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Original article

Adulteration of honey: relation between microscopic analysis and $\delta^{13}C$ measurements

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Abstract – Upon routine microscopic analysis of some honey samples, parenchyma cells, single rings of ring vessels and epidermal cells are found. These cells originate from the sugar cane stem. We investigated whether there was a relation between these plant fragments and the $\delta^{13}C$ value of honey. 17 honey samples and 6 cane sugar samples were analyzed. Microscopic analysis of the samples was done quantitatively by counting the parenchyma cells, rings, and epidermal cells present in 10 g of the sample using polarized light microscopy. Also the repeatability of the microscopic analysis was determined by calculating the standard deviation of the values from the processing and examination of 8 sub-samples from one honey sample For all honey samples in this study, it was found that if more than 150 parenchyma cells and/or 10 rings in 10 g were detected, the samples were adulterated with C_4 sugars (from sugar cane or corn) according to the $\delta^{13}C$ method. Lower microscopic counts indicated honey with suspected adulteration below 7%, the limit of detection of the $\delta^{13}C$ method. Overall, the microscopic procedure was a good screening method for the detection of adulteration of honey with cane sugar products.

honey / adulteration / microscopy / cane sugar / $\delta^{13}C$

1. INTRODUCTION

The official method for analyzing honey adulteration with sugars derived from sugar cane or corn is the $\delta^{13}C$ method. It was first introduced in 1978 and originally about 20% adulteration could be detected with this method [9, 10]. It is based on the principle that the C_4 plants such as sugar cane (*Saccharum officinarum* L.) and corn (*Zea mays* L.)

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absorb relatively more carbon dioxide with $^{13}CO_2$ during their photosynthesis than the C_3 plants from which honey usually originates. The amount of ^{13}C present in the honey is expressed as $\delta^{13}C$, defined as the deviation of this amount from an internal standard material (VPDB) [3]. For analysis, the honey or sugar is burnt and the CO_2 is led into an isotope ratio mass spectrometer (IRMS) in which the $\delta^{13}C$-value is determined. The $\delta^{13}C$ value of pure corn and cane sugar is about –10‰ [2, 9, 10]. Upon analyzing 119 authentic honey samples, White and Doner [9, 10] found $\delta^{13}C$ values between –22.5 and –27.4‰ with a mean value of –25.4. Samples with a $\delta^{13}C$ value less negative than this mean and twice the standard deviation of the series, termed the 2-s limit and equal to –23.5‰, were considered to be adulterated [8].

Later it was found that some honey samples (e.g. *Citrus* honeys) could have slightly less negative $\delta^{13}C$ values [12]. Therefore honeys with values between –21.5 (4-s limit) and –23.5‰ (2-s limit) were only considered to be adulterated if there was a deviation in their thin layer chromatographic sugar spectrum as well [6, 11]. Honey samples with values above –21.5‰, the 4-s limit, were considered as adulterated with a probability of error ≤ 1:25 000, but this still allowed the addition of as much as 20% cane or corn sugar into honey. To improve the effectiveness of this $\delta^{13}C$ method, it was modified by using the honey protein as internal standard [13]. With this new procedure, it became possible to detect down to 7% the addition of corn or cane sugars, while maintaining the same 4-s limit as previously used (probability of error ≤ 1:25 000). This method is at present considered as the official method for the detection of adulteration with this type of sugars in honey.

Microscopic methods can also be useful for the detection of adulteration of honey with cane sugar [5]. The procedure is based on the fact that the raw fibers of the sugar cane, present in all types of cane sugar, posses very characteristic plant cells which can be detected in adulterated honey samples. Characteristic of sugar cane plant cells are the many parts of pitted parenchyma cells (usual range 3 000–30 000 in 10 g), the pitted sclereids, the single rings of ring vessels (a few hundred in 10 g) and parts of epidermal cells (also a few hundred in 10 g). The analysis consists of preparing a microscopic slide of the honey sample in the same way as is done for pollen analysis of honey without acetolysis [7], but this microscopic analysis is preferably done with polarization microscopy [5]. The morphological characteristics of the sugar cane particles have been described previously in detail and illustrated with photographs [5]. Previous studies have analyzed various types of cane sugar from all over the world for the number of plants cells [4, 5]. The presence of high amounts of sugar cane cells in honey coincided with abnormal sucrose and/or HMF contents, which indicated adulteration.

In screening honey for cane sugar plant cells with the above-mentioned microscopic method (Food Inspection Service), some trade honeys were suspected of adulteration as were some other honeys with an abnormal taste, though the other physico-chemical characteristics of these honey samples were normal. The objective of the present study was to determine whether the two methods gave consistent results on these samples, that is whether there was a correlation between the presence and number of sugar cane plant cells on one hand and the $\delta^{13}C$ values on the other.

2. MATERIALS AND METHODS

2.1. Materials

Seventeen honey samples and 6 cane sugar samples were used. Three of the honey samples were singled out from consumers complaints: the samples were bought at a local market in some tropical countries and had an abnormal taste (samples H1 to H3).

Six samples were normal trade honeys (H4 to H7, H12, H13), two were rejected trade honeys (H8, H10) and five were bought by food inspectors in supermarkets (H9, H11, H14-H16). Cane sugar samples were obtained from the sugar collection at the Food Inspection Service Laboratory and from normal consumer packages. Special attention was paid to so called sugar cane honey, obtained by bees from cut or burnt sugar cane stems. Sample H17 was obtained from a company in Venezuela and, according to the producer, partly derived from sugar cane stems.

2.2. Methods of analysis

2.2.1. $\delta^{13}C$-method

$\delta^{13}C$ analysis was carried out at the Centre for Isotope Research in Groningen following AOAC procedures. Older samples were analyzed using method 978.17, that is by measuring solely the $\delta^{13}C$ value of the honey. In 1998, after publication of the validation studies of the internal standard procedure [14], this method – AOAC procedure 991.41 – was followed [1].

About 1 mg honey or protein, obtained from a honey solution by precipitation with tungstic acid sodium salt and sulfuric acid, was burnt in an Elemental Analyser (model 1500 CN; Carlo Erba, Milan, Italy) and the gasses were led into a continuous flow IRMS (Micromass Optima, Manchester, UK). Calibration was done with a reference gas and by burning a reference material (beet sugar) and both reference materials can be traced back to the international VPDB scale. Measurements of the $\delta^{13}C$ value of the samples H8 and H10 were carried out elsewhere.

Calculation of the percentage adulteration for the 12 samples, of which only the $\delta^{13}C$ value of the honey was known, was done by using –22.5 and –23.5‰ as limit value for honey and by using –10‰ for C_4 sugars as mean value. This latter number is based on a value of –9.7‰ for high fructose corn syrup [9, 10], and on a value of about –11‰ for cane sugar [2 and CIO unpublished results]. For the other 5 samples, of which the $\delta^{13}C$ value of honey and protein was known, calculation was done according to AOAC-procedure 991.41 [1]. Adulteration is expressed as percentage added C_4 sugar (corn or cane) on a weight basis.

2.2.2. Microscopic method

Microscopic analysis of honey was performed [5] at the Laboratory of the Food Inspection Service in Amsterdam with a BH microscope (Olympus, Tokyo, Japan) with 2 polarization filters and a first order red retardation plate inserted in the light path. Ten g of honey (or cane sugar) were dissolved in 20 ml water and centrifuged for 10 min at 1 350 *g* (ca. 2 500 rpm). The residue was taken up in 10 ml water, centrifuged again for 10 min at the same acceleration and taken up in 100 µl water. For quantitative analysis, 10 µl of the homogenized suspension was brought with a micropipette onto a 1.0×1.0 cm^2 square on the microscopic slide and spread out. The square was drawn on the slide with a waterproof ink marker. The suspension on the slide was dried at 40 °C and covered with glycerin jelly colored with basic fuchsin. The remaining 90 µl of the suspension was brought quantitatively onto a 1.5×1.5 cm^2 square, spread out, dried and taken up in uncolored glycerin jelly, following to the same procedure. Depending on the number of sugar cane cell elements, counting was done either on the 1.0×1.0 cm^2 square or the 1.5×1.5 cm^2 square.

Sugar cane rings and epidermal cells were identified and counted using crossed polars and a first order red retardation plate at a magnification of 400×. In this way, rings and epidermal cells were identified very easily by their bright yellow and blue polarization colors. Rings usually had a diameter of 20–50 µm, but rings with a diameter as small as 5 µm were also observed. Epidermal

cells had undulating cell walls alternating with small somewhat round, transverse cells or stomata.

Sugar cane parenchyma cells and sclereids were identified and counted by using crossed polars and a rather wide open iris diaphragm. The first order red retardation plate had to be rotated until the background of the microscopic field of view was nearly black. This procedure may not be possible for some types of microscopes and, in such cases, crossed polars may be used without the first order red plate. In this way, the parenchyma cells show slightly blue or yellow interfering colors (or white in the case of only crossed polars) with characteristic small pits with a diameter of 2–4 µm. Each pit has a polarization cross or a single beam with bright surroundings. Because they were both characterized by their pits, the numbers of parenchyma cells and sclereids were counted together. The numbers of part of rings, of complexes or parts of epidermal cells, and of parenchyma cells and sclereids were expressed per 10 g honey.

The repeatability (r) for physico-chemical analyses is defined as the degree of agreement between successive results obtained with identical test material under the same conditions (same operator, same equipment, same laboratory, at the same time or after a brief interval). A measure of the repeatability is the standard deviation (SD) of the results. The repeatability was calculated as $r = 2.8 \times SD$ with a reliability of 95% [8]. The repeatability of the analysis of the sugar cane cells was performed by carrying out the processing and microscopic examination of 8 sub-samples of 10 g from honey sample H9.

3. RESULTS AND DISCUSSION

3.1. δ^{13}C method

Nine honey samples were found adulterated with either corn or cane sugars according to the δ^{13}C methods (samples H1 to H6 and H8 to H10; Tab. I). For 6 honey samples (H1 to H6), they were adulterated independent of the chosen δ^{13}C limit value (–22.5 or –23.5‰). Two more samples (H7 and H11) were suspected of adulteration, but they could not conclusively be considered as such using only the δ^{13}C value of honey with its 20% adulteration limit. When δ^{13}C of honey protein was also measured and used as an internal standard, the detection limit of adulteration was lowered to 7% adulteration and, among the 5 honey samples analyzed by this method, 3 were found adulterated (H8 to H10; Tab. I).

3.2. Microscopic method

All 6 samples of cane sugar contained large numbers of plant cells, regardless of their color and origin (Tab. II). Parenchyma cells and sclereids were the most abundant (range 2 658 to 33 418 per 10 g), followed by ring elements (100 to 1 225 per 10 g) and epidermal cells (60 to 420 per 10 g) and these values are in agreement with those reported previously [4]. It is noteworthy that there was no significant rank correlation between the number of elements in each of these 3 classes in these 6 samples ($P \geq 0.067$; highest value Spearman rank correlation $r_s = 0.771$ between the first two most abundant classes of elements), which may result from different extraction and refining processes.

All 11 samples with confirmed or suspected adulteration based on δ^{13}C (samples H1 to H11) also contained large numbers of sugar cane plant cells (Tab. I). For these samples, there was a significant correlation between the recorded number of parenchyma cells and sclereids and the percentage adulteration based on either the –22.5‰ or the –23.5‰ limit values ($r \geq 0.751$, $n = 11$, $P \leq 0.008$). The correlation was also nearly significant for the number of epidermal cells ($r \geq 0.588$, $n = 11$, $P \leq 0.057$), but not for the number of rings ($r \geq 0.496$, $n = 11$, $P \leq 0.0121$). Over all 17 samples analyzed,

Table I. Results of $\delta^{13}C$ and microscopic analyses for 17 honey samples.

Sample, number & year of analysis	Origin & nominal floral source[1]	$\delta^{13}C$ analysis: $\delta^{13}C$ honey ‰	$\delta^{13}C$ analysis: $\delta^{13}C$ protein ‰	Adulteration[2] %	Microscopic analysis: Parenchyma cells + sclereids (number in 10 g honey)	Microscopic analysis: Rings (number in 10 g honey)	Microscopic analysis: Epidermal cells (number in 10 g honey)
H1/94	Nepal	–11.04		92–92	1 328	32	8
H2/94	Nepal	–11.26		90–91	10 796	36	326
H3/98	Kenya	–12.44		80–82	7830	220	50
H4/96	China (acacia)	–18.29		34–39	707	20	9
H5/95	China (acacia)	–19.05		28–33	2 278	35	30
H6/96	China (acacia)	–20.92		13–19	420	12	0
H7/96	China (acacia)	–22.29		2–9	1 070	18	2
H8/99	China	–23.20	–24.90	11	276	28	6
H9/98	China	–21.99	–23.34	10	562	30	10
H10/99	China	–23.70	–24.70	7	176	34	8
H11/95	China (acacia)	–22.32		1–9	194	9	1
H12/98	China	–22.87	–23.80	nd[3] (<7)	398	26	6
H13/96	China (acacia)	–23.34		nd (0–1)	202	10	2
H14/96	China (rape)	–23.94		nd (0–0)	50	20	0
H15/96	China	–25.58		nd (0–0)	5	5	0
H16/95	Hungary (acacia)	–23.35		nd (0–1)	0	0	0
H17/98	Brazil ('sugar cane honey')	–21.45	–21.96	nd (<7)	0	0	0

[1] Acacia is black locust *Robinia pseudoacacia* L.; rape is *Brassica napus* L.
[2] For 12 samples for which only the $\delta^{13}C$ value of the honey was known, calculation of the percentage adulteration was done by assuming that the unadulterated honey had a $\delta^{13}C$ value of –22.5‰ (first figure) and –23.5‰ (second figure) and the limit value for cane sugar was –10‰. For 5 samples for which the $\delta^{13}C$ values of both honey and honey protein were known, the percentage adulteration was calculated as in AOAC procedure 991.41 [1].
[3] nd = not detectable.

Table II. Results of the microscopic analyses of 6 cane sugar samples.

Sample number and year of analysis	Type and origin	Microscopic analysis (elements per 10 g): Parenchyma cells + sclereids	Microscopic analysis (elements per 10 g): Rings	Microscopic analysis (elements per 10 g): Epidermal cells
S1/96	brown	33 418	560	420
S2/96	raw	21 863	1 225	100
S3/96	brown, Nigeria	5 525	475	275
S4/94	brown	4 082	168	157
S5/94	white, Nepal	3 689	100	60
S6/94	brown	2 658	170	112

there was a significant correlation between the $\delta^{13}C$ value of honey and the number of parenchyma cells and sclereids (r = 0.776, $P = 0.0002$), the number of epidermal cell (r = 0.601, $P = 0.011$) and the number of ring elements (r = 0.544, $P = 0.024$).

Four other samples (H12-H15), which could not proven to be adulterated with the $\delta^{13}C$ methods, contained sugar cane plant cells and could be therefore suspected of adulteration.

Except for samples H16 and H17, the honey samples from Table I were adulterated or suspected of adulteration and contain sugar cane plant cells. It should be stressed that upon investigation of thousands of authentic honey samples from all over the world, including China, by the Food Inspection Service through the years, no sugar cane plant cells were found. H16 (black locust honey from Hungary) is representative of such samples. As for sample H17, which was partly derived from sap collected by honey bees on the stems of sugar cane according to the producer and confirmed by $\delta^{13}C$ analysis, it is noteworthy that it did not contain sugar cane plant cells. Similar lack of sugar cane plant cells were observed with other honeys derived from sugar cane from Cuba, Madeira and Tobago (Food Inspection Service, unpublished data). This suggests that the occurrence of false positive samples with the microscopic method may be very rare.

Up to now, it was not known if adulteration of honeys with C_4 sugars was done from corn or sugar cane, but microscopic analysis seems to indicate that cane sugar syrup is used. From our results, honey samples with more than 150 parenchyma cells and sclereids and/or more than 10 rings in 10 g may be suspected of adulteration. Honey samples with lower number of these cells may be suspected as well, but they may not be proven to be adulterated using the latest $\delta^{13}C$ method with honey protein as internal standard due to its detection threshold of 7% adulteration. This suggests that the microscopic method may be more sensitive than the $\delta^{13}C$ method as suggested by the large number of cane sugar plant cells in cane sugar samples (Tab. II). Even assuming a low value of 3 000 parenchyma cells and sclereids in 10 g for cane sugar, a 1% w/w adulteration of honey with cane sugar could easily be detected as it would result in 30 parenchyma cells and sclereids per 10 g in the adulterated product. These results indicate that the microscopic method could be used reliably as a screening method for the detection of cane sugar products in honey. It should be stressed that these results are based on the isolation of pollen and plant cells from a honey solution without acetolysis. If the acetolysis procedure is used for pollen analysis, then the sugar cane plant cells may be destroyed as their cell wall consist mainly of cellulose.

3.3. Validation of microscopic counts

There was a wide range in the counted numbers because the particles to be counted are not as discrete as pollen grains. Upon counting of parenchyma cells especially, small and large fragments are counted and this resulted in a repeatability of only 41% (Tab. III). With the same extraction method, counting the absolute amount of pollen in a sample Nigerian honey with about 10 000 pollen grains in 10 g gave the repeatability figure of 25% (unpublished data). For rings and epidermal cells, the repeatability was lower due to the smaller numbers of these particles.

4. CONCLUSION

All honey samples which contained large numbers of sugar cane plant cells were suspected of adulteration according to the $\delta^{13}C$ methods, with a correlation coefficient of 0.776 between the $\delta^{13}C$ value and the number of parenchyma cells and sclereids, and 0.60 between the $\delta^{13}C$ value and the number of epidermal cells. The microscopic method cannot be used to detect adulteration with sugars derived from corn, but it was useful for screening honey samples for C_4 sugars derived from sugar cane and, upon routine microscopic analysis of honey, special

Table III. Determination of the repeatability of the microscopic method based on 8 repeated analyses from honey sample H9.

Parameter	Parenchyma cells + sclereids	Rings	Epidermal cells
	Elements per 10 g		
Minimum	467	19	6
Maximum	710	37	11
Mean	562	30	10
SD	83	5.9	2.8
Repeatability (r = 2.8 SD)	232	16.5	7.8
Repeatability in % of mean	41	55	77

attention should be paid for the presence of these sugar cane plant cell elements. Whenever such elements are found, the honey may be suspected of adulteration with cane sugar since no false positive sample was found, not even with honey harvested in areas where honey bees had access to sugar cane stems. The microscopic method appears very sensitive as calculation based on the cell content of pure cane sugar suggest that down to 1% adulteration could be normally detected. As the present standard method for adulteration of honey, the $\delta^{13}C$ internal standard method, can only detect adulteration of 7% or more, a honey sample cannot be rejected upon finding only a few sugar plant cell elements. The results from this study indicate that when more than 150 parenchyma cells and sclereids and/or more than 10 rings are found in 10 g honey, an adulteration of more than 7% may be expected. Further work is needed with cane sugar and experimentally adulterated honeys to set more definite limits for plant cell element figures.

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Résumé – Falsification du miel : relation entre l'analyse microscopique et les mesures de $\delta^{13}C$. La méthode officielle pour détecter la falsification du miel par des sucres provenant de plantes en C_4, telles que la canne à sucre ou le maïs, est la méthode du $\delta^{13}C$ de l'AOAC [1]. Pour des échantillons de miel non falsifiés, les valeurs de $\delta^{13}C$ sont comprises entre –22,5 et –27,4 ‰ (valeur moyenne –25,4). Des échantillons avec une valeur moins négative que la valeur limite de 2 fois l'écart-type (2s), –23,5 ‰, sont considérés comme falsifiés [9, 10]. Récemment la protéine du miel a été utilisée comme standard interne [13] et cette amélioration permet de détecter jusqu'à l'addition de 7 % de sucres de maïs ou de canne. Des éléments cellulaires caractéristiques provenant des fibres de canne à sucre, tels que le parenchyme ou les cellules scléreuses, se trouvent dans le sucre de canne et ces éléments peuvent être retrouvés par analyse microscopique dans le miel falsifié avec ce sucre [5]. Nous avons comparé les méthodes au $\delta^{13}C$ et l'analyse microscopique et déterminé si elles donnaient des résultats cohérents.

Dix-sept échantillons ont été analysés par les deux méthodes et six échantillons supplémentaires de sucre de canne analysés au microscope. L'analyse au $\delta^{13}C$ a été faite selon la méthode de l'AOAC. L'analyse

microscopique a été réalisée sur des échantillons de 10 g (miel ou sucre). Des anneaux de canne à sucre provenant des vaisseaux annelés et des cellules épidermiques ont été identifiées et comptées à l'aide de polariseurs croisés et d'une lame à retard de phase dans le rouge au grossissement de 400×. Des cellules du parenchyme de canne à sucre et des scléréides ont été identifiées et comptées à l'aide de polariseurs croisés et d'un diaphragme à large ouverture. La répétabilité du procédé de comptage a été faite en traitant et analysant huit sous-échantillons d'un unique échantillon de miel.

La méthode au δ^{13}C a détecté six miels falsifiés avec des sucres de maïs ou de canne (H1 à H6, Tab. I) et deux miels ont été soupçonnés de falsification (H7, H11) ; la méthode au δ^{13}C avec la protéine de miel comme norme interne a détecté trois miels falsifiés (H8-H10, Tab. I). Les six échantillons de sucre de canne contenaient un grand nombre d'éléments cellulaires végétaux, quelle que soit la couleur et l'origine du sucre (Tab. II). Les 11 échantillons falsifiés, ou soupçonnés de l'être d'après l'analyse au δ^{13}C (H1 à H11), renfermaient aussi une grande quantité de cellules de canne à sucre (Tab. I) et il y avait une corrélation positive entre le nombre de cellules du parenchyme et de scléréides comptées et le pourcentage de falsification sur la base de la valeur limite de 2s, soit–23,5 ‰, ($r = 0{,}756$, $n = 11$, $P = 0{,}0071$). Pour l'ensemble des 17 échantillons de miel, il y avait une corrélation significative entre la valeur de δ^{13}C du miel et le nombre de cellules de parenchyme ou de scléréides ($r = 0{,}776$, $P = 0{,}0002$).

Quatre autres échantillons (H12-H15) contenaient des cellules de canne à sucre et pouvaient donc être suspectées de falsification, bien que les méthodes au δ^{13}C ne l'aient pas prouvé. Un échantillon de miel (H17), qui était partiellement issu de sève récoltée par les abeilles sur les tiges de canne à sucre, ne contenait pas de cellules de cette plante ce qui suggère que la présence d'échantillons faussement positifs peut être très rare avec la méthode microscopique. La répétabilité du comptage des cellules de parenchymes et des scléréides sous le microscope n'a été que de 41 % (Tab. III) parce que, contrairement aux grains de pollen qui ont une taille bien définie, les cellules du parenchyme peuvent être soit grandes soit petites ou comportent des éléments grands et petits.

La méthode microscopique peut être utile pour rechercher dans des échantillons de miel la falsification par des sucres de canne à sucre. Lorsque l'on trouve plus de 150 cellules de parenchymes et de scléréides et/ou 10 éléments d'anneaux, la méthode au δ^{13}C ne peut pas être utilisée pour confirmer la falsification. Néanmoins la méthode microscopique ne peut être utilisée pour détection des fraudes avec des sucres issus de maïs.

miel / falsification / analyse microscopique / sucre de canne / δ^{13}C

Zusammenfassung – Verfälschung von Honig: Vergleich von mikroskopischer Analyse und δ^{13} C Messungen. Die offizielle Methode zum Nachweis von Honigverfälschungen mit Zuckern, die aus C_4 Pflanzen wie Zuckerrohr oder Mais stammen, ist die δ^{13} C Methode. Bei unverfälschten Honigen liegen die Werte von δ^{13} C zwischen –22,5 und –27,4 ‰ (Mittelwert –25,4 ‰). Proben mit Werten, die weniger negativ sind als der 2s -Grenzwert (–23,5 ‰) werden als verfälscht gewertet [9, 10]. In letzter Zeit wurde der Proteingehalt des Honigs als interner Standard [13] genutzt und auβerdem ist es mit einer verbesserten Methode möglich, den Zusatz von Mais- oder Rohrzuckern bis zur Konzentration von 7 % nachzuweisen. In Zucker aus Zuckerrohr werden charakteristische Zellreste der Fasern wie Parenchym- oder Sklerenchymzellen gefunden. Diese Elemente können auch bei einer mikroskopischen Untersuchung von mit Rohrzucker verfälschten Honigen nachgewiesen werden [5]. Deshalb wurden die δ^{13} C Methode und die mikroskopische Analyse miteinander

verglichen und bestimmt, ob die Messungen übereinstimmen.

Siebzehn Honigproben wurden mit beiden Methoden analysiert und 6 Proben von Rohrzucker wurden zusätzlich mikroskopisch untersucht. Die δ^{13} C Methode wurde nach der Beschreibung der AOAC [1] durchgeführt. Die mikroskopische Analyse erfolgte mit 10 g Honig bzw. Zucker. Die Ringe der Ringgefäβe und die Epidermalzellen wurden identifiziert und mit Hilfe von Kreuzpolaren und einem Rotfilter erster Ordnung bei einer 400 fachen Vergröβerung gezählt. Die Parenchymzellen und Zellwandteile wurden mit Kreuzpolaren und einem weit geöffneten Irisblende identifiziert und gezählt. Die Zuverlässigkeit der Zählungen wurde durch die jeweilige Bearbeitung und mikroskopische Analyse von 8 Einzelproben einer einzelnen Honigprobe gewährleistet.

Bei 6 Honigproben wurde eine Verfälschung mit Mais oder Zuckerrohr mit der δ^{13} C Methode nachgewiesen (H1-H6, Tab. I), bei 2 Proben bestand der Verdacht der Verfälschung (H7, H11) und 3 weitere Proben, bei der die δ^{13} C Methode mit Honigprotein als internem Standard benutzt wurde, erwiesen sich als verfälscht (H8-H10, Tab. I). Alle 6 Proben von Zuckerrohrzucker enthielten groβe Mengen von Pflanzenzellresten, unabhängig von Farbe oder Ursprung des Zuckers (Tab. II). Die 11 Honige, bei denen eine Verfälschung nachgewiesen bzw. vermutet wurden, enthielten eine groβe Anzahl von Zellresten (Tab. I) und es ergab sich eine positive Korrelation zwischen der Anzahl der Parenchym- und Sklerenchymzellen und dem Prozentsatz der Verfälschung, der mit dem 23,5 ‰ 2s – Grenzwert bestimmt wurde (r = 0,756, n = 11, P = 0,0071). Bei allen 17 Proben fand sich eine signifikante Korrelation zwischen dem δ^{13} C Wert des Honigs und der Anzahl an Parenchym- und Sklerenchymzellen (r = 0,776, P = 0,002).

Vier weitere Proben (H12-H15), die nach der δ^{13} C Methode als nicht verfälscht eingestuft wurden, enthielten Zellen der Zuckerrohrpflanze und es besteht daher der Verdacht auf eine Verfälschung. Eine Honigprobe (H17), die zum Teil vom Pflanzensaft stammte, der von den Honigbienen von den Stengeln gesammelt wurde, enthielt keine Zellreste. Das bedeutet, dass mit der mikroskopischen Methode sehr selten irrtümlich positive Proben auftreten könnten. Die Wiederholbarkeit bei der Zählung der Zellen vom Parenchym und Sklerechym unter dem Mikroskop betrug nur 41 % (Tab. III). Anders als Pollenkörner, die eine recht gut definierte Gröβe haben, bestehen besonders die Parenchymzellen sowohl aus groβen wie aus kleinen Fragmenten.

Die mikroskopische Methode kann für eine Reihenuntersuchung von Honigproben auf Verfälschung mit Zucker des Zuckerrohrs sehr nützlich sein. Wenn mehr als 150 Zellreste und/oder 10 Ringelemente gefunden werden, sollte die δ^{13} C Analyse durchgeführt werden, um die Verfälschung zu bestätigen. Leider kann die mikroskopische Analyse nicht zum Nachweis für eine Verfälschung mit Zuckern benutzt werden, die von Mais gewonnen wurden.

Honig / Verfälschung / Mikroskopie / Rohrzucker / δ^{13} C

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