Lightnovo

APPLICATION NOTE

ApN-009

miniRaman Spectrometer for Analisys of Honey and Syrups

INTRODUCTION

People consume sweet foods mainly because the body associates sweet taste with the presence of substances that can be quickly converted into energy (sucrose, fructose, glucose) [1]. The main industrial sweet food product is regular sugar, which consists almost entirely of sucrose. However, due to its high calorie content and metabolic peculiarities, excessive consumption of sucrose leads to the development of serious diseases (type II diabetes, cardiovascular diseases, obesity, dental caries, etc.). Over the past decade, the problem of sucrose overconsumption has reached the proportions of a global pandemic. Accordingly, consumers and foodstuff producers are increasingly seeking to use regular sugar substitutes. The low-calorie artificial sweeteners (saccharin, aspartame, etc.) are well-known since the 1980s, but debate about their negative impact on the body (liver and bladder toxicity, carcinogenicity, etc.) is still ongoing, so they often cause consumer mistrust. Against this background, natural sweeteners are becoming increasingly popular, first of all, bee honey, containing only ≤ 5% sucrose [2], and plant-based compounds (sorbitol, erythritol, xylitol, steviol glycosides, glycyrrhizin, sweet-tasting proteins, etc.).

Humans do not have complete control over the production of honey by bees, so monitoring its composition, especially the content of contaminants (pesticides, antibiotics, etc.), is mandatory [2]. Also, large-scale falsification of honey is currently observed all over the world, usually with various syrups (malt, rice, corn, inverted sugar), which makes the task of authenticity checking especially relevant. In turn, composition analysis is necessary in the production of plant-based sweeteners and the development of food products based on them [3]. Moreover, it cannot be ruled out that the problem of falsification will also occur here. Adulterations by sucrose/glucose may be particularly dangerous for people suffering from diabetes, insulin resistance, atherosclerosis, etc.

To solve the above-mentioned problems, Raman spectroscopy can be used, which has already proven its effectiveness in the analysis of honey [2], natural [3] and artificial sweeteners [4].

This Application Note aims to demonstrate the potential of Lightnovo® miniRaman® spectrometer [5], the most compact serial Raman instrument, for the analysis of liquid sweet food products, in particular, for the differentiation of high-sucrose (sugar syrup) and low-/non-sucrose ones (honey, as well as syrups based on natural sugar substitutes).

SAMPLES

The following substances were tested in this study.

1. **Natural bee honey:** a late summer honey from linden, white clover, and wildflowers. The honey originated from Lolland, Denmark.
2. **Maple syrup based on natural sugar substitutes** (hereinafter "maple syrup"): a commercial product, consisting of corn fiber, sweeteners (sorbitol, erythritol and steviol glycosides), molasses, salt and flavouring agent.
3. **Syrup based on natural sugar substitutes** (hereinafter "sweet syrup"): a commercial product of the same manufacturer as the previous one, consisting of corn fiber, sweeteners (sorbitol, erythritol and steviol glycosides), molasses and salt.
4. **Sugar syrup:** a homemade homogeneous solution of commercial white sugar in water, which were taken in a proportion of 2:1 (by volume).

Sugar, maple and sweet syrups were purchased from a supermarket in Birkerød, Denmark, while honey was purchased from local manufacturer in Denmark.

MEASUREMENTS AND DATA PROCESSING

Lightnovo miniRaman Power 785 nm spectrometer [5] equipped with Middle Working Distance Probe (10mm) mounted in Precise Z Focusing Stage accessory was used for the measurements, Figure 1(a). Lightnovo® Miraspec®, the spectrometer's bundled software installed on a personal computer, was used to control measurement process, data collection and postprocessing, Figure 1(b). The measurement parameters: output power (on the sample) 63 mW, image sensor gain 0, exposure 250 ms, number of averages 10 (per spectrum), background correction ON.

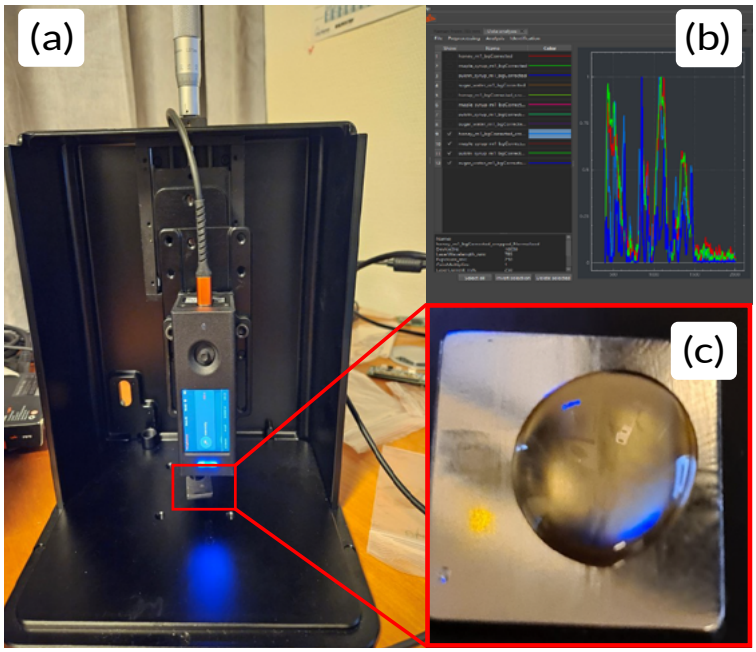

Figure 1.
(a) The setup used for the measurements: miniRaman power 785 nm spectrometer mounted on the Z focusing stage;
(b) Lightnovo Miraspec software with the spectra of the samples (baseline subtracted, normalized);
(c) honey sample on a stainless-steel substrate.

The measurement procedure was as follows. A sample – a drop (~5 ml) of the analyzed substance – was deposited onto a stainless-steel substrate, Figure 2(c). The last one was placed on the base of Z Focusing Stage in such a way that the laser radiation fell approximately into the middle of the drop. The stage's micrometric head was used to obtain the maximum level of Raman signal. For each sample 10 spectra were measured on the same laser spot location.

The spectra were stored in raw and postprocessed forms using Miraspec software. For processing, the program first applied the rolling-circle filter with radius 4000 and scaleY 0.1 to correct baseline, and then the min-max algorithm to normalize Raman intensity to the range [0…1], Figure 1(b).

Finally, to differentiate the samples, their baseline-subtracted and normalized spectra were subjected to the Principal Component Analysis (PCA). For this purpose, the Scikit-Learn Python package [6] were applied with preliminary data standardization using the StandardScaler algorithm.

RESULTS

Figure 2 shows the raw spectra measured 10 times subsequently for each sample. Each spectrum is marked in the order it was acquired by a number from m1 to m10 and a specific color. Raman intensity is expressed here in arbitrary units corresponding to the percentage of the saturation level of the spectrometer's CMOS image sensor.

As it can be seen from Figure 2, the raw spectra are quite reproducible in terms of curve shape. All the samples provide distinct Raman features. Honey, maple syrup, and sweet syrup also exhibit significant fluorescence, which however does not yield to the saturation of the detector, meaning that the fluorescence background could be removed by applying baseline subtraction algorithm. On the other hand, sugar syrup exhibited minimal fluorescence and showed clearly resolved Raman peaks.

If examined carefully, it can be seen that there is a general trend in all the measurements: the fluorescence background goes down with each subsequent measurement. This could be explained by the effect of photobleaching (reduction of fluorescence in time when exposed to the laser). However, the first measurement stands out of this trend in all the cases, i.e. there is a rapid increase of fluorescence between the first (m1) and second (m2) measurement followed up by slow decay of fluorescence at each subsequent one (m3, …, m10) due to photobleaching. The possible explanation of this initial increase of fluorescence could be the thermal effect of laser radiation on the sample, diffusion in the sample, and sample inhomogeneity.

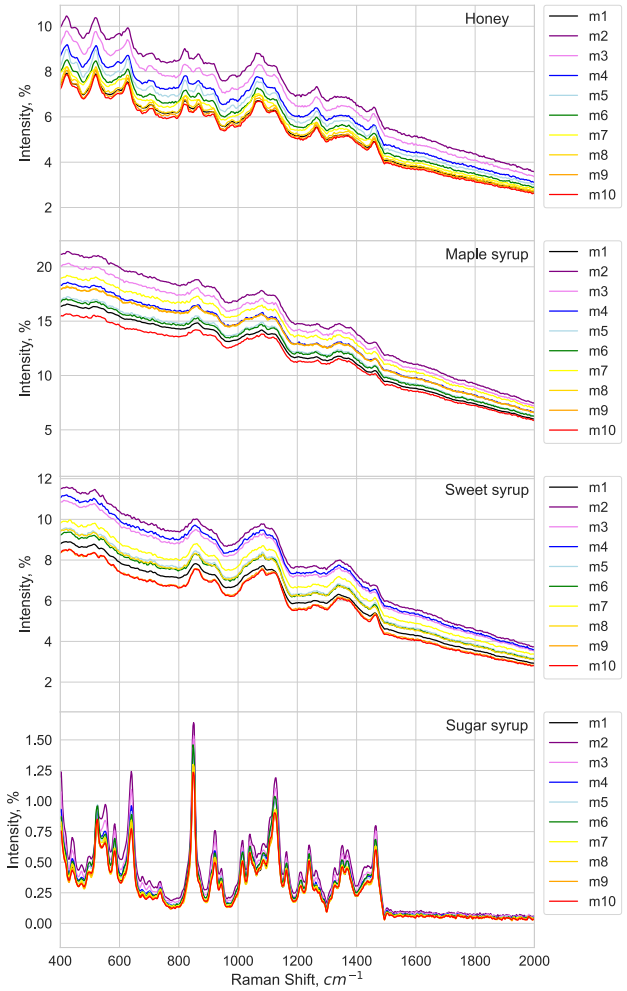

Figure 2. Raw spectra of honey, maple, sweet and sugar syrups. For each sample, spectra m1, …, m10 were measured sequentially.

The postprocessed spectra are provided in Figure 3. As it can be seen, baseline correction allows to effectively remove the effects of both fluorescence and photobleaching, and the resulting normalized spectra show good reproducibility between measurements. To better evaluate the postprocessing effect, each set of spectra m1, ..., m10 from Figures 2 and 3 was averaged to improve its data quality and enhance signal-to-noise ratio, and the resulting mean spectra for pre- and postprocessed data are compared respectively in aligned Figures A1 and A2 (Annex 1).

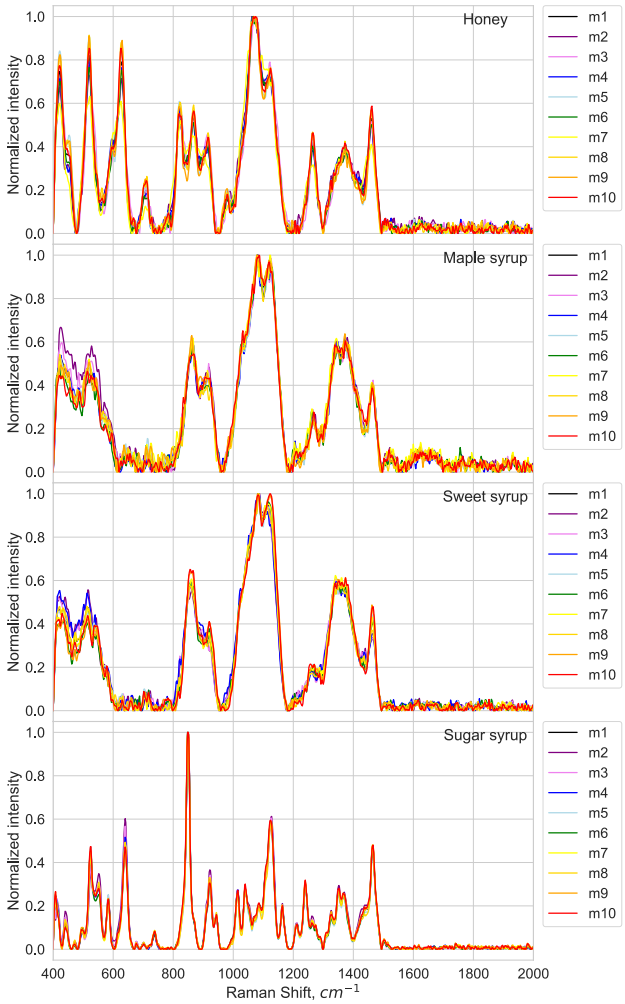

Figure 3. Baseline-corrected and normalized spectra of honey, maple, sweet, and sugar syrups. The spectra numbers m1, ..., m10 are the same as in Figure 2.

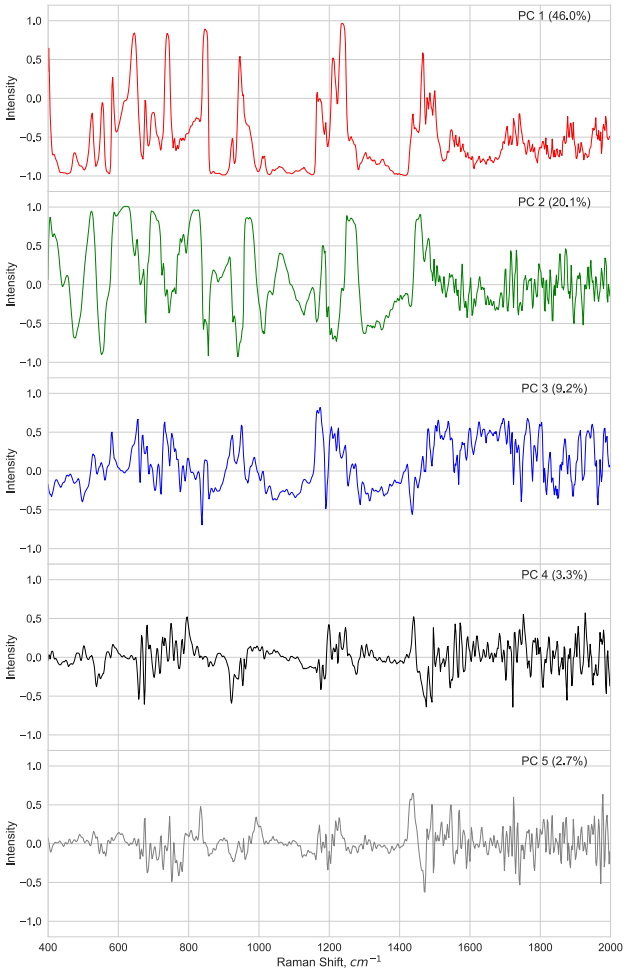

Figure 4. Loading plots (spectra) of first five principal components derived from PCA of the spectra in Figure 3.

For all samples in Figure 3, the most intensive Raman peaks fall into the wavenumber range (400 ... 1600) cm^{-1}. The spectra of honey, maple and sweet syrups contain a lot of overlapping peaks, which is obviously due to complex compositions of these substances. On the contrary, relatively simple sugar syrup produces many narrow and well-defined peaks. The spectra of honey, syrups based on natural sweeteners and sugar syrup have own unique spectral features. Therewith, the curves for maple and sweet syrups, as expected, look very similar to each other.

Figure 4 shows the loading spectra for the first five principal components (PCs) calculated withing PCA of the entire data set (40 spectra) from Figure 3. The plots for PC1, PC2 and PC3 demonstrate intensive peaks in the range of interest (400 ... 1600) cm^{-1}, while for PC4, PC5 and higher-order PCs (not shown in Figure 4) the major peculiarities are located chiefly at >1600 cm^{-1}. Thus, considering PC1, PC2, and PC3 should be sufficient to cluster the data and differentiate the samples.

This inference is supported by Figure 5, which shows the impact of the PCs number on (a) the cumulative explained variance, and (b) the explained variance ratio, i.e. the fraction of the total variance that falls on a specific PC. Together, PC1, PC2 and PC3 can explain 75.2% of the data variation. At the same time, other PCs have such a small contribution that their consecutive addition to the analysis increases this value by only a few percent.

The presence of an «elbow» in Figure 5 (b) is in good agreement with the heuristic «elbow rule» used in cluster analysis: the point, at which the rate of change of the explained variance drops sharply, determines the optimal number of clusters. In this case, it is obviously equal to the number of samples under study, i.e. 4.

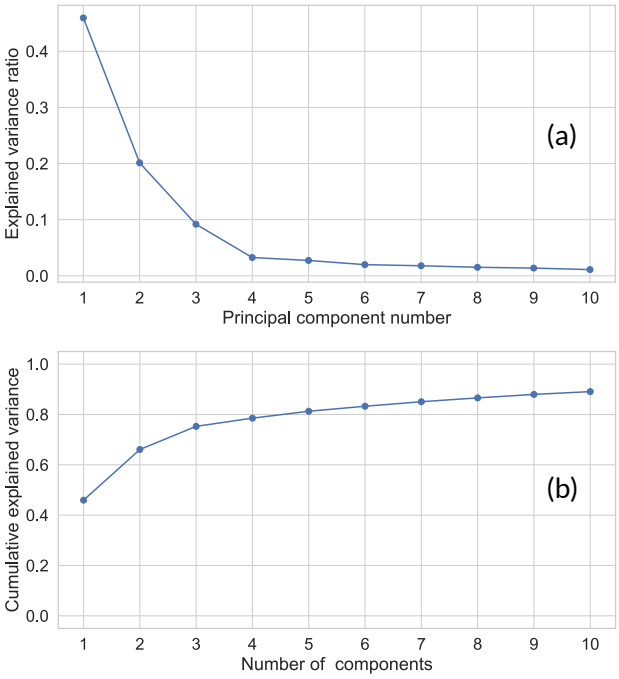

Figure 5. Impact of principal component number on: (a) the cumulative explained variance; (b) the explained variance ratio.

Figures 6 (a) and (b) show, respectively, the 2D scatter plots of scores for PC2 and PC3 against PC1, while Figure 6 (c) show the 3D scatter plot of scores for all three these components.

It is clear from Figure 6 that the data for different types of the samples used in this study are well clustered and can be linearly separated already based on two main principal components PC1 and PC2. The cluster for sugar syrup is located quite far from the others along the PC1 axis. Obviously, this is due to the clear difference between its spectra and spectra of other samples, see Figures 3, whereas, according to PCA approach, PC1 is calculated in such a way as to correspond to the maximum dispersion of the data. In turn, maple and sweet syrups, as well as honey, have more similar spectra, Figures 3, and the distance between their clusters along PC1 is significantly smaller, Figure 6. Moreover, the clusters of the last two even overlap along this axis.

Surprisingly, the maple and sweet syrups, which nominally have minor compositional differences, can also be differentiated because their clusters are clearly separated along the PC1 axis. This can be explained by the presence of a flavoring agent in maple syrup, which probably provides some spectral peculiarities that are not evident during a cursory inspection. Moreover, the inclusion of the third component PC3 into consideration allows to even better separate their clusters, and also make it possible to simultaneously differ them (with a small error) from honey one, Figure 6 (b).

The honey cluster is very distant from the other ones along the PC2 axis, Figure 6 (a). This can probably be explained by the fact that the complex composition of honey includes some compounds producing unique but not intense enough spectral peaks. At the same time, the clusters for honey and sugar syrup are located far from each other both along PC1 and PC2 axes, Figure 6, which can have important practical significance for the honey authentication checking.

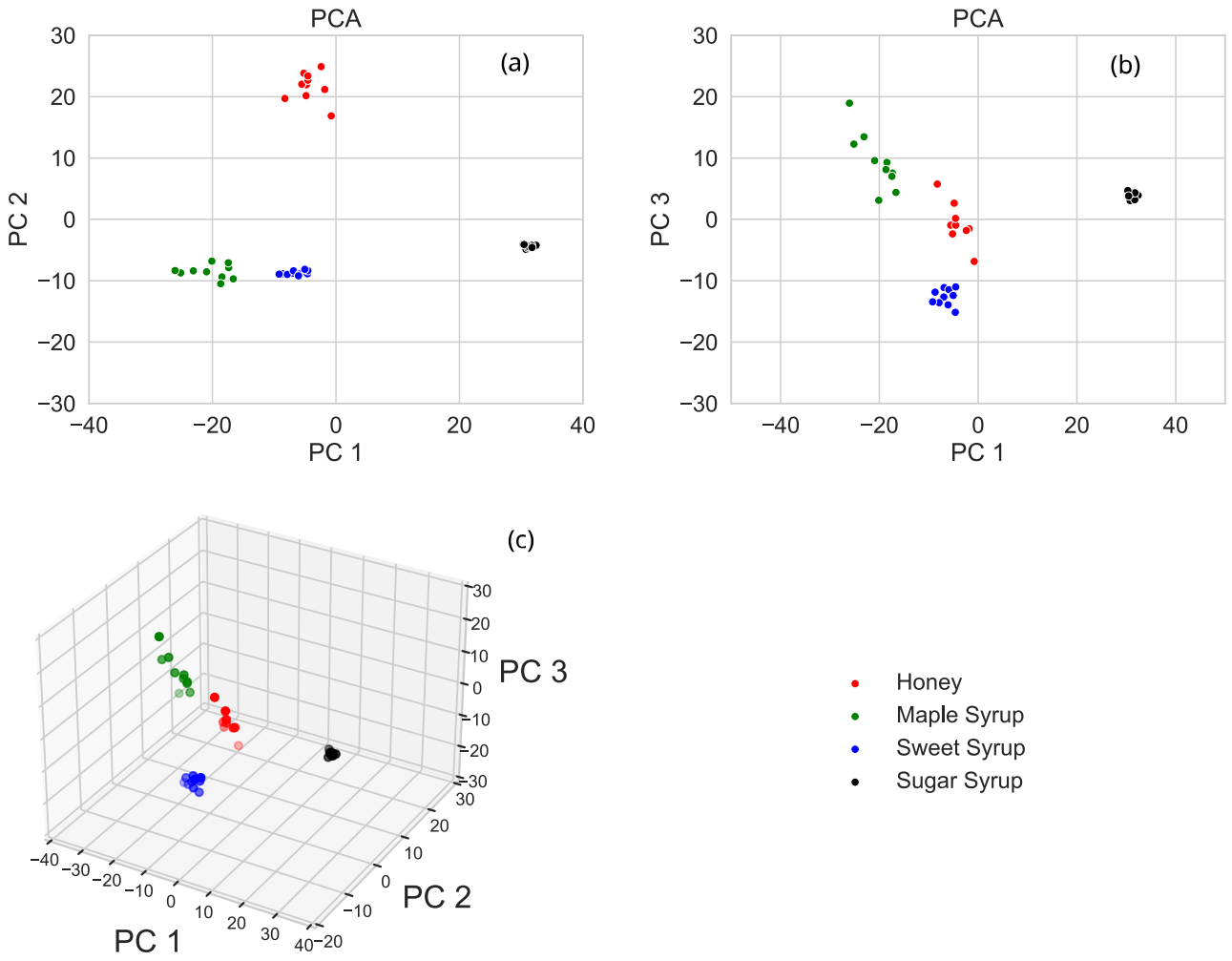

Figure 6. Score plots of the first three principal components derived from PCA of the spectra in Figure 3: (a) PC2 vs. PC1; (b) PC3 vs. PC1; (c) PC3 vs. PC2 vs. PC1 (3D scatter plot).

An interesting feature is that the spread of data points within each cluster definitely correlates with the compositional complexity of the studied compound. E.g., sugar syrup, besides water, contains only sucrose, and its cluster has smallest size for all three PCs. On the contrary, honey and maple syrup have sophisticated compositions and give the widest cluster, which also have an elongated form. All this may indicate, for example, diffusion processes in the sample during measurements, or inhomogeneity of analyzed substance, e.g., due to fractionating.

CONCLUSIONS

- Lightnovo miniRaman 785 nm spectrometer is capable of directly, i.e., without sample preparation, measuring Raman spectra of honey, natural sweeteners-based and sucrose-based syrups.
- The software supplied with miniRaman spectrometer allows to efficiently remove the intense background fluorescence signal, as well as the photobleaching effect, from Raman spectra of honey and the natural sweeteners-based syrups. Therewith, excellent reproducibility of measurements is provided.
- Conventional Principal Component Analysis of Raman spectra allows to clearly differentiate of honey, the natural sweeteners-based and sucrose-based syrups using only three first principal components.
- This all indicates that Lightnovo miniRaman spectrometer is a powerful tool for analysis of honey and syrups, and in particular could be used to detect possible adulteration of honey or natural sugar substitutes, as well as to control the manufacturing processes of liquid food products based on them.

REFERENCES

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ANNEX 1.

EFFICIENCY OF POSTPROCESSING IN MIRASPEC SOFTWARE

For all samples, each of the sets (m1, ..., m10) of the raw spectra, Figure 2, and Miraspec postprocessed spectra, Figure 3, were averaged. A special script written in Python programming language was used for this purpose. Averaging improves data quality and enhances the signal-to-noise ratio, allowing better inspection of spectral features and evaluation of the effect of postprocessing. Figures A1 and A2 show the raw and postprocessed averaged spectra, respectively.

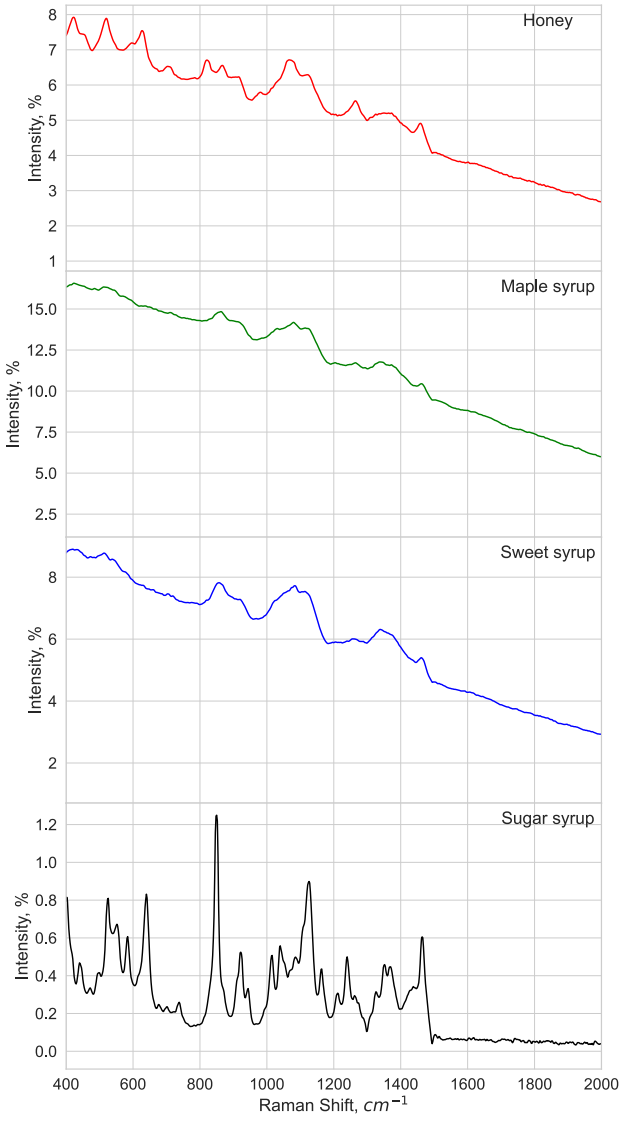


Figure A1. Averaged raw spectra of honey, maple, sweet and sugar syrups.

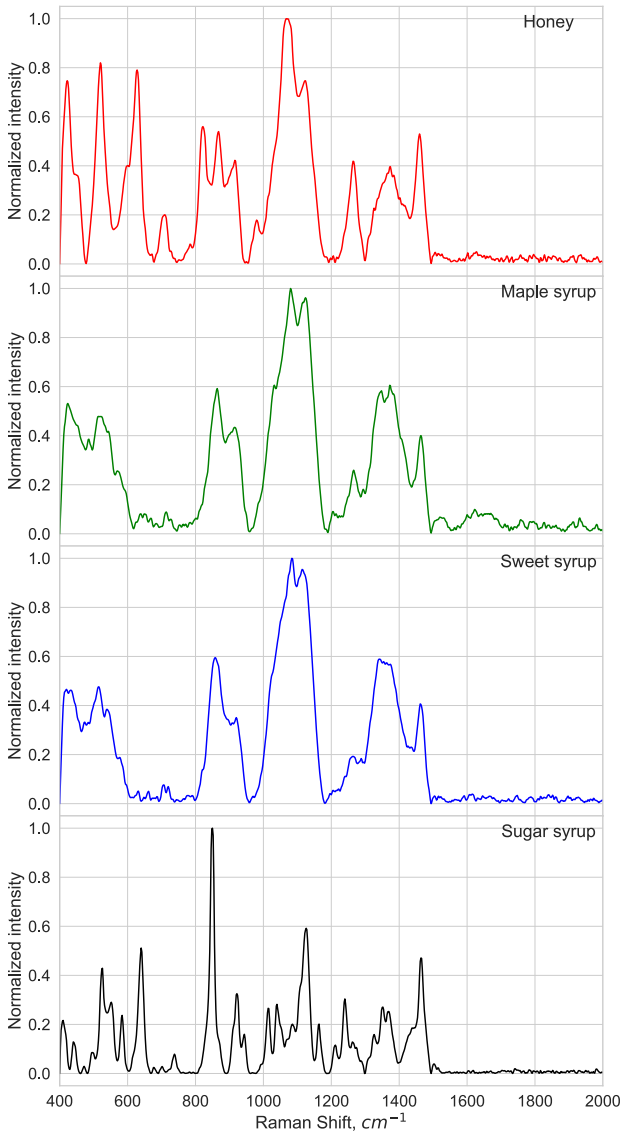
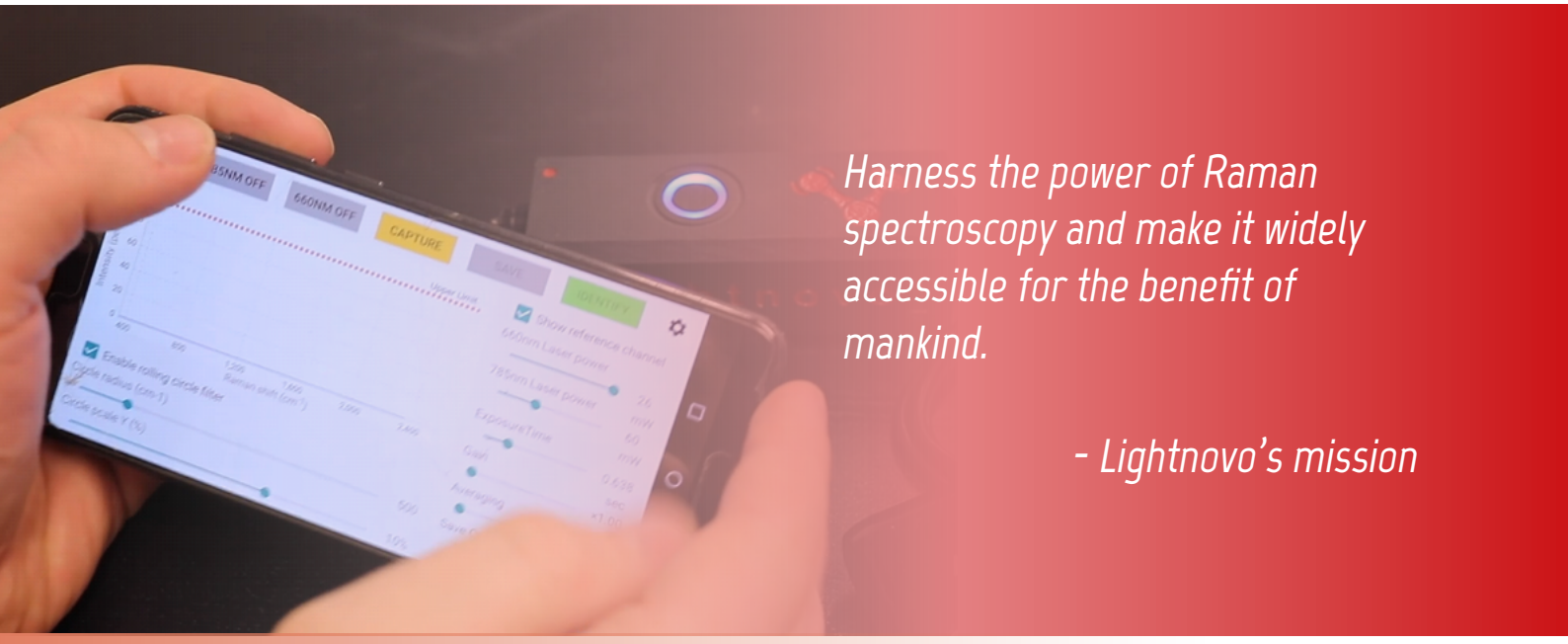


Figure A2. Averaged baseline-subtracted and normalized (Miraspec) spectra of honey, maple, sweet and sugar syrups.

The raw spectra in Figure A1, despite the fluorescent background, have some unique peaks that could hypothetically be used to differentiate honey, syrups based on natural sweeteners (as a class) and sugar syrup. However, this approach does not allow to distinguish between maple and sweet syrups, which have very similar spectra due to the resembling chemical compositions. Of course, in this particular case, the problem may be solved by the fact that maple syrup gives almost twice the strong fluorescence signal, which is most likely due to the presence of flavouring agent. Nevertheless, it is obvious that, in the general case, for deep analysis and/or further processing of spectra, fluorescence must be completely rejected.

Figure A2 demonstrates that Miraspec software copes with this task perfectly, completely eliminating the slope of the baseline. At the same time, no noticeable distortion of the Raman peaks is observed, which is clearly visible in the spectrum of sugar syrup. This sample produces relatively weak fluorescence that doesn't significantly suppress its Raman peaks, allowing easy comparison of raw and postprocessed spectra.



Harness the power of Raman spectroscopy and make it widely accessible for the benefit of mankind.

- Lightnovo's mission

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