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Original article

Microscopic detection of adulteration of honey with cane sugar and cane sugar products

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Summary — A microscopic procedure is described to detect adulteration of honey with cane sugar, acid-hydrolized cane sugar syrup or with 'honey' obtained from feeding sugar to bees. The method consists of preparing a microscope slide of the honey sample and taking up the sediment in glycerin jelly in the same way as in classical pollen analysis. Microscopic analysis is preferably done by polarization microscopy using crossed polars and a first-order red retardation plate. Adulteration of honey with white or brown cane sugar and syrups derived from cane sugar is shown by the presence of many parenchyma and sclereid cells, single rings from ring vessels and epidermis cells. These cells are very characteristic and originate from the sugar cane stem. Even a low percentage of cane sugar (products) may be detected in this way. Analysis of 10 selected samples of highly adulterated honey from the Philippines and Nepal is described. Upon further study it turned out that no false-positive or false-negative results were obtained. Sugar cane honeys did not contain sugar cane plant cells and so no false positives were observed, even in sugar cane honey.

honey / adulteration / cane sugar / microscopy

INTRODUCTION

Adulteration of honey is a well-known problem and many methods of analysis are available to detect falsification with various types of sugars and with inexpensive sugar syrups. In some tropical countries, honey for sale on the local market may be adulterated by directly adding crystallized cane sugar, cane sugar syrup, invert sugar syrup obtained by heating slightly acidified cane sugar, or 'honey' obtained from feeding sugar to bees. By carrying out the usual chemical determinations, such as the glucose, fructose, sucrose and hydroxymethylfurfural (HMF) content and the diastase index, these adulterations of honey can easily be detected (Codex, 1989; White, 1979).

However, in many developing countries a laboratory for the above-mentioned routine analyses is not always available. However, by simple microscopic analysis it is possible to detect adulteration of honey with cane sugar and products derived from cane sugar. Cane sugar has a built-in indicator of origin; it contains many characteristic particles, originating from the sugar cane stem: parenchyma, sclereid and epidermis cells, single rings of ring vessels and sugar cane starch (Kerkvliet, 1982). These particles are present in raw (brown) cane sugar, refined white cane sugar and dark brown (B-type) molasses cane sugars from all over the world, but not in beet sugar, maple sugar and Indonesian palmtree sugar.

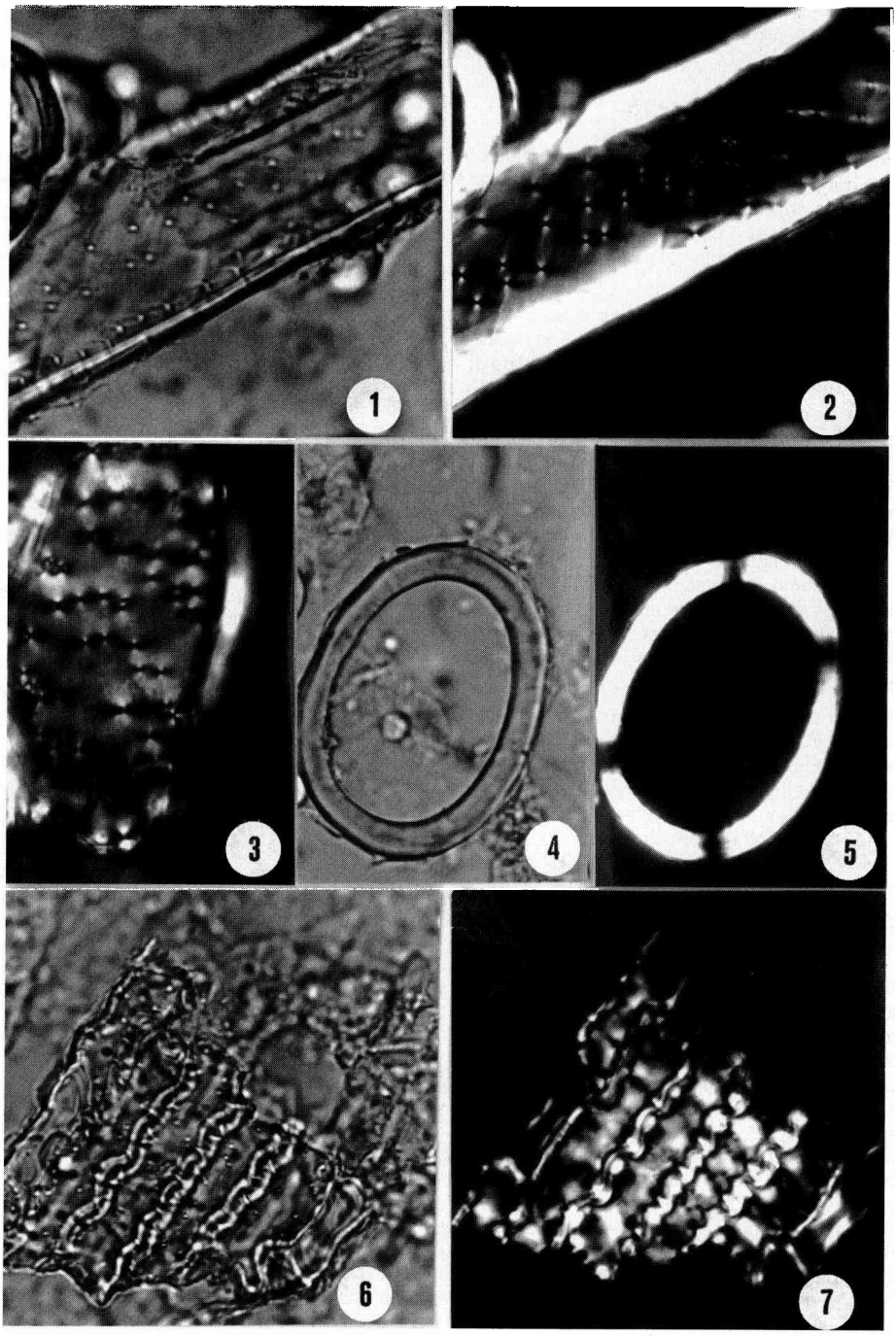
In the literature no specific method for the microscopic analysis of honey of cane sugar particles is described. Some authors mention the presence of other structured particles besides pollen in the honey sediment, but detailed identification of vegetable cells is not given (*eg,* Evenius and Focke, 1967; Sancho *et al,* 1991).

By centrifuging a 1:2 honey/water solution, as used in classical pollen analysis, in cases where cane sugar and cane sugar products are used for adulteration, the residue obtained contains the characteristic sugar cane plant cells in addition to pollen, honeydew elements and oxalate crystals. After mounting the water washed residue in glycerin jelly, microscopic analysis is carried out. Using crossed polars and a first-order red retardation plate, the presence of cane sugar is immediately revealed by a bright lightening of the characteristic sugar cane plant cells. Typical forms are pictured in figures 1–7. The acetolysis method (Louveaux *et al,* 1978), a well-known procedure in melissopalynology in which the pollen sediment is acetolysed by acetic anhydride/sulphuric acid mixture to obtain clear pollen cell walls, cannot be used for detection of these plant cells because of destruction of the cells during the procedure.

Polarization microscopy is especially useful for this type of analysis. Between crossed nicols, vegetable cells with cell walls consisting mainly of cellulose show first-order white interference colours on a black background. Many crystals and native starch grains are also visible. However pollen, yeasts and fungal spores are not optically active and hence cannot be seen between crossed nicols. By inserting a first-order red retardation plate (red I plate), the black background changes to to wine-red. Pollen, yeasts and fungal spores can be distinguished but are not lit up. However the optically active plant cells (and crystals and native starch) do give second-order bright blue (red I plus white I) or first-order yellow (red I minus white I) interference colours, depending on their orientation in the microscopic field (Czaja, 1971).

In this study the results of the microscopic analyses for sugar cane fragments are compared with the glucose, fructose and sucrose content of the honey and with the HMF values. In some (sub)tropical countries bees forage on sap exuding from cut or burnt sugar cane (*Saccharum officinarum*) stems, therefore, microscopic analysis of some sugar cane honeys was also included in this study.

Fig 1. Sclereid cells of cane sugar in honey, normal light, 750 x. **Fig 2.** Sclereid cells of cane sugar in honey, crossed nicols, 750 x. **Fig 3.** Parenchyma cells of cane sugar in honey, crossed nicols, 750 x. **Fig 4.** Single ring of cane sugar in honey, normal light, 750 x. **Fig 5.** Single ring of cane sugar in honey, crossed nicols, 750 x. **Fig 6.** Epidermis cells of cane sugar in honey, normal light, 750 x. **Fig 7.** Epidermis cells of cane sugar in honey, crossed nicols, 750 x. Note: The photographs in figures 2, 3, 5 and 7 are taken with crossed nicols to obtain good black/white prints. However in practical work an additional red I plate is recommended to obtain the described interference colours.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MATERIALS AND METHODS

Samples

Honey samples were obtained from the Philippines during a survey carried out in 1987, in the course of a development project. Two highly suspected samples, bought on the local market, were analysed at the Food Inspection Service (Haarlem), The Netherlands.

The other 8 honey samples in this study originated from Nepal (Kathmandu, Terai). They were encountered during chemical and microscopic routine analyses at the laboratories of BETRESP, a joint development project set up by His Majesty's Government of Nepal and The Netherlands Development Organization with the aims of training beekeepers and improving honey yield and honey quality. By analyzing more than 300 honey samples, BETRESP has laid the basis for establishing a national honey standard in Nepal. Because some of the samples contained sugar cane fragments they were also analyzed at the Regional Inspectorate for Health Protection Food Inspection Service laboratories, Amsterdam, The Netherlands.

Cane sugar samples were bought in local shops in Kathmandu, Nepal. Pure sugar cane honey samples were obtained from the honey collection at the Food Inspection Service, Amsterdam, The Netherlands. One sample originated from Cuba, the other from Madeira.

Methods of analysis

Microscopic analyses were carried out according to the methods published by the International Commission for Bee Botany (Louveaux *et al,* 1978) by dissolving 10 g honey (or cane sugar) in 20 ml water, centrifuging, washing out the residue with water, centrifuging again and taking up the residue in 100 µl water. For quantitative analysis, a 10 µl suspension was placed on 1 cm^2 of the microscopic slide, dried and taken up in glycerin jelly. Cane sugar particles were counted by using crossed polars and a first-order red retardation plate at a magnification of 400 x. The remaining part of the suspension was placed on a second slide, dried and also taken up in glycerin jelly. This slide was used for qualitative identification and, if necessary, for low counts in quantitative work using the same microscopic technique.

Parenchyma cells of cane sugar are roughly rectangular with various dimensions; the length of a cell is mostly 50–100 µm, the width approximately 40–60 µm. More round forms are also present. Sclereid cells (stone cells) are rectangular, length approximately 100–200 µm, width 40–50 µm. Both type of cells possess optically active cell walls with blue (orientation north-east/south-west) and yellow (orientation north-west/south-east) polarization colours. The surface of the parenchyma and the sclereid cells is characterized by many small pits with a diameter of about 2–3 µm. Each pit has a polarization cross or a single beam with optically active surroundings (yellow and blue) (figs 1–3).

Because they are characterized by their pits, the number of parenchyma and sclereid cells were counted together for quantitative work and expressed as the total number in 10 g honey. Clusters and parts of cells were each counted as one cell.

Single rings of ring vessels of cane sugar are also very characteristic. Their diameter is about 30–50 µm, and the thickness of the rings is 4–5 µm (fig 4). They show blue and yellow polarization colours in a single ring; the colours are interchanged by small red (fig 5: black) bands. Single rings were counted and expressed as the total number in 10 g honey.

Epidermis cells of cane sugar are characterized by their undulatory long cell walls; dimensions of one cell are about 15 µm by more than 150 µm. The long cell walls show bright blue polarization colours in the orientation north-east/south-west. The colour between the blue cell walls is bright yellow. In the other direction (north-west/south-east) the polarization colours are reversed (figs 6 and 7). Epidermis cells are mostly present in the honey residue as clusters. Single cells and clusters are counted and expressed as the total number in 10 g honey.

HMF was determined by the high pressure liquid chromatography (HPLC) technique (Jeuring, 1980); sugar determinations (glucose, fructose and sucrose content) were also carried out by an HPLC method. pH and electrical conductivity were measured in a 20% (m/m) honey (or cane sugar) solution in distilled water (Vorwohl, 1964), the electrical conductivity is expressed in µSiemens/cm on the honey as such (not on dry matter). Water content was determined by the refractometer method.

RESULTS

The results of the chemical and microscopic analyses of 10 adulterated honey samples from the Philippines and Nepal are shown in tables I and II. These tables also give the results of the analyses of a typical crystallized normal quality Nepalese cane sugar along with 2 types of finely powdered minor quality molasses cane sugars.

The sugar cane honey samples were dark-brown or black with a taste reminiscent of cane sugar. Upon microscopic analysis no sugar cane plant cells were observed. The total number of pollen grains in sample R 128 had a normal value; most pollen were from a (wind-pollinated?) *Palmeae* species. Sample A 485 did not contain pollen, but did have lots of calcium oxalate crystals.

DISCUSSION

Honey samples

From the chemical data of all 10 honey samples, it is evident that they are wholly or partly adulterated or at least strongly heated. From the microscopic data it follows that the samples are adulterated. There is no direct correlation between the number of cane sugar fragments and sucrose content, but all 8 Nepalese samples do have a high number of cane sugar fragments and a large

Table I. Chemical characteristics of the honey and cane sugar samples

Sample	*Country of origin*	*Water (% m/m)*	*pH*	*Elect cond* [a] *(µS/cm)*	*Glucose (% m/m)*	*Fructose (% m/m)*	*Sucrose (% m/m)*	*HMF (mg/kg)*
Honey samples								
I 156	Philippines	26.2	4.99	438				1.9
I 157	Philippines	23.8	4.91	402	17.3	16.4	32.4	2.4
A 227	Terai,Nepal		3.90	550	32.2	29.0	17.9	401
A 228	Terai,Nepal		3.80	495	36.9	31.7	7.4	582
A 229	Terai,Nepal		3.90	518	36.5	33.6	4.9	283
A 230	Terai,Nepal		3.85	700	35.5	31.1	11.3	555
A 307	Nepal	22.5	3.20	670	41.3	32.4	0	1 110
A 386	Nepal		3.65	455	28.5	25.5	21.0	183
A 387	Nepal	17.2	3.35	540	39.4	37.2	0	1 258
A 412	Nepal		3.80	400	23.1	20.3	32.7	151
Sugar cane honey samples								
R 128	Cuba		3.84	505				
A 485	Madeira		4.75	5 990				< 40
Normal cane sugar sample								
A 368	cane sugar		4.95	116				
Molasse cane sugar samples								
A 370	cane sugar		5.45	2 160				
A 371	cane sugar		5.40	2 150				

[a] Electrical conductivity.

Table II. Microscopical characteristics of the honey and cane sugar samples.

Sample	Country of origin	Number in 10 g honey/sugar				Main pollen types/other microscopic characteristics
		Parenchyma + sclereid cells	Rings	Epidermis cells	Pollen	
Honey samples						
I 156	Philippines	1 665	454	2	1 800	*Cocos nucifera, Citrus, Tilia, Litchi chiensis;* wheat starch, corn starch,
I 157	Philippines	455	272	91	0	Wheat starch, corn starch
A 227	Terai, Nepal	2 558	426	512	0	Wheat starch, wheat hairs
A 228	Terai, Nepal	3 751	68	< 68	48 740	*Brassica napus, Compositeae, Castana sativa*
A 229	Terai, Nepal	7 845	1 025	340	677 000	*Brassica napus, Eupatorium, Castanea sativa, Acanthaceae*
A 230	Terai, Nepal	2 864	136	205	261	*Cupressaceae/Taxaceae, Castanea sativa*
A 307	Nepal	3 337	10	2	0	High amount of wheat starch
A 386	Nepal	4 691	< 10	114	14 074	*Brassica* sp, *Toona ciliata*
A 387	Nepal	1 892	10	20	0	
A 412	Nepal	564	8	4	72	*Cupressaceae/Taxaceae, Brassica* sp, *Compositeae, Eucalyptus* sp
Sugar cane honey samples						
R 128	Cuba	0	0	0	34 873	*Palmeae* (82%), *Mimosa* sp, *Compositeae,* some honeydew elements
A 485	Madeira	0	0	0	0	Many calcium oxalate crystals, some yeasts
Normal cane sugar samples						
A 368	cane sugar	5 902	6	6	0	Some fungi, some wheat starch
Molasse cane sugar samples						
A 370	cane sugar	46	4	12	0	Many sand particles
A 371	cane sugar	40	22	0	0	Many wheat starch grains, some rice starch, wheat bran

amount of HMF, an indication that they are adulterated with acid-hydrolyzed cane sugar syrup. This does not hold for the 2 Philippine honeys, which contain practically no HMF (and they have very low diastase numbers, not inserted in the table) and are probably adulterated by mixing honey directly with cane sugar.

The presence of many wheat starch grains along with sugar cane fragments in some of the honey samples may indicate that they are adulterated with minor quality molasse cane sugar syrup.

Microscopic analyses

The use of polarization microscopy (crossed nicols with first-order red retardation plate) makes the identification of cane sugar fragments very easy. In this way, using a total magnification of 100 x a whole slide can be

screened for parenchyma and sclereid cells, single rings and epidermis cells. Especially, the rings can be seen at the first glance. For proper identification of epidermis and especially parenchyma and sclereid cells, it is better to use a higher magnification (400 x) with the same microscopic technique or with crossed nicols only. In this way the characteristic pits in the parenchyma cell walls can easily be detected.

The mean value of parenchyma and sclereid cells in this 10 adulterated honey samples together with the standard deviation and the range is shown in table III.

The number of parenchyma and sclereid cells in a typical white cane sugar sample from Nepal is 5 902 in 10 g. In an earlier study (Kerkvliet, 1982), it was found that in various types of cane sugar originating from different countries, the number of single rings ranged between 76 and 595 in 10 g and the number of epidermis cells between 25 and 222 in 10 g. Parenchyma and sclereid cells are present in a far greater number, usually between 3 000 and 6 000 in 10 g. It was also found that cane sugars contained parenchyma, sclereid and epidermis cells and rings. From this observation it seems highly unlikely that false-negative results are obtained in screening honey samples for sugar cane fragments.

False positive results are highly unlikely. In the honey samples from beekeepers who are under supervision of the Nepalese BETRESP project, no sugar cane fragments were found upon microscopic analysis. Chemical analyses also showed normal values.

To make an estimation of the limit of detection of the presence of cane sugar in honey, a minimum of about 30 parenchyma and sclereid cells in 10 g of honey can easily be detected by screening the 1 cm^2 area of the microscopic slide of the sample. From this data and taking into account the abovementioned natural variation in the number of parenchyma and sclereid cells in cane sugar, it follows that even 1 or 2 percent cane sugar can be detected in a simple way by this method.

CONCLUSIONS

Microscopic screening of honey samples for sugar cane fragments is an additional method to detect even a minor addition to honey of cane sugar, inverted cane sugar syrup and 'honey' from cane sugar fed to bees. As the investigated sugar cane (*S officinarum*) honey samples did not contain the plant cells from sugar cane described in this

Table III. Mean number of parenchyma and sclereid cells, rings and epidermis cells along with the standard deviation and range in 10 adulterated honey samples.

Cell type	*Number in 10 g honey*		*Range*
	Mean	*Standard deviation*	
Parenchyma + sclereid cells	2 962	2 176	455–7 845
Rings	242	325	8–1 025
Epidermis cells	136	171	2–512

study we may conclude that the presence of sugar cane fragments shows that the honey sample is adulterated. By counting the sugar cane fragments an indication of the amount of cane sugar in the honey sample can be obtained. The method is especially usuful in the analysis of honey from developing countries and can be done with simple equipment. In combination with the HMF value the microscopic method may differentiate between heating and adulteration.

Résumé — Diagnostic en microscopie de la falsification du miel par du sucre de canne et des produits à base de sucre de canne. On décrit une méthode microscopique pour détecter les falsifications de miel avec du sucre de canne, du sirop de sucre de canne et du «miel» de sucre. La méthode a été mise au point pour des miels locaux provenant de régions (sub)tropicales. Le sucre de canne raffiné, le sucre de canne brut et les sirops de sucre inverti préparés avec du sucre de canne renferment toujours un grand nombre de cellules parenchymateuses des tiges de canne à sucre, des cellules scléreuses, des anneaux isolés des vaisseaux annelés et des cellules de l'épiderme. Ces particules sont absentes du sucre de betterave, du sucre d'érable et du sucre de palme. On ne les a pas trouvées non plus dans les 2 échantillons analysés de miel de canne à sucre (*Saccharum officinarum*) (tableau II). Ces cellules végétales caractéristiques ont été mises en évidence sur une préparation microscopique de miel réalisée selon la méthode classique (Louveaux *et al*, 1978). Le résidu est repris dans 100 µl d'eau. 10 µl de ce volume sont étalés sur une lame, le reste sur une seconde lame et les 2 lames sont séchées. Les résidus sont montés dans de la gélatine glycérinée de Kaiser et étudiés au microscope. L'étude a été faite en partie au microscope polarisant avec une plaque rouge I. Des cellules parenchymateuses, scléreuses, les anneaux et les cellules de l'épiderme ont été identifiés. Une analyse quantitative est également possible sur une préparation de 1 cm^2. Les cellules parenchymateuses, de forme généralement rectangulaire ou ronde, mesurent 50–100 µm de long et 40–60 µm de large. Les cellules scléreuses, rectangulaires, mesurent environ 100–200 µm de long et 40–50 µm de large. Les 2 types de cellules possèdent des parois optiquement actives avec les couleurs de polarisation bleue (orientation nord-est/ sud-ouest) et jaune (orientation nord-ouest/sud-est). La surface des cellules est garnie de nombreuses ponctuations (2–3 µm de diamètre), chaque ponctuation possédant une croix de polarisation ou une bande avec un entourage optiquement actif (jaune et bleu) (figs 1-3). Les anneaux des vaisseaux annelés ont 30–50 µm de diamètre, leurs parois une épaisseur de 4–5 µm. Les anneaux présentent des couleurs de polarisation bleue et jaune avec d'étroites bandes rouges (figs 4–5). Les cellules de l'épiderme, ±150 x 15 µm, possèdent de longues parois ondulées et présentent des couleurs de polarisation bleu clair et jaune (figs 6–7). Dans les 10 miels falsifiés analysés, provenant des Philippines et du Népal, on a trouvé entre 455 et 7 845 cellules parenchymateuses et scléreuses, de 8 à 1 025 anneaux et de 2 à 512 cellules de l'épiderme dans 10 g de miel (tableau II). Cette méthode permet de détecter des falsifications de miels locaux avec seulement 1 à 2% de sucre de canne (ou de produits à base de sucre de canne).

miel / falsification / sucre de canne / microscopie

Zusammenfassung — Mikroskopische Methode zum Nachweis von Verfälschungen von Honig mit Rohrzucker und Rohrzuckerprodukten. Es wird über eine mikroskopische Methode zum Nachweis von Verfälschungen des Honigs mit Rohrzucker, Rohrzuckersirup oder Rohrzucker-fütterungshonig berichtet. Die Methode

wurde zur Untersuchung von lokalen Honigen aus (sub)tropischen Gebieten entwickelt. Weisser und brauner Rohrzucker sowie aus Rohrzucker bereitete (Invert)Zuckersirupe enthalten immer eine grosse Anzahl Parenchymzellen des Zuckerrohrstengels, Sklereidzellen, einzelne Ringe von Ringgefässen und Epidermiszellen. Rübenzucker, Ahornzucker und Palmzucker besitzen derartige Bestandteile nicht; auch in zwei Proben von Zuckerrohrhonigen (*Saccharum officinarum*) wurde keines dieser Fragmente gefunden. Zum Nachweis dieser charakteristischen pflanzlichen Zellen wird ein mikroskopisches Präparat des Honigs nach der klassischen Methode hergestellt (auflösen von 10 g Honig in 20 ml Wasser, zentrifugieren, Rückstand mit Wasser auswaschen, zentrifugieren). Der ausgewaschener Rückstand wird in 100 µl Wasser aufgenommen, 10 µl werden auf 1 cm^2 des Objektglasses aufgetragen und getrocknet; das übrige Volumen wird auf ein zweites Objektglas aufgetragen und ebenfalls getrocknet. Beide Rückstände werden zur mikroskopischer Untersuchung in Glyceringelatine nach Kaiser aufgenommen. Zur Untersuchung erwies sich die Polarisationsmikroskopie mit der Rot I Platte als vorteilhaft. Identifiziert werden Parenchymzellen+Sklereidzellen, Ringe und Epidermiszellen; auch eine quantitative Auszählung auf dem 1 cm^2 Präparat ist möglich. Parenchymzellen sind meistens rechwinklig oder rund, 50–100 µm lang und 40–60 µm breit. Sklereidzellen sind rechtwinklig, ungefähr 100–200 µm lang und 40–50 µm breit. Beide Zelltypen besitzen optisch aktive Zellwände mit blauen (Lage nordost/südwest) und gelben (Lage nordwest/südost) Polarisationsfarben. Die Oberfläche der Zellen hat viele Tüpfel (2–3 µm Durchmesser) die ein Polarisationskreuz oder einen Balken mit optisch aktiver Umgebung (gelb und blau) aufweisen (Abb 1-3). Einzelne Ringe von Ringgefässen haben einen Durchmesser von 30-50 µm, die Dicke der Ringwände beträgt 4-5 µm. Die Ringe zeigen blaue und gelbe Polarisationsfarben mit schmalen roten Balken (Abb 4,5). Epidermiszellen haben Abmessungen von ±150 x ±15 µm und besitzen gewölbte lange Zellwände. Sie zeigen helle blaue und gelbe Polarisationsfarben (Abb 6,7). In 10 Proben von (teilweise) verfälschten Honigen von den Philippinen und aus Nepal wurden 455 bis 7845 Parenchymzellen + Sklereidzellen, 8 bis 1025 Ringe und 2 bis 512 Epidermiszellen pro 10 g Honig gefunden. Verfälschungen von lokale Honigen mit nur 1 oder 2 Prozenten Rohrzucker(produkten) sind mit dieser Methode nachweisbar.

Mikroskopie / Honig / Rohrzucker / Verfälschung

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