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Original article

Infrared spectroscopy in routine quality analysis of honey

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Abstract – Fourier-transform infrared spectroscopy (FT-IR) was used as a rapid, simple and reliable method for quality analysis of honey. More than 1600 samples of honey were analysed using FT-IR and reference methods to develop a partial least-square regression based calibration model for the major components of honey (sugars, proline, free acids, invertase, moisture, hydroxymethylfurfural, pH and electrical conductivity). The coefficient of determination R^2 ranging from 0.84–0.98 indicates an acceptable calibration for most of the parameters. Statistical verification of the spectral analysis in routine analysis showed a high correlation (0.81–0.99), good repeatability (0.84–0.99), no environmental influences ($P > 0.05$) and no significant statistical differences to the reference methods. This study shows that not only chemical composition but also the physical properties can be determined by FT-IR. The calibrations can be adapted to different analytical standards and honey sources.

Fourier-transform infrared spectroscopy / FT-IR / honey quality/ sugar analysis

1. INTRODUCTION

The natural source of honey means that there is a broad diversity of components to be analysed. The European Community fixed the limits for the quality criteria of commercial honey in the European honey directive (Council directive 74/409/EEC, 1974) and most of them were standardized by the German Institute of Norms (DIN 10751-10759). Almost all standard methods for determining the chemical composition and physical properties of honey are time-consuming and expensive. Consequently, only a limited fraction of honey produced is checked using these methods. To achieve a high quality standard for honey, it is necessary to develop a new method that is simple, fast and reliable.

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Infrared spectroscopy (Stuart, 1996) is used for analysing in various areas in the food industry (Kuchenbecker, 1997; Schindler et al., 1998) and for clinical applications (Ng and Simmons, 1999).

Other researchers (Cho and Hong, 1998; Ha et al., 1998; Quiu et al., 1999; García-Alvarez et al., 2000) have already shown that it is possible to analyse the main sugars and moisture in honey by using near-infrared spectroscopy (NIR). Quiu and coworkers failed to determine the free acids by NIR and published no results for the physical parameters such as electrical conductivity and pH. NIR measurements are performed at wavelengths close to the visible region (800–2500 nm). At higher wavelengths the absorption increases at a given concentration. Absorption bands become defined and more specific. This means that in the mid infrared region (2500–2.5 × 10^4 nm or 400–4000 cm^{-1}) more information about the ingredients of the samples can be collected and more components can be analysed. We used an FT-IR interferometer facility calibrated for analysing dairy products and wine that is available completely automated. We tried to develop new calibrations for the determination of the most important quality criteria of honey. The aim of our study was to find a new method that needs neither traditional sample preparation nor expensive or aggressive reagents so that it can be used for routine analysis.

2. MATERIALS AND METHODS

2.1. Honey samples

821 samples from 1999 and 886 from 2000 were mainly blossom honey and came from beekeepers in Germany. Using FT-IR, about 800 of these were analysed for calibration, the others for verification and routine analysis.

Depending on the parameter, about 1000 to 1700 of the samples were analysed using official methods as reference values for the calibration model or for verification of the FT-IR data in routine analysis.

2.2. Spectroscopic analysis

Milkoscan Ft 120 (Foss Electric, Hillerod, Denmark) uses an FT-IR interferometer in combination with a flow system. Standardisation of the instrument, filling in the honey sample and washing were performed automatically. 10 g of a honey sample was diluted prior to analysis with 50 ml S-6060 Zero Liquid (Foss Electric, Hillerod, Denmark) with the aid of a balance option of the Milkoscan Ft 120-software.

The FT-IR unit scans the infrared transmission spectrum from 926 to 5012 cm^{-1}. Two spectral scans were taken for each calibration sample. Figure 1 shows a plot of the entire FT-IR absorbance spectrum of a honey sample. The two water absorption areas (wave number 1543–1717 cm^{-1} and 2971–3627 cm^{-1}) were not used for filter selection because of the strong noise of the signal. In the next step of the calculation procedure the most reliable wave numbers (15–25) for each component in this sample set were selected usually between wave number 926 cm^{-1} and 2971 cm^{-1}. This is the characteristic absorption area of most of the functional groups in infrared spectroscopy.

The stability of the FT-IR determination was ensured by analysing a standard honey sample at regular intervals.

2.3. Reference analysis

Chemical analysis, physical properties and determination of the botanical origin using light microscopy was performed according to the German DIN-Norms (10751–10756,10758, 10759, Louveaux et al., 1970a, 1970b) and the European Honey Commission (Bogdanov et al., 1997). The sugar content was determined by HPLC, and moisture was measured

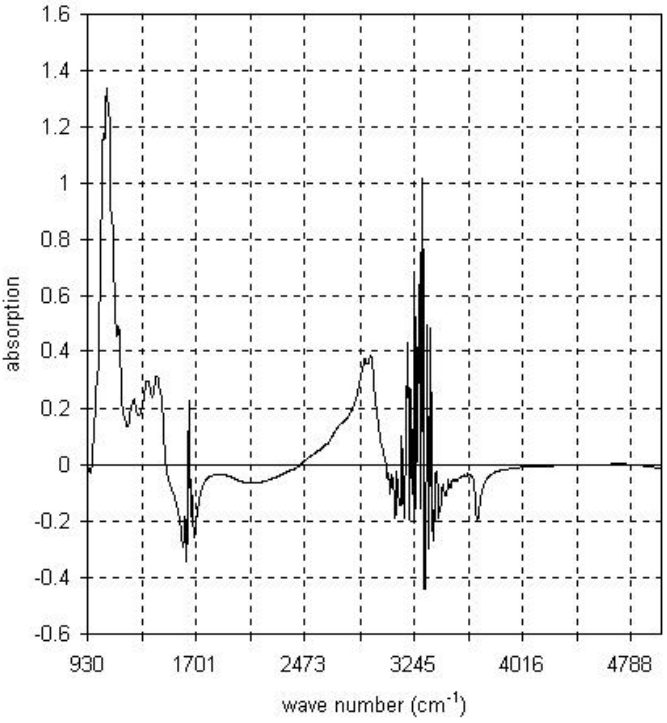

Figure 1. Absorbance spectrum of a honey sample. The two areas of water absorption (1543–1717 cm^{-1}, 2971–3627 cm^{-1}) were not used for calibration.

using a refractometer. The determination of the amount of hydroxymethylfurfural (HMF), proline and the invertase activity was performed using a spectrophotometer. The free acids were measured by equivalence point titration.

2.4. Data analysis

All calculations were carried out using the Application Module of the software package provided by the manufacturer (Foss Electric Ft 120 Software Version 2.1.3, 2000). Calibration was performed by assigning the assessed spectra to the reference values and selection of the most reliable wavelength ranges (appendix). For exact description of the calculation procedure see Milkoscan FT 120 reference manual (Foss Electric, 2000).

Since the absorption of infrared radiation is influenced by other compounds, partial least squares (PLS) regression is used for calibration which leads in this case to a more reliable calibration than the multiple linear regression (MLR).

After elimination of outliers, from the lowest cross validation error (CVE), the optimum number of factors was determined and the number of filters was reduced to a minimum to avoid an over fitting of the equations. The internal validation of the calibration was performed in 4 steps with 25% of the data not used for calibration.

Finally, the slope and intercept adjustment was performed during calibration procedure, which should often be repeated in order to ensure that the system is performing optimally.

Parts of routine analysis measured samples were examined by standard methods. For statistical evaluations we used SAS (1998) to determine the coefficient of correlation, error probability, reproducibility and possible influences of environmental conditions.

3. RESULTS AND DISCUSSION

3.1. Calibration

The optimal number of filters and PLS factors in the regression equation lead to the lowest CVE and the highest coefficient of determination (R^2). In Table I the statistical parameters of the calibration models for honey analysis are shown. 25% of the analysed samples (n) were used for internal validation of the calibration.

The FT-IR instrument was not only calibrated to determine the chemical composition of honey (main sugar components, moisture, free acidity, proline, HMF and invertase) but also indirect parameters such as physical properties e.g. electrical conductivity and the pH with significant results. We obtained R^2 values between 0.8 and 0.98. The low R^2 for maltose was the result of the inaccuracy of the reference method for this sugar and will be improved.

The measurements can be adapted to seasonal and laboratorial differences by inclusion of additional samples for adjusting the calibration parameters.

3.2. Verification

After calibration, the precision of the FT-IR analysis during routine use was verified. Each sample was measured twice:

Table I. Statistical data of FT-IR calibration (ft-120-software).

	n[a]	R^2 [b]	SEC[c]	bias[d]	CVE[e]	factors
fructose (%)	599	0.84	0.850	0.0010	0.890	9
glucose (%)	639	0.97	0.850	0.0007	0.910	9
sucrose (%)	599	0.94	0.550	0.0006	0.590	8
turanose (%)	663	0.91	0.200	–0.0002	0.220	12
maltose (%)	655	0.80	0.330	0.0000	0.370	8
trehalose (%)	653	0.91	0.075	0.0008	0.078	5
isomaltose (%)	702	0.92	0.077	–0.0004	0.077	9
erlose (%)	569	0.93	0.158	0.0017	0.165	8
moisture (%)	172	0.95	0.240	–0.0094	0.280	9
el. conductivity (mS/cm)	287	0.98	0.020	–0.0020	0.027	10
pH	217	0.90	0.190	0.0002	0.230	8
free acidity (mVal/cm)	54	0.98	1.340	0.0040	2.250	10
proline (mg/kg)	128	0.96	37.50	0.2700	62.40	17
invertase (U_G)	137	0.88	2.640	0.0067	3.990	16
HMF (mg/kg)	84	0.95	4.970	0.0014	6.260	12

[a] Number of analyses, [b] coefficient of determination, [c] standard error of calibration, [d] systematic error, [e] cross validation error.

using the official methods and FT-IR. The results are shown in Table II. Most of the parameters still have valuable correlation coefficients of more than 0.85 between the standard methods in honey analysis and the FT-IR determinations. The error probability of the correlation is highly significant ($P < 0.001$). Some of the data are presented in a scatter plot (Fig. 2).

3.2.1. Sugars

The results for fructose and sucrose were better than expected from the calibration model. For some of the sugars that usually occur at less than 2%, the analysis is still problematic due to small variations of the concentrations in the sample set used for calibration.

3.2.2. Proline and invertase activity

The amount of the amino acid proline and the activity of the enzyme invertase show a good correlation for most of the honey samples (r = 0.96 and 0.92) but there were some cases where the determination of these parameters failed. Maybe factors that have not yet been identified, such as colour, consistency, or type of honey influenced the IR spectrum. We solved a comparable problem for the analysis of free acidity. The best calibration for all honey samples regardless of their consistency was obtained by calibrating free acidity with only those which were nearly white (sensory testing) and crystallized.

3.2.3. Hydroxymethylfurfural

The analysis of HMF, an indicator for improper storage, is still problematic. Since about 96.5% of the HMF-concentrations determined by the reference method according to Winkler (1955) were below 15 mg/kg some of the honey samples were heated. In spite of the promising calibration

Table II. Comparison of the results of FT-IR and standard method, statistical data.

	Standard method			FT-IR			
	n	mean	SD[a]	mean	SD[a]	r[b]	*P*[c]
fructose	71	37.3	2.55	36.6	2.44	0.94	***
glucose	70	28.9	4.44	28.9	3.96	0.96	***
sucrose	72	3.02	3.61	3.29	3.82	0.99	***
turanose	72	1.50	0.51	1.73	0.51	0.85	***
maltose	70	2.16	0.51	1.94	0.63	0.76	***
trehalose	55	0.66	0.38	0.88	0.41	0.91	***
isomaltose	55	0.83	0.33	0.79	0.37	0.71	***
erlose	55	1.16	0.81	1.31	0.78	0.88	***
moisture	47	16.1	1.37	16.4	1.22	0.73	***
el. conduct.	77	0.34	0.19	0.34	0.18	0.95	***
pH	184	4.08	0.40	4.06	0.43	0.81	***
free acidity	93	14.7	8.16	14.7	9.37	0.92	***
proline[d]	67	345	146	347	152	0.96	***
invertase[d]	67	16.0	6.50	16.3	7.27	0.92	***

[a] standard deviation, [b] correlation, [c] error probability of the correlation (* $P < 0.5$; ** $P < 0.01$; *** $P < 0.001$), [d] not for all types of honey.

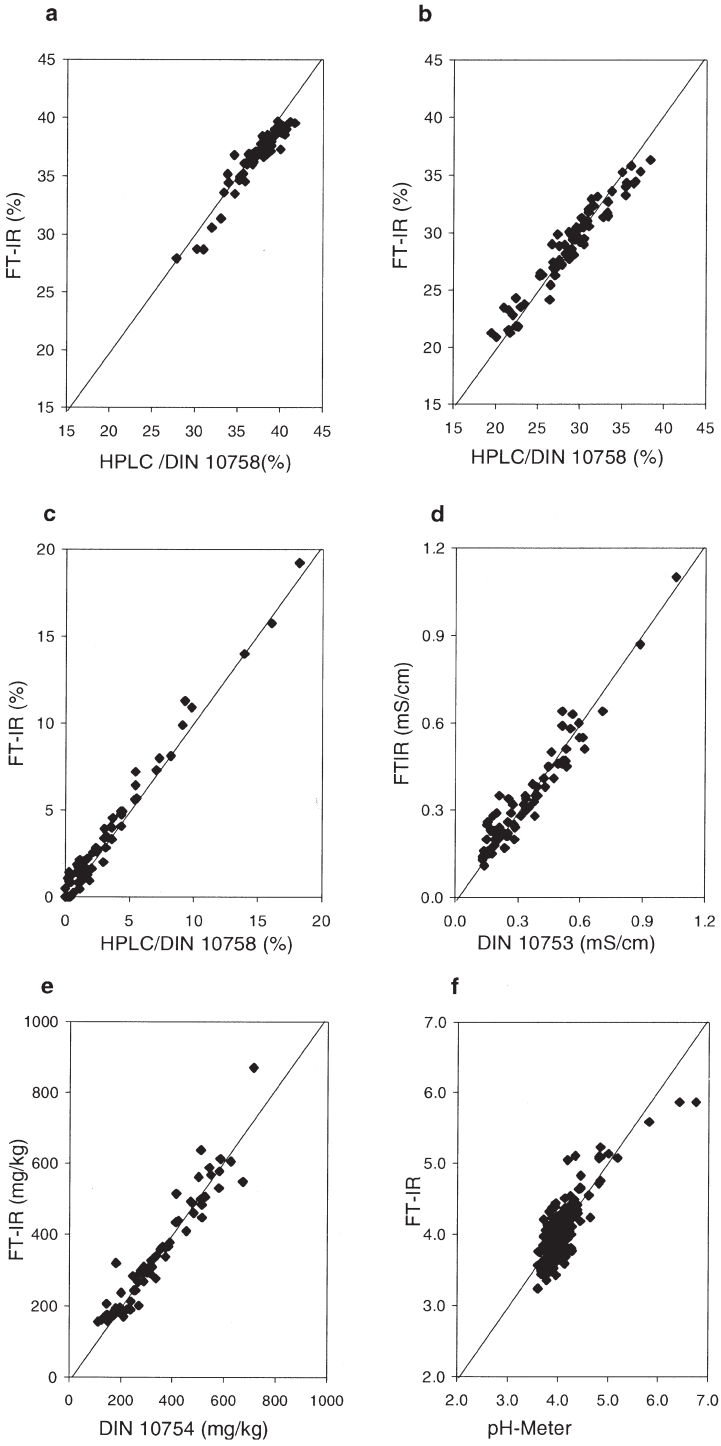

Figure 2. Verification of FT-IR data: fructose, glucose, sucrose, electrical conductivity, proline (not all types of honey), free acidity; presented in scatter plots FT-IR vs. official methods.

for HMF (R^2 = 0.95) especially at higher concentrations it was not possible to obtain reliable results with the FT-IR.

It may be necessary to split the FT-IR analysis into two calibrations: one to determinate if the HMF-concentration below 15 mg/kg or higher and the other to determine the exact concentration under this critical level. Higher values have to be analysed by the standard method.

The standard procedure (Winkler, 1955) is based on the "Stenhouse" reaction that determines the amount of HMF in a colorimetric test. This reaction is influenced by other aldehydes which are found in honey at various levels. On the other hand, increasing the temperature increases not only the HMF levels but also the levels of other furfurals which also undergo the "Stenhouse" reaction (Wotton and Ryall, 1985). There is no reliable method for determining HMF especially at higher concentrations and this is a possible explanation for problems with this calibration.

3.2.4. Moisture

Until now the quality of determination of moisture has been poor. One possible reason is that the area of extensive infrared absorption of water is omitted from the spectral scan and cannot be used for evaluation. Since the analysis of moisture is the simplest procedure in honey analysis and can be easily carried out by beekeepers themselves it is not necessary to be determined by FT-IR.

3.3. Repeatability

Other samples (n = 23, each measured four times) were used for evaluation of the repeatability (w, factor for reliability of repeated measurements). Values between 0.84 and 0.99 confirm the quality of the FT-IR determination of the main sugar

Table III. Repeatability and influence of environmental conditions on the measurements, statistical data.

	[a]n	[b]w	F(day)	F(operator)
fructose	92	0.98	0.03 [c]ns	0.08 ns
glucose	92	0.99	1.23 ns	0.80 ns
sucrose	92	0.99	0.02 ns	0.02 ns
turanose	92	0.96	0.57 ns	0.37 ns
maltose	92	0.86	1.40 ns	1.30 ns
trehalose	92	0.84	0.36 ns	0.01 ns
isomaltose	92	0.92	0.04 ns	0.40 ns
erlose	92	0.97	0.28 ns	0.22 ns
moisture	92	0.82	0.31 ns	0.01 ns
el. conductivity	92	0.95	0.10 ns	0.23 ns
pH	92	0.96	0.34 ns	0.09 ns
free acidity	92	0.97	0.20 ns	0.04 ns
proline	14	0.94	[d]nd	0.01 ns
invertase	56	0.94	nd	0.01 ns

[a] Number of analyses, [b] repeatability, [c] not significant, [d] not determined.

components, electrical conductivity, pH, free acidity, proline and invertase activity and show that the measurements are reproducible and not influenced by environmental conditions. The operator and the time of the analysis do not have any effect on the results (Tab. III; $P > 0.5$).

4. CONCLUSIONS

Fourier transformed infrared spectroscopy is a useful tool in quality control of honey. We have chosen a half-automated equipment including a flow system because it was our aim to develop a routine method.

Since honey comes from different floral sources, it is a complex product with a broad diversity of components, colour and consistency. Due to this fact great demands are made on the methods of analysis. The samples used for calibration should represent the whole matrix of variations in the concentration of the components and honey types. For this reason we analysed more than 1600 samples of honey of different botanical origin. However, we used European standard methods for reference analysis, the FT-IR approach can also be adapted to other analytical standards and honey sources.

With FT-IR it is possible to measure all the calibrated quality criteria of a honey sample at the same time rapidly (about 2 minutes) with a minimum of sample preparation prior to analysis. Only a few chemicals are required, and there is no expensive waste disposal necessary.

Even indirect measurable physical parameters such as the pH and the electrical conductivity can be analysed.

The calibration for some "minor" sugars (present at less than 2%), moisture, proline, invertase and HMF (hydroxymethylfurfural) need to be improved to be more precise for all honeys independent of their origin and composition.

The diversity of components, colour and consistency of honey may influence the infrared spectrum and must be taken into account. Therefore we could not use standard spectra from a library but had to develop the optimal absorption wavelengths for each parameter during the calibration procedure.

Since FT-IR with our instrumentation is a rapid method developed for routine use, allowances should be made for some parameters, especially when the reference method is disputed with regard to its reliability (Wootton, 1985; White, 1994). Accurate results from FT-IR are based on the quality of the reference analysis.

Other researchers (Cho and Hong, 1998; Ha et al., 1998; Quiu et al., 1999; García-Alvarez et al., 2000) have shown that near infrared spectroscopy could be method for honey analysis. They obtained similar results for the determination of the sugar components. Due to different statistical evaluations it is not possible to make exact comparisons.

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Résumé – Spectroscopie infrarouge pour l'analyse en routine de la qualité du miel. L'analyse de la qualité du miel selon les méthodes traditionnelles demande beaucoup de temps et coûte cher. La spectroscopie infrarouge est connue pour être une méthode rapide, fiable et polyvalente, disponible sous forme d'un équipement (semi) automatique. Dans cette étude nous avons utilisé la spectroscopie infrarouge transformation de Fourier (FT-IR) pour essayer d'analyse les principaux composés et les propriétés physiques d'échantillons de miel

en un minimum de temps. L'appareil à spectroscopie FT-IR (Foss Mikroscan FT-IR 120) a été étalonné pour l'analyse du miel. La qualité des mesures a été vérifiée lors de l'analyse en routine.

Environ 1600 échantillons de miel ont été analysés par les méthodes standard. Les spectres infrarouges ont été enregistrés par la spectroscopie FT-IR (Fig. 1). La régression PLS a été utilisée pour calculer à partir de ces données les étalonnages des différents paramètres du miel. Les valeurs de R^2 comprises entre 0,8 et 0,98 indiquaient un bon étalonnage (Tab. I).

Au cours de l'analyse en routine la précision des mesures par la spectroscopie FT-IR a été améliorée par rapport aux méthodes de références. La spectroscopie FT-IR est une méthode fiable pour déterminer les principaux sucres, le pH, la conductibilité électrique et l'acidité libre (Fig. 2). Les coefficients de corrélation entre les méthodes de référence et la spectroscopie FT-IR étaient compris entre 0,85 et 0,99 (Tab. III). Actuellement l'acide aminé proline et l'activité de l'enzyme invertase ne peuvent pas être analysés dans tous les types de miel. Ces étalonnages, ainsi que ceux de l'hydroxymethylfurfural (HMF), de l'humidité et des sucres en concentration inférieure à 2 % doivent être améliorés. Il serait alors possible d'analyser tout type de miel quelles que soient son origine et sa composition.

La qualité de la spectroscopie FT-IR a été confirmée en déterminant la fiabilité de mesures répétées. L'analyse par spectroscopie FT-IR est reproductible (0,84–0,99) et indépendante des conditions du milieu (Tab. III). Puisque les étalonnages peuvent être adaptés à différentes conditions de laboratoire et à différents échantillons de miel, cette méthode peut être utilisée partout. Cette étude montre que la spectroscopie FT-IR est une méthode de routine qui convient pour analyser la qualité du miel, qu'elle n'est pas aussi coûteuse ni gourmande en temps que les méthodes standard. Même des paramètres mesurés indirectement, tels que le pH et la conductibilité électrique, ont été analysés de manière fiable.

spectroscopie infrarouge transformée Fourier / analyse qualité / miel / glucides

Zusammenfassung – Infrarotspektroskopie als Routinemethode zur Qualitätskontrolle von Honigen. Qualitätsuntersuchungen beim Honig mit Standardverfahren sind zeitaufwendig und teuer. Mit der Forderung nach neuen (halb)automatisierten Verfahren bietet sich die Infrarotspektroskopie als schnelle und vielseitige Methode an. In dieser Arbeit sollte versucht werden, mittels Fouriertransformierter Infrarotspektroskopie (FT-IR) von Honigproben mit minimalem Zeitaufwand die wichtigsten chemischen und physikalischen Eigenschaften zu bestimmen.

Das FT-IR-Gerät (Foss Milkoscan FT 120) musste zunächst für Honig kalibriert werden. Die Qualität der Messungen wurde dann in der Routine überprüft.

Alle Honigproben (ca. 1600) wurden zunächst mit den gängigen Labormethoden untersucht. Anschließend wurden mittels FT-IR die Infrarotspektren aufgezeichnet (Abb. 1). Mit der Berechnung einer PLS-Regression aus diesen Daten konnten Kalibrierungen für die einzelnen Parameter etabliert werden. R^2-Werte zwischen 0,8 und 0,98 wurden erreicht (Tab. I).

Die Genauigkeit der FT-IR-Messungen wurde anschließend in der Routine im Vergleich zu den Standardlabormethoden überprüft. Die wichtigsten Zucker, der pH-Wert, die elektrische Leitfähigkeit und die freien Säuren lassen sich zuverlässig mit FT-IR bestimmen (Abb. 2). Der Korrelationskoeffizient zwischen FT-IR- und Referenzmethode erreicht dabei Werte von 0,85 bis 0,99 (Tab. II). Die Messung der Invertaseaktivität und der Aminosäure Prolin ist noch nicht bei allen Honigen möglich. Diese Kalibrierungen und die des Hydroxymethylfurfurals (HMF), Wassergehaltes und der Zucker, die in nur sehr geringen Mengen vorkommen, müssen noch überarbeitet werden, so dass jeder Honig gemessen

werden kann, unabhängig von seiner Herkunft und Zusammensetzung.
Bei allen kalibrierten Komponenten zeigte die Wiederholbarkeit bei Mehrfachmessungen Werte zwischen 0,84 und 0,99. Dieses Ergebnis und die personelle und zeitliche Unabhängigkeit der Messungen bestätigen die Qualität der Honiganalyse mittels FT-IR (Tab. III). Da die Kalibrierungen an unterschiedliche Laborbedingungen und Honige angepasst werden können, ist diese Methode überall einsetzbar.

Es konnte gezeigt werden, dass sich die FT-IR als Routineverfahren zur schnellen und kostengünstigen Qualitätskontrolle von Honigen eignet. Auch indirekt messbare Größen wie pH-Wert und elektrische Leitfähigkeit lassen sich zuverlässig mit dieser Methode bestimmen.

Fourier-transformierte Infrarotspektroskopie / FT-IR / Honigqualität / Zuckeranalyse

Appendix. Selected wave numbers [cm^{-1}] of the calibrated parameters.

	fructose		**glucose**		**sucrose**		**turanose**		**maltose**		**el. cond.**		**pH**		**free ac.**	
No.	*from*	*to*	*from*	*to*	*from*	*to*	*from*	*to*	*from*	*to*	*from*	*to*	*from*	*to*	*from*	*tp*
1	1184	1184	1142	1146	1134	1142	1146	1150	1142	1142	1150	1150	1744	1748	1146	1150
2	1146	1150	2863	2863	1370	1370	2863	2863	2863	2863	1181	1184	2708	2708	2855	2855
3	1138	1138	1181		1154	1154	1181	1181	1169	1169	2863	2866	1134	1142	1736	1736
4	1532	1532	1532	1535	1262	1262	1138	1138	1242	1242	1138	1138	1543	1543	1181	1181
5	1366	1370	1740	1740	1184	1184	1161	1169	1154	1154	1543	1543	1917	1917	1539	1539
6	1740	1740	999	1003	1736	1748	1736	1738	995	999	1890	1894	1169	1169	999	999
7	1165	1165	1157	1161	1161	1165	1404	1404	1543	1543	1277	1277	1277	1277	1134	1134
8	1281	1285	1273	1273	1397	1397	995	995	1740	1740	1161	1161	1184	1184	1273	1277
9	2809	2812	1200	1200	1528	1532	1524	1543	1281	1281	1725	1728	1474	1474	1404	1404
10	988	1003	2222	2222	999	999	1235	1238	1188	1200	1771	1771	1890	1894	1157	1157
11	1481	1481	1134	1138			1273	1277	2272	2272	1408	1408	1393	1393	1798	1798
12	2218	2218	1404	1404			1084	1084	1412	1412	1238	1238	2257	2257	1254	1258
13			1481	1481			1119	1127			1929	1929	1775	1775	1111	1115
14							1775	1775			995	999	1238	1238	1829	1829
15							1362	1366			1443	1447	1728	1728	1377	1381
16															1080	1080
17															1532	1532
18															1030	1034
19															1092	1096
20															1200	1200
21															1304	1304
22															2990	2990
23															992	992

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