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Quantitative analysis of sugar composition in honey using 532-nm excitation Raman and Raman optical activity spectra

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Raman spectroscopy based on the 1064-nm laser excitation was suggested as a handy non-invasive technique allowing to quickly determine sugar content in honey and similar food products. In the present study, the green 532-nm laser radiation is explored instead as it provides higher-quality spectra in a shorter time. The sample fluorescence was quenched by purification with activated carbon. For control mixture decomposition of Raman spectra to standard subspectra led to a typical error of the sugar content of 3%. Raman optical activity (ROA) spectra that could be measured at the shorter excitation wavelength as well provided a lower accuracy (~8%) than the Raman spectra because of instrumental sensitivity and noise limitations. The results show that Raman spectroscopy provides elegant and reliable means for fast analyses of sugar-based food products. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: honey; sugar mixtures; spectral decompositions; Raman spectroscopy; Raman optical activity

Introduction

Sugar-rich products are of interest in a large segment of the food industry, which generates the need for fast, accurate and economic means of analysis of their chemical composition. For honey, variations in the ratio of the main sugar components, sucrose, glucose, fructose and maltose, may be used to trace the origin of this natural product and detect some counterfeiting attempts.[1–3] Detailed sugar composition is also important for many physical properties, such as viscosity, hygroscopic affinity, granulation and energy value.[4,5]

Standard methods to monitor sugar composition include nuclear magnetic resonance (NMR),[6] infrared absorption (IR),[6,7] high-performance liquid chromatography (HPLC)[5,6,8,9] and gas chromatography.[10] The authenticity of honey has also been checked by monitoring of the non-saccharidic components, and using enzymatic and biological methods including analysis of the remaining floral components, such as flavonoids or pollen particles.[3,8]

Also the Raman spectroscopy has been investigated as an alternative, non-invasive and reasonably fast method to monitor approximate sugar content in honey. The Raman spectrum (unlike NMR, for example) is to a very good approximation a simple sum of individual components present in the sample. Percentages of the sugars can thus be obtained by decomposition into standard sub-spectra.[2,3,11] The method can be used to other sugar mixtures as well, as shown in a recent Raman study on soft-drinks.[12]

However, the non-saccharidic fluorescent honey components including aromatic aminoacids, niacin, pyridoxine and other vitamins[13] prevent measurement with laser excitations within the visible spectral range, where the Raman signal is hidden in a fluorescence background. Instead, near infrared (NIR, usually 1064 nm) laser must be used, causing a negligible fluorescence only.[2,3,11] On the other hand, the efficiency of the Raman scattering is very low at NIR; the intensity of the scattered light is proportional to λ^{-4}, where λ is the wavelength.[14,15]

In the present study the performance of Raman spectroscopy with the 532-nm laser excitation radiation is tested with samples pre-purified by active carbon. The activated carbon efficiently absorbs the fluorescent components without a significant change of the sugar content. Although the purification stage slows down the analysis, this obstacle is compensated by a higher quality of the spectra and shorter accumulation times. In addition, Raman optical activity (ROA) can be measured at this excitation as well, which would be difficult to do at the longer wavelengths. The ROA signal is proportional to λ^{-5}, and only few attempts were done to extend this technique to near infrared region.[16,17]

ROA monitors a small difference in the scattering of the right- and left-circularly polarized light.[14,18] ROA bands thus can be both positive and negative, which often allows resolving transitions that overlap as Raman bands. In addition, ROA spectra are sensitive to chirality (e.g. to the enantiometric excess) and fine variations in molecular geometry including conformational states. In the past, the technique was applied to a variety of systems including small organic molecules, inorganic complexes, peptides, proteins, nucleic acids and even whole viruses.[15,19]

Measurement of ROA is difficult because the low ratio of the ROA and Raman signal (so-called circular intensity difference, CID, about 10^{-4} for usual organic molecules). Nevertheless, the sensitivity is

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expected to improve with future instrumentation advances, and saccharides in general are naturally suitable for ROA measurements. Their chirality cannot be usually studied using the more frequent electronic circular dichroism (CD) technique because they usually lack a chromophore absorbing in typical operational range (180–800 nm) of CD spectrometers. Many saccharides are well-soluble in water, which is an ideal solvent for Raman spectroscopy; finally, they provide many strong Raman and ROA bands within a wide wavenumber region. Previous ROA studies comprised, for example, monosaccharides,[20–23] disaccharides[24] and polysaccharides[22] including cyclodextrins[25] and the glucosaminoglycan heparin.[26] Lately, the technique was used to analyze interactions of glycan and yeast external invertase[27] and entanglement-induced conformational changes in the oligosaccharide chains of mucin.[28] Thus although ROA intensities currently cannot be measured with the same precision as the Raman ones, we find it important to investigate also the quantitative performance of ROA spectroscopy for its future development.

Materials and methods

Samples

Standard solutions were prepared from fructose, sucrose, glucose and maltose powders (Sigma-Aldrich) dissolved in distilled water to concentrations of 0.5 g/ml (~1.5–3 mol/l). For sugar mixtures used for calibration each component was weighted separately and then dissolved in water to achieve the same total sugar concentration of 0.5 g/ml.

Honey samples (Table 1) were obtained in a supermarket or from local farms. Product sold as 'wheat syrup' was included for comparison. The samples were mixed with distilled water at 50 °C in a 1:1 volume ratio and purified twice using activated charcoal (carbon). About 0.05 g of the charcoal was added to each ml, the solution was stirred 20 min at 50 °C, centrifuged (14 000 rpm, 10 min) and filtered. Next 0.025 g/ml of the carbon was added, the sample was stirred 30 min at 20 °C, centrifuged and filtered. Remaining particles were removed using membrane filter (PTFE, 0.45 μm).

Spectral acquisition

Raman and ROA spectra were measured with a backscattering SCP BioTools μ-ChiralRAMAN-2X instrument based on the design of W. Hug[29,30] equipped with a diode-pumped solid-state laser operating at 532 nm. Laser power was set to 450 mW at the source (~200 mW at the sample) and acquisition time was about 3 min for Raman and 12–33 h for the ROA spectra. Because the ROA acquisition took so long, ROA spectra were measured for selected samples only. During the measurement the samples were contained in 4 × 3 mm fused silica rectangular cell at 30 °C; minimal volume was about 60 μl. Raman spectrum of the cell filled with water was subtracted from all Raman spectra, and a minor polynomial baseline correction was also applied to the ROA spectra. Sugar samples were measured later than 24 h after preparation so that different sugar forms (α-β anomers) were equilibrated.

Table 1. Measured honey samples

Sample	Origin
I	'Med květový' (flower honey), Medokomerc sro., Čestín, Bohemia
II	'Med luční' (meadow honey), Medokomerc sro. Čestín, Bohemia
III	'Med lesní' (forest honey) Medokomerc sro. Čestín, Bohemia
IV	'Akazien Honig' (Acacia honey), Hans Plümer Nachf., Niedersachsen
V	'Wabenquell' (mountain honey) Hans Plümer Nachf., Niedersachsen
VI	'Wald Honig' (forest honey), Hans Plümer Nachf., Niedersachsen
VII	honey, Dolní Dobrouč, small farm in East Bohemia
VIII	honey 'biomed', Jan Kolomý, Staré Město, small farm in Moravia
IX	small farm, Františkovy Lázně, west Bohemia
X	small farm, Šternberk, Moravia
XI	'Med květový', Tesko (commercial)
XII	wheat syrup, 'Country life', Slovakia

Spectral decomposition

Experimental spectra $S(\omega)$ were decomposed into subspectra of pure fructose, sucrose, glucose and maltose,

$$S(\omega) = \sum_{i=1}^{N} c_i S_i(\omega),$$

using minimization of $\int_{\omega_1}^{\omega_2} \left(S(\omega) - \sum_{i=1}^{N} c_i S_i(\omega) \right)^2 d\omega + A \rightarrow \min$, where $N = 4$, $\omega_1 = 200\,\mathrm{cm}^{-1}$, $\omega_2 = 1600\,\mathrm{cm}^{-1}$ and $A = \sum_{i=1}^{4} \alpha (c_i - c_{ave})^2$. The parameter α was chosen to be small and avoid negative coefficients c_i, forcing them to an average value. In most cases this was not needed, and $\alpha = 0$. The subspectra were normalized to a standard measurement time; sample spectra were not normalized and the decomposition coefficients were renormalized ($\sum_{i=1}^{4} c_i = 1$) after the decomposition. Other saccharides and compounds contributing to the Raman intensities were neglected, as their contents are supposed to be beyond the sensitivity limit of the method (<1%, http://www.honey-well.com/composit.html).

Results and discussion

The effect of purification

For the measurements with the 532-nm laser excitation the removal of the fluorescent impurities is essential. This is documented in Fig. 1, where the Raman spectra of raw and purified honey are

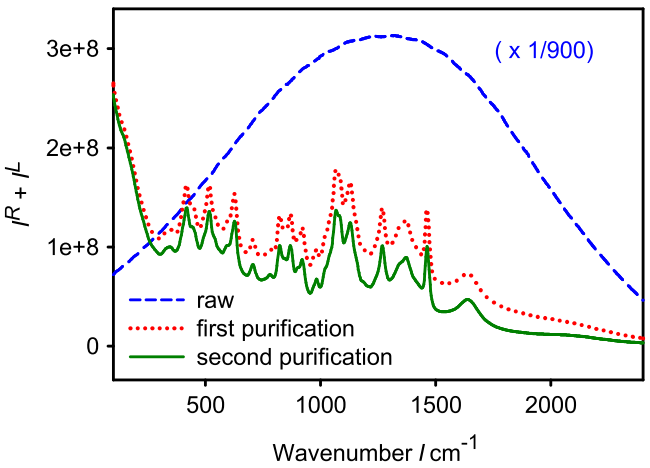

Figure 1. Raman spectrum (532 nm) of honey before and after the purification with activated charcoal.

compared. In the raw sample the fluorescence is about 1000 times stronger than the true Raman signal. However, already the first purification (0.05-g active carbon per milliliter, see the methods) eliminated the fluorescence almost completely, and the second purification (0.025 g of active carbon) led to a minor decrease of the fluorescent background only. The remaining background signal is largely caused by the water[31] and fused silica cell,[32] and can be subtracted as a baseline obtained with distilled water.

Raman and ROA spectra of pure monosaccharides

Experimental spectra of pure D-glucose, D-fructose, D-maltose and sucrose are plotted in Fig. 2. A detailed assignment of Raman and ROA spectral features to vibrational normal modes has been discussed in numerous previous studies.[2,12,20,23,24,33] Various wavenumber regions are differently sensitive to various structural details; thus one can talk about the low-wavenumber region (<600 cm^{-1}) sensitive to disaccharide linkage and other torsional modes, anomeric region (600–920 cm^{-1}) reflecting configuration on the anomeric chiral center, fingerprint (920–1200 cm^{-1}) and CH_2 and COH deformation (>1200 cm^{-1}) regions.[24] A more detailed interpretation requires complex simulations involving molecular hydration and dynamics[33–35] and reveals contributions of individual vibrational motions to spectral intensities, such as of the OH twist (~200–400 cm^{-1}), C—C/C—O stretches and ring deformations (~900–1000 cm^{-1}), ring torsions (1300–1450 cm^{-1}) and CH bending (1200–1500 cm^{-1}), etc. Especially at the low-wavenumber region water molecules heavily participate in the sugar vibrations as well.

Because of the similar structure of all saccharides, Raman intensity profiles are similar, too. In particular, the spectra of D-glucose and D-maltose exhibit striking similarities in the entire wavenumber region. The D-fructose Raman spectrum appears as the most distinct in the quartet. The ROA spectra exhibit much larger variability with respect to the sugar type including sign changes, similarly as for other chiral molecules.[36–38]

Decomposition of arbitrary sugar mixtures

The decomposition method based on the Raman and ROA spectra is exemplified in Fig. 3, where the spectra-based sugar ratios are plotted against the concentrations calculated from sugar weights. Six two-component (D-glucose/D-fructose) one three (D-glucose/D-fructose/D-maltose) and two four (D-glucose/D-fructose/D-maltose/sucrose) trial mixtures were analyzed; detailed ratios are listed in Table 2. Clearly, the Raman spectra (bottom panel in Fig. 3) provide reliable sugar ratios with a high correlation coefficient of 0.986 and average error of 3%. Taking into account the expected experimental

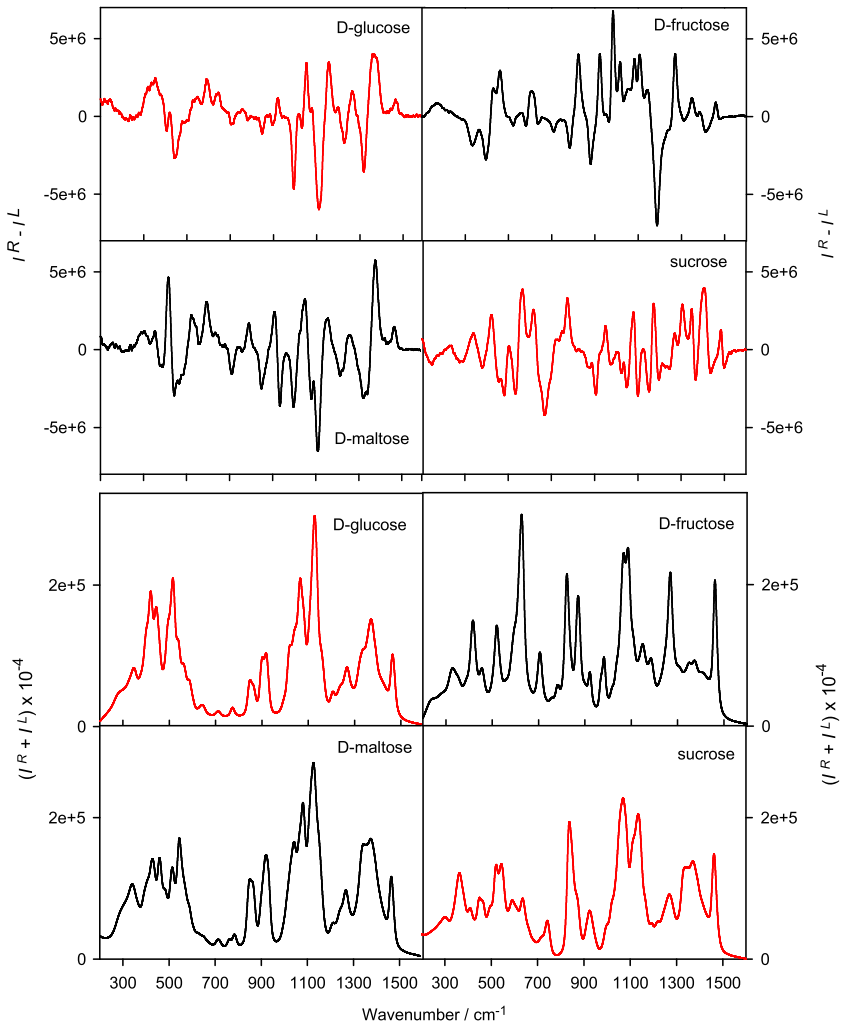

Figure 2. Experimental ROA ($I^R - I^L$, top four panels) and Raman ($I^R + I^L$, bottom) spectra of glucose, sucrose, fructose and maltose.

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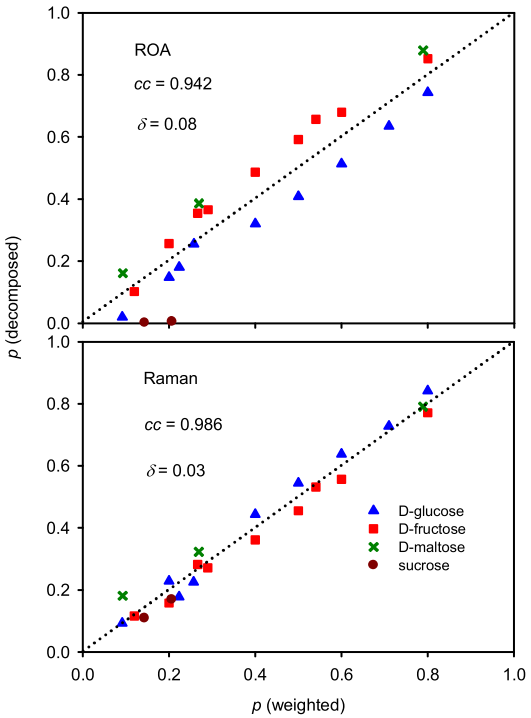

Figure 3. Comparison of sugar molar fraction (p) obtained by the ROA (top) and Raman (bottom) spectra decomposition of test sample to ratios calculated from sample weights. Correlation coefficients (cc) and average absolute errors (δ) are indicated.

Table 2. Comparison of weighted sugar ratios with the values obtained from the ROA and Raman spectra. The ratios are in %, for D-glucose, D-fructose (F), D-maltose (M) and sucrose (S)

	Weighted				ROA				Raman			
Sample	G	F	M	S	G	F	M	S	G	F	M	S
s1	80	20			74	26			84	16		
s2	60	40			51	49			64	36		
s3	50	50			41	59			54	46		
s4	40	60			32	68			44	56		
s5	20	80			15	85			23	77		
s6	71	29			63	37			73	27		
s7	9	12	79		2	10	88		9	12	79	
s8	22	54	9	14	18	66	16	0	18	53	18	11
s9	26	27	27	21	25	35	39	1	23	28	32	17

error associated with preparation of the small-volume solutions the spectroscopy thus reproduces the sugar content in the samples virtually exactly. The performance of the decomposition does not seem to deteriorate for the multi-component mixtures.

The performance of the decomposition based on the ROA spectra (upper panel in Fig. 3) is significantly worse than for the Raman analysis, although the sugar molar fractions are still reasonably close to the reference values with an average absolute error of 8% and correlation coefficient of 0.942. Rather discouraging appear the too low sucrose contents determined from the spectra. The reason of the higher error of ROA-based spectral decompositions can be illustrated in Fig. 4, where for a trial sample (D-glucose/D-fructose in 6:4 molar ratio) experimental Raman and ROA spectra are overlapped with the fit. The experimental and fitted Raman spectra nearly overlap.

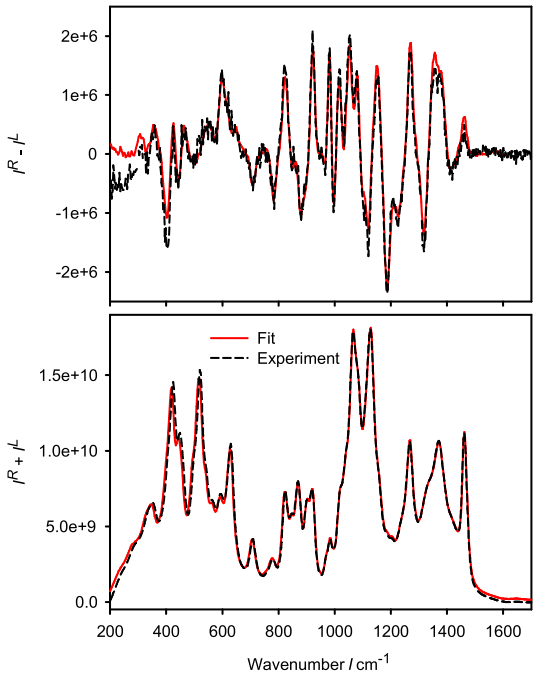

Figure 4. Example of spectral decomposition. Experimental ROA (top) and Raman (bottom) spectra of D-glucose and D-fructose mixture (6:4 molar ratio, dashed line) and the fit (solid line) obtained from the subspectra in Fig. 2. The decomposition ratios are given in Table 2, sample s2.

For ROA, they significantly deviate, particularly in the lowest-energy region (<300 cm^{-1}) where the baseline is very unstable. (Nevertheless, in this case omitting this region provided virtually the same decomposition ratios as the fit of whole spectrum.) In addition, the ROA intensities can be measured with a smaller accuracy than the Raman ones, and the ROA signal is hampered by instrumental noise and occasional 'spikes', i.e. random sharp peaks originated in background cosmic radiation. The noise level at any point of ROA and Raman spectrum is approximately proportional to the square root of number of Raman counts (proportional to the counts registered by the CCD detector).[18] Because the ratio of ROA and Raman signal is so small, the noise thus affects the decomposition based on the ROA spectra much more than for the Raman spectra. A non-linear dependence of the spectral intensities on sugar concentration was not observed. Nevertheless, such finer effects cannot be completely excluded at this time, and more detailed studies are desirable in the future. Potential variations of sugar forms/conformations in the mixtures could also explain some of the differences between the Raman and ROA results, because the ROA spectra are intrinsically more sensitive to the structure.

Application to the honey samples

The sugar composition in the 12 honey samples listed in Table 1 as determined by the decomposition of the ROA and Raman spectra is summarized in Fig. 5. As in Fig. 3, we see that the ROA method systematically over-estimates the fructose content, by about 10%, and underestimates the molar ratios of glucose, otherwise roughly follows the values obtained from the Raman spectra. The variation of the sugar content found by (more reliable) Raman spectra decomposition in various honeys corresponds to those found in previous studies.[3,8,11] Interestingly, samples obtained from small farms (VII–X) tend to exhibit higher content of maltose and/or sucrose that those from the supermarket. The wheat syrup (sample

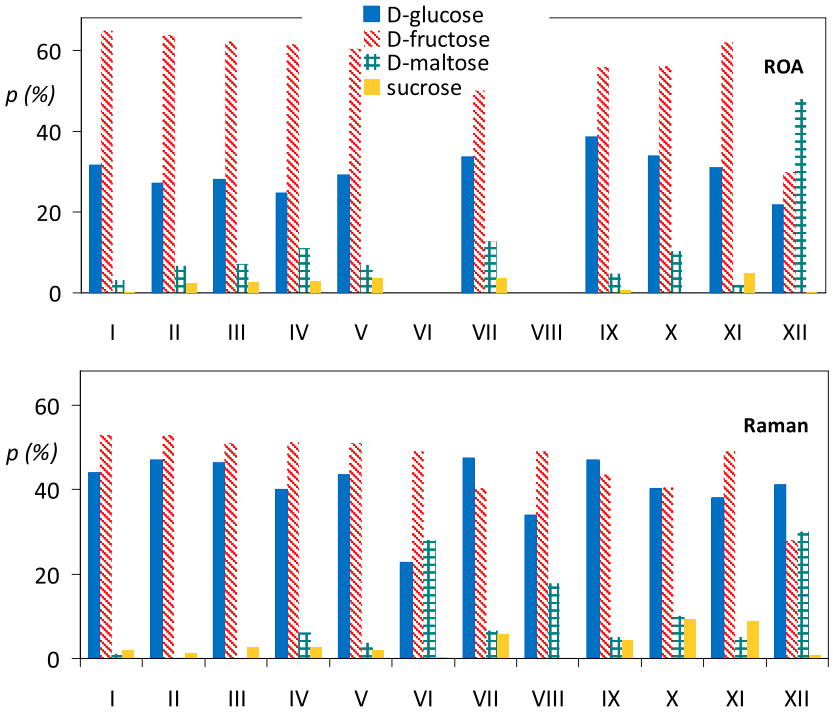

Figure 5. Molar sugar ratios determined by the decomposition of ROA (top) and Raman (bottom) spectra in the honey samples in Table 1 (samples VI and VIII were not used for ROA).

XII) differs from most honey samples by the rather even ratio of glucose, fructose and maltose.

Conclusions

We explored the Raman spectroscopy with the 532-nm laser radiation as a tool to determine the sugar composition in honey and similar sugar mixtures. High-quality spectra were obtained when the fluorescence in the samples was quenched by the purification with activated carbon, and the molar ratios could be reproduced with an accuracy of few %, similarly as for the more usual 1064-nm Raman spectroscopy. For the first time, the ROA has been used for honey analysis as well. It provided lower accuracy of the sugar content than the Raman spectroscopy. Thus future improvements in instrumental stability and sensitivity are desirable. We nevertheless find the results encouraging as they show that a quantitative analysis using ROA is possible and increases the analytical potential of this technique so sensitive to fine details in molecular structure. The Raman spectroscopy provides an elegant and reliable means for fast analyses of sugar-based food products.

Acknowledgements

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References

[1] R. S. Singhal, P. R. Kulkarni, D. V. Rege, *Handbook of Indices of Food Quality and Authenticity*, Woodhead Publishing Limited, Cambridge, **1997**.
[2] B. Özbalci, I. H. Boyaci, A. Topcu, C. Kadılar, U. Tamer, *Food Chem.* **2013**, *136*, 1444.
[3] M. M. Paradkar, J. Irudayaraj, *Food Chem.* **2001**, *76*, 231.
[4] J. S. Bonvehi, *Anales de Bromatologia* **1989**, *41*, 41.
[5] S. Ouchemoukh, P. Schweitzer, M. S. Bey, H. Djoudad-Kadji, H. Louaileche, *Food Chem.* **2010**, *121*, 561.
[6] L. G. Justino, M. Caldeira, V. M. S. Gil, M. T. Baptista, A. P. da Cunha, A. M. Gil, *Carbohydrate Polym.* **1998**, *34*, 431.
[7] J. Irudayaraj, S. Sivakesava, *J. Sci. Food Agric.* **2001**, *81*, 683.
[8] E. de la Fuente, A. I. Ruiz-Matute, R. M. Valencia-Barrera, J. Sanz, I. M. Castro, *Food Chem.* **2011**, *129*, 1483.
[9] C. D. Romero, A. B. Manzanares, Z. H. García, B. R. Galdón, E. R. Rodríguez, *Food Chem.* **2011**, *126*, 664.
[10] V. Kaskoniene, P. R. Venskutonis, V. Ceksteryte, *Acta Aliment* **2011**, *40*, 205.
[11] J. Irudayaraj, M. M. Paradkar, *Food Chem.* **2002**, *76*, 231.
[12] K. Ilaslan, I. H. Boyaci, A. Topcu, *Food Control* **2015**, *48*, 56–61.
[13] J. M. Graham, *The Hive and the Honeybee*, Dadant&Sons, Hamilton, IL, **1992**.
[14] L. D. Barron, *Molecular Light Scattering and Optical Activity*, Cambridge University Press, Cambridge, **2004**.
[15] V. Parchaňský, J. Kapitán, P. Bouř, *RSC Adv.* **2014**, *4*, 57125.
[16] M. Unno, T. Kikukawa, M. Kumauchi, N. Kamo, *J. Phy. Chem. B* **2013**, *117*, 1321.
[17] K. Hiramatsu, M. Okuno, H. Kano, P. Leproux, V. Courderc, H. Hamaguchi, *Phys. Rev. Lett.* **2012**, *109*, 083901.
[18] L. Nafie, *Vibrational Optical Activity: Principles and Applications*, Wiley, Chichester, **2011**.
[19] E. W. Blanch, I. H. McColl, L. Hecht, K. Nielsen, L. D. Barron, *Vib. Spectrosc.* **2004**, *35*, 87.
[20] L. D. Barron, A. R. Gargaro, Z. Q. Wen, *Carbohydr. Res.* **1991**, *210*, 39.
[21] A. F. Bell, L. Hecht, L. D. Barron, *J. Raman Spectrosc.* **1993**, *24*, 633.
[22] A. F. Bell, L. Hecht, L. D. Barron, *J. Raman Spectrosc.* **1995**, *26*, 1071.
[23] Z. Q. Wen, L. D. Barron, L. Hecht, *J. Am. Chem. Soc.* **1993**, *115*, 285.
[24] A. F. Bell, L. Hecht, L. D. Barron, *J. Am. Chem. Soc.* **1994**, *116*, 5155.
[25] A. F. Bell, L. Hecht, L. D. Barron, *Chem-Eur. J.* **1997**, *3*, 1292.
[26] T. R. Rudd, R. Hussain, G. Siligardi, E. A. Yates, *Chem. Commun.* **2010**, *46*, 4124.
[27] C. Johannessen, R. Pendrill, G. Widmalm, L. Hecht, L. D. Barron, *Angew. Chem. Int. Ed.* **2011**, *50*, 5349.
[28] L. Ashton, P. D. Pudney, E. W. Blanch, G. E. Yakubov, *Adv. Colloid Interface Sci.* **2013**, *199–200*, 66.
[29] W. Hug, G. Hangartner, *J. Raman Spectrosc.* **1999**, *30*, 841.

[30] W. Hug, *Appl. Spectrosc.* **2003**, *57*, 1.
[31] D. M. Carey, G. M. Korenowski, *J. Chem. Phys.* **1998**, *108*, 2669.
[32] M. Dračínský, L. Benda, P. Bouř, *Chem. Phys. Lett.* **2011**, *512*, 54.
[33] A. Melcerová, J. Kessler, P. Bouř, J. Kaminský, *Phys. Chem. Chem. Phys.* **2016**, *18*, 2130.
[34] J. R. Cheeseman, M. S. Shaik, P. L. A. Popelier, E. W. Blanch, *J. Am. Chem. Soc.* **2011**, *133*, 4991.
[35] F. Zielinski, S. T. Mutter, C. Johannessen, E. W. Blanch, P. L. A. Popelier, *Phys. Chem. Chem. Phys.* **2015**, *17*, 21799.
[36] C. R. Jacob, S. Luber, M. Reiher, *Chem. Phys. Chem.* **2008**, *9*, 2177.
[37] J. Kessler, J. Kapitán, P. Bouř, *J. Phys. Chem. Lett.* **2015**, *6*, 3314.
[38] E. W. Blanch, L. Hecht, L. D. Barron, *Methods* **2003**, *29*, 196.