1. Relationship between invertase and diastase activity.

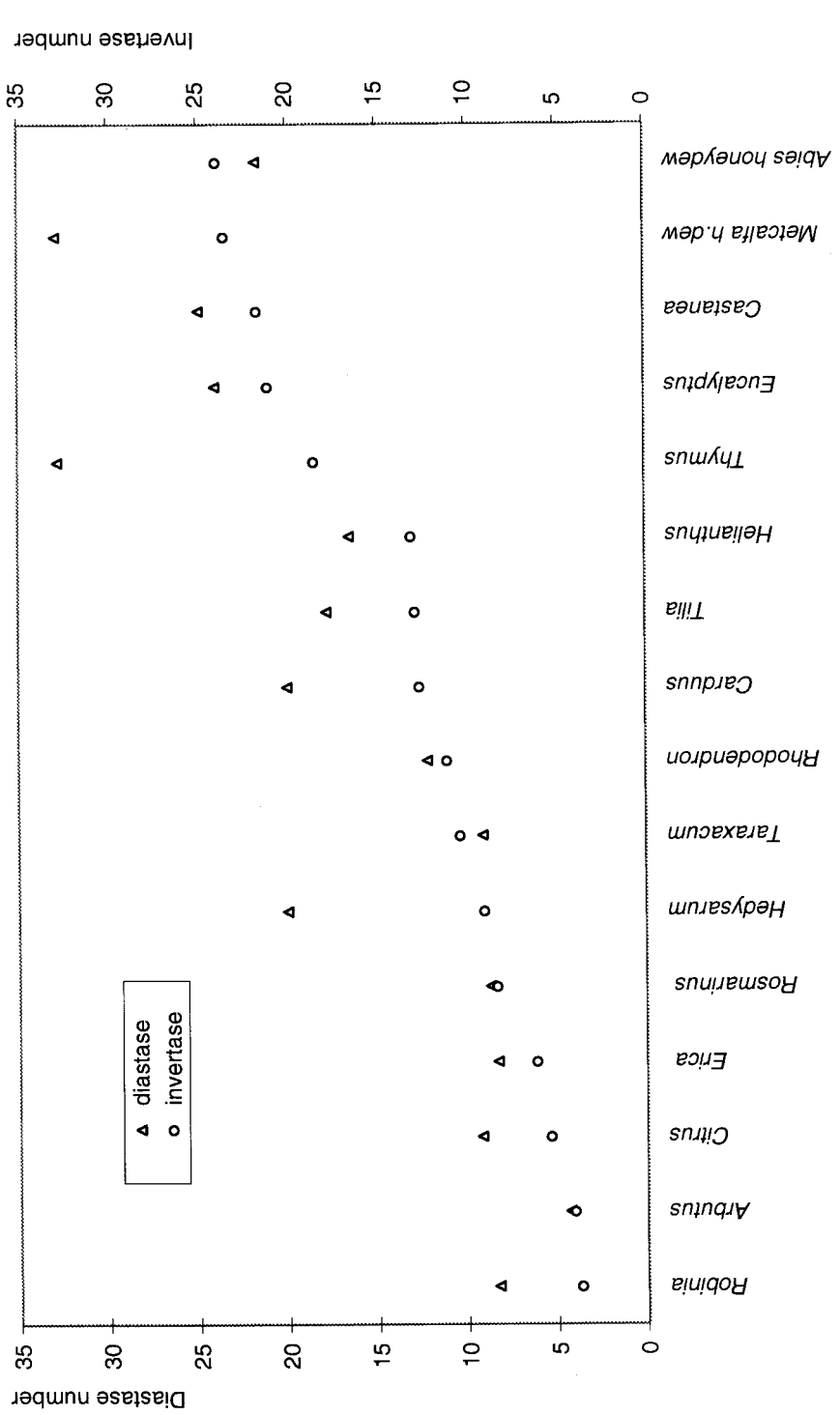

Figure 2. Invertase and diastase average values in different unifloral honeys.

such as acidity. As already mentioned, the two enzymes are added by the honey bee and their activity decreases in old or heated honey.

HMF and diastase are included as international quality standards for honey [6, 9], but invertase is considered better than diastase as a freshness index because it is more sensitive to heating [7, 8, 11, 24, 32]. It therefore seems to be a more suitable parameter for the evaluation of specially labelled high quality honeys.

However, in an attempt to establish limits for this parameter, its variability from one type of honey to another must be taken into account. Our data on multifloral and unifloral samples suggest that the invertase content of most honeys falls in the range 5–20 IN. A reasonable general limit could therefore be 'not less than 5 IN', but a different value should be prescribed for low-enzyme honeys (for example 0.5 IN for *Robinia* and *Arbutus*, 1 IN for *Citrus* and *Erica*, 4 for *Rosmarinus*). On the other hand, a limit of 5 IN could be too permissive for honeys with a high invertase content (*Thymus, Eucalyptus, Castanea* and the two honeydew honeys) for which a limit of 'not less than 13 IN' would be more suitable. In short, any limit could be unfairly severe for some honeys and too permissive for others. When the invertase activity of a sample is measured, it is hard to interpret the result in terms of freshness: since the initial value is unknown, there is no correct starting point from which to evaluate possible overheating or ageing effects.

The same problem obviously exists for diastase [20, 27, 28]. White [31] strongly criticises the use of diastase as quality standard for honey: "Where can one set the starting point from which to calculate a heat damage or so-called partial destruction? The entire approach is in error because of this factor". The same argumentation can be applied to the introduction of invertase as quality standard.

Also the invertase/diastase ratio does not seem to give better information. Some authors [1, 16, 22] studied the invertase/diastase ratio in fresh and stored or heated honeys. Since invertase is more susceptible to temperature than diastase, the ratio is supposed to be the best measure for honey freshness. In particular, according to Kiermeier and Köberlein [16], the value of IN/DN ratio should be greater than 0.5 in fresh honey, and between 0.2 and 0.5 in commercial honeys. In our research we did not analyse old or heated samples, but our results on IN/DN ratio, based on fresh honeys, show such a wide range (0.1–2), that it seems hard to draw any conclusion about the use of this parameter for detecting honey freshness.

In conclusion, HMF remains the more reliable indicator for the evaluation of honey freshness. It starts from nil in all honeys and, once a limit is established, more or less strict according to the required quality level, it has the same significance for all honeys. Enzymes can be a useful complement, but mostly as a parameter to characterise unifloral honeys.

Résumé – Activité de l'invertase dans les miels. On a mesuré l'activité de l'invertase (α-glucosidase) dans 499 miels (27 miels multifloraux et 472 miels monofloraux de *Arbutus, Carduus, Castanea, Citrus, Erica, Eucalyptus, Hedysarum, Helianthus, Rhododendron, Robinia, Rosmarinus, Taraxacum, Thymus, Tilia*, de miellat de sapin et de miellat produit par *Metcalfa pruinosa*) afin de déterminer la variabilité et d'établir le domaine d'activité pour chaque type de miel. Les résultats (*tableau I*) montrent que l'activité de l'invertase varie d'un type de miel à l'autre : l'indice d'invertase (IN) est compris entre 0,5 et 30. Les miels de *Robinia, Arbutus, Citrus, Erica* et *Rosmarinus* ont les valeurs les plus basses, en général inférieures à 10 ; les miels d'*Hedysarum, Taraxacum, Rhododendron, Carduus, Tilia* et *Helian-*

thus ont des valeurs faibles à moyennes, entre 5 et 20 ; ceux de *Thymus, Eucalyptus* et *Castanea* des valeurs moyennes à fortes, entre 14 et 30 et les deux miels de miellat ont les valeurs les plus élevées, supérieures à 18. L'indice des miels multifloraux est compris entre 7 et 28.

On a également mesuré la diastase (α-amylase) des mêmes échantillons pour comparer la teneur des miels en ces deux enzymes (*tableau I*). On observe une certaine corrélation entre l'activité de l'invertase et celle de la diastase ($r = 0,835$, $P < 0,001$). Les miels qui ont une faible teneur en invertase ont généralement aussi une faible teneur en diastase et vice versa (*figure 2*). Le rapport indice d'invertase/indice diastasique (IN/DN) présente une large variabilité, que ce soit entre les divers types de miels qu'à l'intérieur d'un même type (*tableau I*); il va de moins de 0,1 à plus de 2.

La variabilité de l'activité enzymatique est probablement due à un ensemble de facteurs tels que l'abondance de la miellée et sa teneur en sucres, le stade physiologique de la colonie, l'âge des abeilles, etc... La discussion porte sur le rôle de l'invertase, plus sensible à la chaleur que la diastase, et du rapport IN/DN dans l'évaluation de l'état de fraîcheur du miel. Il faut prendre en compte cette variabilité lorsqu'on détermine des valeurs extrêmes de ces paramètres comme normes de qualité.

miel monofloral / invertase / diastase / activité enzymatique / contrôle qualité

Zusammenfassung – Invertaseaktivität im Honig. Für 499 Honige wurde die Invertaseaktivität bestimmt, um die Variabilität zu bestimmen und um den Bereich der Aktivität für jeden Honig festzulegen. Untersucht wurden 27 multiflorale und 472 uniflorale Honige von *Arbutus, Carduus, Castanea, Citrus, Erica, Eucalyptus, Hedysarum, Helianthus, Rhododendron, Robinia, Rosmarinus, Taraxacum, Thymus, Tilia*, Tannenhonigtau und Honigtau von *Metcalfa pruinosa*..

Die Ergebnisse (*Tabelle I*) zeigen, daß die Invertaseaktivität in den verschiedenen Honigensorten unterschiedlich ist (von weniger als 0,5 bis mehr als 30 IN). *Robinia, Arbutus, Citrus, Erica* und *Rosmarinus* haben die niedigsten Werte (meist weniger als 10 IN); Honige von *Hedysarum, Taraxacum, Rhododendron, Carduus*, and *Helianthus* zeigen niedrige bis mittlere Werte (5–20); Honige von *Thymus, Eucalyptus* and *Castanea* mittlere bis hohe (zwischen 14 und 30), und die beiden Honigtauhonige die höchsten Werte (mehr als 18 IN). Die multifloralen Proben erreichten 7 bis 28 IN.

Von denselben Proben wurde zusätzlich die Diastaseaktivität bestimmt, um den Gehalt der beiden Enzyme zu vergleichen (*Tabelle I*). Es ergab sich eine gewisse Korrelation (*Abb. 1*) zwischen der Invertase- und Diastaseaktivität ($r = 0.835$, $P < 0.001$). Honige mit einem niedrigen Invertasegehalt haben normalerweise auch einen geringen Anteil von Diastase und vice versa (*Abb. 2*). Das Verhältnis von IN/DN (*Tabelle I*) weist eine große Variabilität von weniger als 0.1 bis mehr als 2 auf, sowohl zwischen als auch innerhalb der verschiedenen Honigsorte.

Diese Variabilität der Enzymaktivität hängt wahrscheinlich von vielen Faktoren ab, wie der Häufigkeit des Nektarangebots und seines Zuckergehaltes, dem physiologischen Zustand des Volkes, dem Alter der Bienen, usw.

Die Bedeutung von beiden Enzymen, Invertase (diese ist empfindlicher gegen Erhitzung als Diastase) und des IN/DN Verhältnis für eine Bewertung der Frische eines Honigs wird diskutiert. Bei der Erstellung von möglichen Grenzwerten durch diese Parameter als Qualitätsstandard muß ihre Variabilität berücksichtigt werden.

Uniflorale Honige / Invertase / Diastase / Enzyme / Frische des Honigs

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