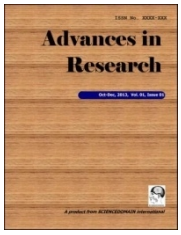


Advances in Research
2(2): 95-108, 2014, Article no. AIR.2014.004

SCIENCEDOMAIN *international*
www.sciencedomain.org

Fluorescence Spectroscopy as Method for Quality Control of Honey

Kr. Nikolova[1*], T. Eftimov[2] and A. Aladjadjiyan[3]

[1]University of Food Technologies, Department Mathematics and Physics, 4002 Plovdiv, Bulgaria.
[2]Plovdiv University, Department Experimental Physic, 4000 Plovdiv, Bulgaria.
[3]Agricultural University, Department Mathematics, Informatics and Physics, 4000 Plovdiv, Bulgaria.

Authors' contributions

This work was carried out in collaboration between all authors. Author KN choice the samples, measured its sugar content and fluorescence spectra, performed the mathematical analysis of the obtained data. Author TE construct basic configuration for the observation of fluorescence spectra. Author AA managed the discussion and final manuscript. All authors read and approved the final manuscript.

Original Research Article

Received 21st October 2013
Accepted 14th November 2013
Published 5th February 2014

ABSTRACT

The present work aims in exploring the possibilities of fluorescent spectroscopy for quality control of honey, particularly to distinguish types of honey and to distinguish the natural honey from that with artificial additives - sweeteners.
24 samples of the most often encountered sorts of honey in Bulgaria distributed in 7 groups have been studied. The samples have been excited with light-emitting diodes (LEDs) emitting in the interval from 375 to 450 nm. Fluorescence spectra of all investigated honey samples have a peak at λ = 490 nm, and for some honey samples with iso sweet an additional one at λ = 505 nm. The ratio I_{425}/I_{375} of the peak intensities of fluorescence spectra can be used for differentiation between natural honeys (from 1.07 to 3.35) and honey with sweeteners and honeydew About (greater than 4).
The first derivatives of the fluorescence spectra of the blossom honeys and honeydew show two peaks at about 422 nm and 480 nm. However, the first derivatives for samples with sweeteners have many different maxima and smaller amplitudes in the variations but the most clearly expressed are those around 393 nm, 480 nm and 533 nm for iso sweet

**Corresponding author: E-mail: kr.nikolova@abv.bg;*

and 493 nm and 479 nm for glucose additions.
The obtained results show that the investigation of fluorescence spectra opens the possibility for distinguishing honey samples with added artificial sweeteners from natural ones except for dew honey.

Keywords: Fluorescence spectroscopy; honey; high performance liquid chromatography (HPLC).

1. INTRODUCTION

The recent global scale industrial food production is based on the use of artificial additives. Sometimes this fact causes serious problems because of the allergies and the predisposition of consumers to some other diseases which makes the development of methods for food control very important [1].

Fluorescence is short-time photoluminescence that is expressed as a secondary emission of light spectra from objects preliminarily irradiated with electromagnetic radiation [2].

Honey is a natural sweet substance produced by bees through transforming natural nectar of plants. It is well known and favorable for its valuable nutritional and medicinal qualities [3]. Honey composition depends on its type and origin. Honey contains monosaccharides, macro- and micronutrients, antioxidants, free amino acids, organic acids, vitamins, enzymes and minerals [4]. The content of these components varies in different sorts of honey. Sometimes sweeteners are added in industrially produced honey, which compromises its quality, as all unfamiliar substances added to honey falsify its quality.

The content of water, glucose, fructose, saccharose, the hydroxymethyl furfural (HMF) content, acidity, conductivity, diastase activity, essential and toxic metal ions are major parameters defining the quality of honey and they are related to its physical properties [5]. Parameters with stronger impact on consumers are the color, the crystallization state and the fermentation grade of honey; these parameters affect optical properties of honey [6,7].

As it is well known, sugar content is one of the basic parameters for the evaluation of the quality of honey. Honey contains the most widely spread glucose, fructose and sacharose in following percentages: 31.3%, 38% and 8%, respectively [8]. The variety of components in bee honey is an important criterion for quality and it is related to some significant particularities of the corresponding sample. For example, honey with high water content is susceptible to fermentation [9]. The protracted storage of the product leads to an increase of the inverted sugar and the acidity of the product [10]. The mineral content is related to the botanic origin of honey and it is known that herbs honey is with poorer mineral content than honey dew [11].

The presence in honey of different quantities of aromatic amino acids, vitamins, phenolic components, enzymes and minerals makes fluorescent spectroscopy technique applicable to distinguish different groups of honey [12].

Conventional methods (such as high performance liquid chromatography HPLC) for detecting adulteration are based on monitoring the ratio of the relative concentration of the different forms of the carbohydrates, which are different for natural honey and adulterates

[13]. The other method used for monitoring the authenticity of honey is mass spectrometry [14]. The present work aims at exploring the possibilities of fluorescent spectroscopy for quality control of honey, particularly to distinguish types of honey and to distinguish the natural honey than that with artificial additives –sweeteners.

Generally fluorescence is used in food industry to investigate the quality of fish, oils, meat and other products since the year 2000. There are however, only a few works investigating honey quality [10]. The sources traditionally are based on Xenon lamps and bulk monochromators which limits the methods to laboratory analysis. The proposed fluorescence method is perspective, time saving, faster and easier to implement, without using chemical treatments. It is low cost because it uses LEDs in visible region and thus it is perspective for field analysis. The use of LEDs and first derivatives of fluorescence spectra is an improvement of the fluorescence methods used in food analysis to the moment.

2. MATERIALS AND METHODS

2.1 Samples

About 24 samples of honey were collected from different localities in Bulgaria, sub-divided into seven groups according their origin: linden, acacia, sunflower, wild herb, dew, thistle, and polyfloral honeys with sweeteners (Table 1). In each group excluding the one with sweeteners, samples were purchased directly from commercial and domestic producers. Samples were stored at room temperature and kept in dark for a maximum of 20 days prior to analysis. Approximately 200 g of each sample were heated in a water bath at 40ºC to melt existing crystals. Then honey samples to be tested were left to cool at room temperature for 1 hour.

Table 1. Geographical and botanical origin of honey samples

Sample no.	Geographical origin	Predominant botanical origin	Year of harvesting
1	Tryavna	Honeydew	2009
2	Elena	Honeydew	2009
3	Karlovo	Honeydew	2009
4	Asenovgrad	Lime (*Tilia*)	2009
5	Montana	Lime *(Tilia)*	2010
6	Karlovo	Lime (*Tilia)*	2010
7	Asenovgrad	Acacia (*Robinia pseudoacacia*)	2008
8	Karlovo	Acacia (*Robinia pseudoacacia*)	2009
9	Galabovo	Acacia (*Robinia pseudoacacia*)	2010
10	Asenovgrad	Thistle (*Cardus nutans*)	2008
11	Montana	Sunflower (*Helianthus annuus*)	2009
12	Montana	Sunflower (*Helianthus annuus*)	2009
13	Montana	Sunflower (*Helianthus annuus*)	2008
14	Sliven	Polyfloral	2008
16	Samokov	Polyfloral	2009
17	Troian	Polyfloral	2009
18	Triavna	Forest honey	2010
19	Elena	Forest honey	2008
20	Triavna	Forest honey	2009
21	Undefined	Polyfloral with glucose	2009
22	Undefined	Polyfloral with glucose	2008
23	Undefined	Polyfloral with isosweet	2010

2.2 Methods

The content of sugars was determined by high performance liquid chromatography (HPLC) method after filtering the honey solution.

The sugar content of honey samples (monosaccharide content) has been determined using an HPLC-chromatographic system WATERS with a refractometric detector Waters R401 (UK) and an Aminex HPX – 87H column (300 x 7.8 mm, BioRad). The analytic parameters were as follows: mobile phase 0.004 mol/l H_2SO_4, flow rate 0.5 ml/min, temperature of 30ºC. The sensitivity of the method is 1g/kg and the repeatability was determined after an analysis of 3 samples within a day and expressed by the relative standard deviation RSD=0.06 ÷ 2.12 for the different representative samples [15]

The water content has been determined by measuring the refraction coefficient of studied sample with an Abbe refractometer (Carl Zeiss, Germany) at temperature 20±0.5ºC. The equivalent water content was determined from the table, given in official methods of analysis [16].

2.3 Used Equipment

A laser diode (LD) is preferable as a light source for measuring the fluorescence because of its narrow spectral width (<1 nm). However, since the range of laser sources having a suitable central wavelength is limited, light emitting diodes (LEDs) with significantly larger spectral widths (~20-40 nm) are often being used. Usually their emission angular distribution lies in a larger angle range of ±30º. In our tests we used low-cost LDs and LEDs (NS 370L-5RL0, LED 395-03V, LED 425-6-30, LED-450-01V, Roithner-laser, Austria).

Specially selected LEDs (roithner-laser) with an integrated lens were used for the present study. Such a lensed LED minimizes the divergence angle to a range of less than ±10º. The LEDs used have been powered by an adjustable DC voltage supply through a limiting resistor of r = 100 Ω branched in series with the LED. LEDs were directly mounted into a lens carrier so as to obtain a collimated beam. The basic experimental configuration for the measurements carried out is presented in Fig. 1.

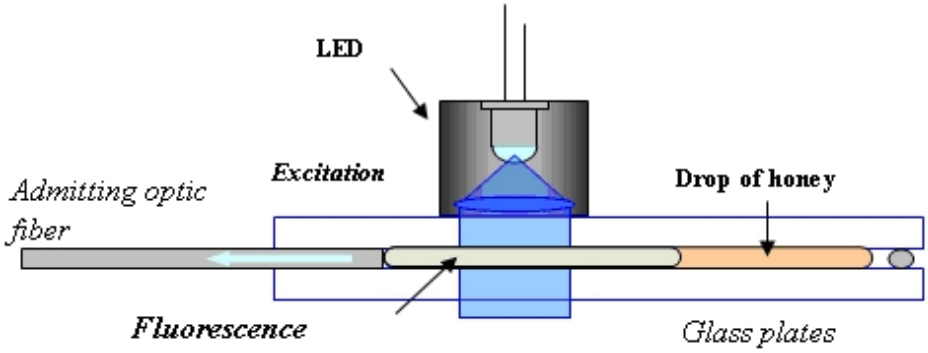


Fig. 1. Basic configuration for the observation of fluorescence spectra

The selected LEDs are directly mounted to the input and their radiation falls into the sample. The information from the receiver is transmitted to the spectrophotometer through an optical

fiber. To measure the fluorescence spectra of the studied samples the same pair of plates was used and the receiving optical fiber was placed between them and dipped in the honey sample. The honey in the area before the receiving fiber was irradiated with a perpendicular parallel beam from selected LEDs. Thus, the optical fiber receives a fluorescent signal perpendicular to the excitation radiation, which minimizes illumination from a diffuse radiation of the source of excitation.

The particular experimental set-up used in our experiments is shown in Fig. 2. Fluorescence and scattering spectra are recorded using fiber-optic spectrometer *Avantes 2048* (Avantes, Netherlands) having a spectral sensitivity within the 250-1100 nm range. The resolution of the spectrometer is $\delta\lambda \approx 5$ nm. An optical fiber with a core diameter of 200μm was used to bring light to the probe and to measure the scattered and fluorescent light. A collimator with a lens of an aperture $d = 5$ mm was used to gather more light and focus it into receiving fiber connected to the spectrometer. Integration time was 50 ms and each spectrum was averaged automatically over 50 independent consecutive measurements. To block stray light the cuvette holder was supplied with a cap.

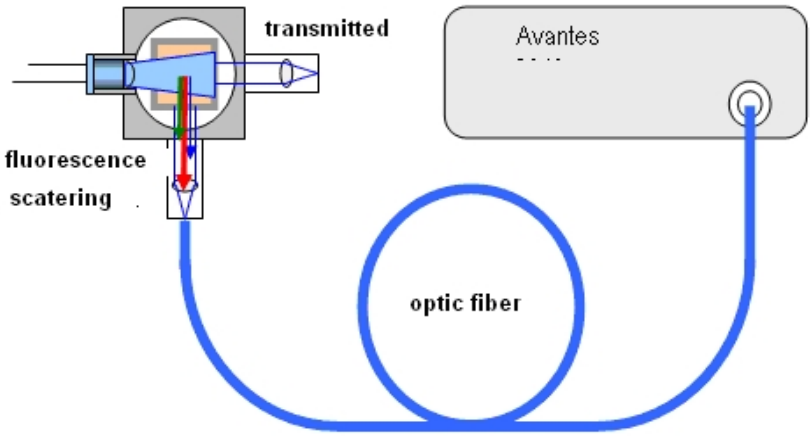


Fig. 2. Experimental set-up for the observation of fluorescence spectra

Recently, instrumental improvements and the availability of specially designed software for extracting the information from spectra have contributed to the development of fluorescence spectroscopy.

All analyses were carried out in triplicate and the average data are presented in tables. Differences between the averages at the 95% (p<0.05) confidence level were considered statistically significant**.** Since the investigated samples of each group were three (except for thistle honey) and the differences between the fluorescence spectra of honey from different regions with the same botanical origin were small, the results for fluorescence spectra were averaged once again for each group of different botanical origin. The fluorescence spectra for each group of samples after averaging the signal of all samples were presented in the figures below.

3. RESULTS AND DISCUSSION

As it is known, one of the key parameters for assessing the quality of the honey is the sugar content therein. Long storage of this honey leads to an increase of the invert sugar and the acidity of the product [10]. The average values of water content *W*, refraction index *n* and the content of glucose, fructose and oligosaccharides for each of investigated seven groups honey is presented in Table 2.

Table 2. Physical - chemical characteristics of natural bee honey and honey with sweeteners (glucose and fructose) from Bulgaria

Number of samples	Predominant botanical origin	n λ = 589.3 nm, t = 20°C	W.%	Sugars, %			
				Oligo-saharides	Saccharose	Glucose	Fructose
3	Honeydew	1.4913 ± 1.10^{-4}	18.13 ± 0.05	2.07 ± 0.06	9.4 ± 0.35	40.63 ± 0.59	47.83 ± 0.21
3	Linden (*Tilia*)	1.4967 ± 1.10^{-4}	15.97 ± 0.57	2.1 ± 0.01	11.43 ± 1.13	36.67 ± 0.43	49.77 ± 0.27
3	Acacia (*Robinia pseudoacacia*)	1.4957 ± 1.10^{-4}	16.33 ± 0.46	3.7 ± 0.14	10.6 ± 0.35	36.07 ± 0.31	49.63 ± 1.27
3	Sunflower (*Helianthus annuus*)	1.4908 ± 6.10^{-4}	18.17 ± 0.23	2.30 ± 0.40	8.5 ± 1.3	40.6 ± 0.41	48.6 ± 0.39
3	Polyfloral	1.4938 ± 1.10^{-4}	17.07 ± 0.58	4.34 ± 0.38	10.1 ± 1.32	35.7 ± 1.44	50.40 ± 0.27
3	Forest honey	1.4934 ± 1.10^{-4}	17.27 ± 1.51	4.57 ± 0.37	12.3 ± 0.56	36.0 ± 0.49	47.13 ± 0.77
1	Thistle (*Cardus nutans*)	1.4940 ± 1.10^{-4}	17 ± 1.31	----------	7.6 ± 0.32	41.3 ± 0.31	51.1 ± 2.12
5	With sweeteners isosweet (invert sugar)	$1.4920 \pm 4 \times 10^{-4}$	17.8 ± 0.2	3.8 ± 0.1	13.8 ± 0.2	35.2 ± 0.3	47.2 ± 0.3
	Glucose	$1.4930 \pm 7 \times 10^{-4}$	17.4 ± 0.4	27.6 ± 0.2	10.9 ± 0.2	31.8 ± 0.2	29.6 ± 0.3

The contents for each type of sugar in percents are calculated relative to the total content of sugars in the samples excluding water content from the chromatographic peaks.

Moisture content in percents in the analyzed honeys ranged from 15.97 to 18.17 %. The water content in honeys depends on various factors, like the harvesting season, the degree of maturity reach in the hive and climatic factors. The maximum amount water contained by honeys is regulated for safety against fermentation. All the samples contained less than 20% water -the maximum amount allowed [17].

One of the main criteria for assessing the quality of honey is its sugar content. According to the criteria of the European Union [17], the content of invert sugar (glucose + fructose)

should not be less than 60% and that of saccharose not more than 5%. According to the data in Table 1, all samples meet the first condition for exporting honey to the European Union; in some of them, however, the saccharose content exceeds 5%. The latter fact can be explained by the honey extraction prior to its maturity [18]. The addition of glucose-fructose syrup to herbal honey results in a drastic increase of the oligosaccharides to 27.6%, while the presence of isosweet in honey does not result in a significant difference in the percentage of the oligosaccharides. They are in the same range as that of unadulterated honey. The addition of sweeteners to honey increases the water content to 18 - 19%, which falls within the standards for quality of honey [19], still creates conditions for a faster crystallization of the product. Since HPLC is a relatively expensive and time consuming method requiring also chemical reagents, an attempt was made to use fluorescent spectroscopy as a rapid and effective method to distinguish natural honey samples from those with added sweeteners.

Fluorescence spectra of honey samples are obtained through irradiation by LEDs (Roithner-laser, Austria) with center wavelengths respectively at λ =375 nm, λ =395 nm, λ =425 nm and λ =450 nm.

The spectra are presented in Figs. 3 and 4 for excitation wavelengths of respectively 375 nm and 425 nm. Fluorescence spectra for 395 nm and 450 nm are very similar to those of 375 nm and 425 nm and by this reason they are not shown.

Fluorescence peaks for most of the samples is observed at λ *=490 nm* and λ *=505 nm*, as can be seen from Figs. 3 and 4.

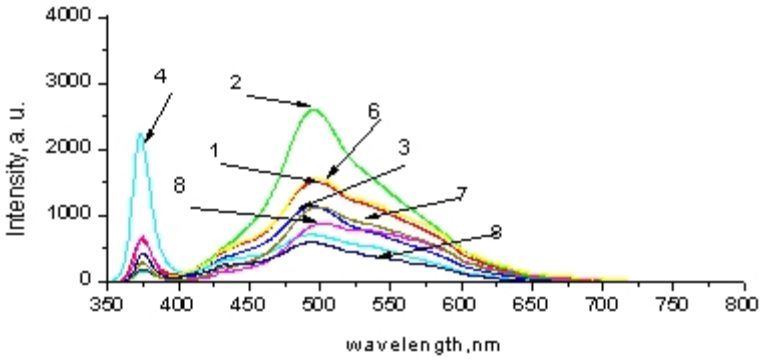


Fig. 3. Fluorescence spectra of honey at excitation wavelength of 375 nm
1-linden; 2- forest honey; 3-acacia; 4-sunflower; 5-thistle; 6- honey with isosweet; 7-honey with glucose; 8- dew honey.

The highest intensity of fluorescence peak was accounted for samples of forest honey, and the lowest one – for these of trade marks that are counterfeited with glucose. In all samples, except for dew honey a maximum fluorescence peak is observed at about 493-496 *nm*. For samples of dew honey peaks appear respectively at λ = *503 nm*. The latter fact can be attributed to the higher content of amino acids and phenolic components in that type of honey. This is confirmed by the literature [20]. According to [19-21] the shape of fluorescent spectra depends on the content and type of amino acids in honey and this fact can be used as a tool for distinguishing the botanical origin of honey.

The similar result is obtained in [15] at the excitation wavelength of 373 nm. The emission spectra of acacia and dew honey have the similar shape with those obtained in our investigation (Fig. 3). They have the fluorescence maximum at about $\lambda = 450\text{nm}$, Bulgarian honeys from the same type show fluorescence maximum at about $\lambda = 495nm \div 505nm$. This is caused by the numerous fluorescing compounds in the various honey types in different concentrations and environments for the samples of same botanical origin, but from different countries. In contrast, the chestnut honey shows a different peak from those of Bulgarian honeys at about 380nm. This observation can be explained with the assumption that this type of honey compared to Bulgarian types analysed in this study contains high amount of cafeic, *p*-coumaric and ferulic acids [22-23]. It is known that it contains more phenylalanine that the sunflower, lime and honeydew honeys.

The best differentiation of fluorescence spectra for different groups was observed in the exposure of samples to λ*=425 nm*. Fig. 4 presents these spectra.

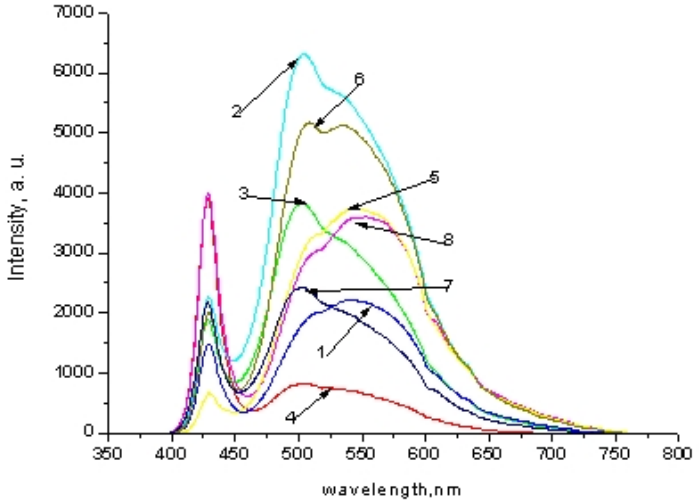


Fig. 4. Fluorescence spectroscopy of honey at excitation wavelength 425 nm.
1-linden; 2- forest honey; 3-acacia; 4-sunflower; 5-thistle; 6- honey with isosweet; 7-honey with glucose; 8- dew honey.

At that wavelength, not only the intensity of the fluorescent peaks but also their shape is different for the different honey groups at λ_{exc} = 425 nm. At that wavelength the spectral maxima of thistle honey and dew honey have almost the same intensity and shape, while for acacia honey and honey with glucose the maximum is shifted to shorter wavelengths. This observation may be attributed to the high water content in the last two honey groups. Only the sample with isosweet has two very close fluorescence maxima at about 510 nm and 550 nm. The observed differences in spectra may be linked to the water content, which depends on the climate, season and humidity in the plant from which the nectar is gathered [24]. There is also a shift of the wavelength at which the fluorescent peak occurs. As it was established by Sanz et al. [24], it may be due to the emergence of substances such as furosine and hydroxymethylfurfural (HMF) components. These substances can be used as an indicator for the presence of sweeteners in commercial samples.

The ratio of the fluorescence peak intensity *I′* (in arbitrary units) to the intensity of scattered light of the excitation radiation *I* (at the wavelength of excitation) is determined for investigated samples. This ratio does not allow to clearly distinguish natural honey samples from those with artificial sweeteners. It allows the clear distinction the honey of wild plants from the one of sunflower as a cultivated plant. The ratio *I′⁄I* for the samples of monofloral or polyfloral herb honey lays in the interval 1÷16.4 , while the same ratio for sunflower honey is very low – between 0.2 and 0.4. Last observation can be attributed to the high content of water in sunflower honey, due to irrigation which leads to comparatively lower concentration of polyphenols and amino acids that are the main sources of photoluminescence in bee honey.

Data about fluorescence spectra are presented in Tables 3 and 4, for λ = 425 and 375 nm, respectively.

From the data presented in Tables 3 & 4 it can be seen, that for the irradiation with wavelength λ = 425 nm, except for the polyfloral forest honey, the sample with isosweet has the highest fluorescence intensity. Similar observation is commented by Glosh [13] for samples of chestnuts honey with added artificial sugar syrup.

Table 3. Main characteristics of the fluorescent spectra of samples of honey from Bulgaria at *λ=425 nm*

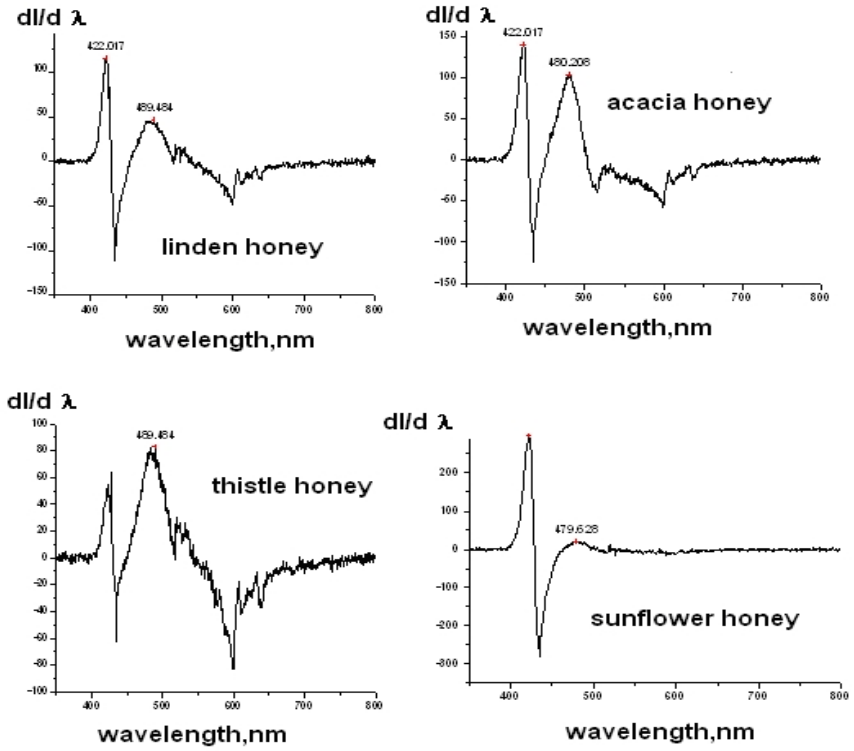
Honey samples	λ, nm	I', **a.u.**	I, **a.u.**	I'/I r.u.
Acacia	503.4	3828.8	1884.9	2.0
Linden	541.5	3828.8	1884.9	2.0
Sunflower	520	762.2	3910	0.2
Thistle	553	3601	666.3	5.4
Polyfloral forest honey	503.4	6311.1	2265.7	2.8
Dew	553	3601.9	3984.7	0.9
Honey with glucose-fructose syrup	502.8	2420.6	2400	1
Honey with isosweet	500.5	5169.7	2012.7	2.6
	536	5134.3		2.5

Table 4. Main characteristics of the fluorescent spectra of samples of honey from Bulgaria at *λ=375 nm*

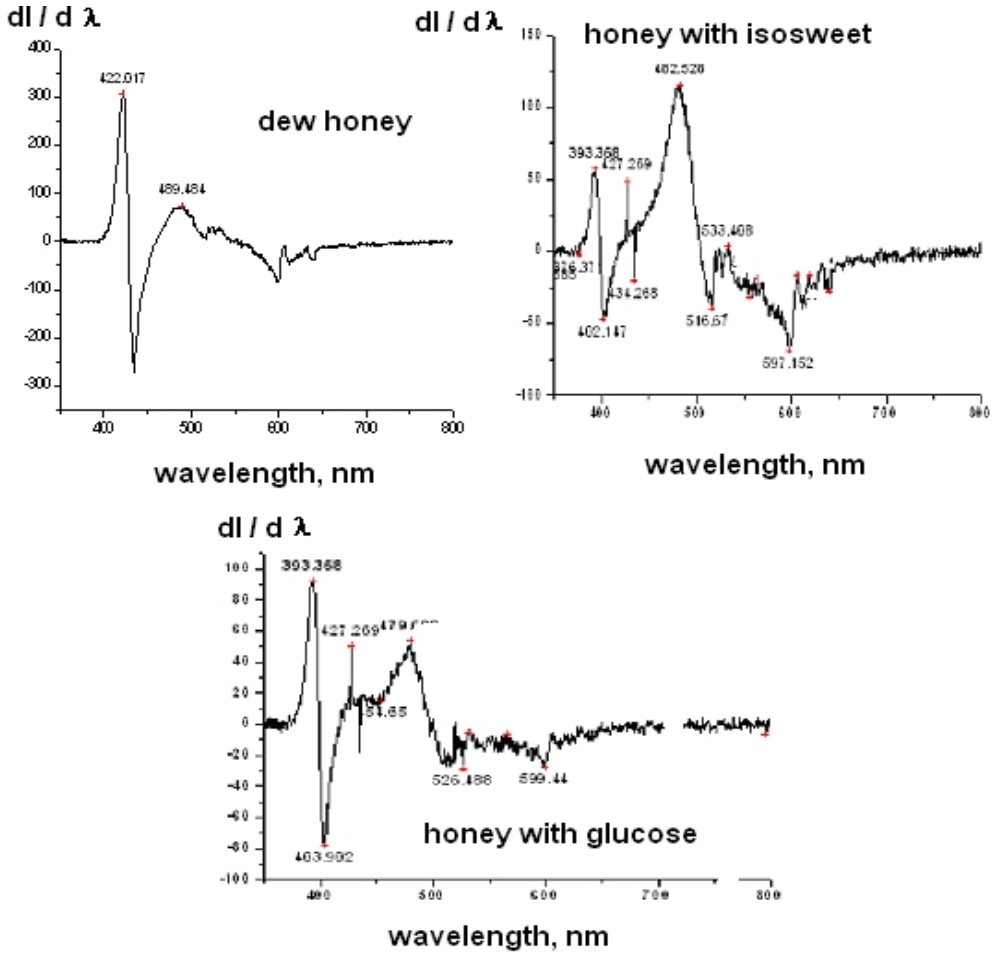
Honey sample	λ, nm	***I*, a.u.**	***I*, a.u.**	***I′/I* r.u.**
Acacia	495.3	1142.8	1184.92	1.04
Linden	496	1515.3	637.1	2.38
Sunflower	494	711.3	2231.3	0.32
Thistle	496	1559	296	5.26
Polyfloral forest honey	495	2599.63	158.93	16.36
Dew	503.4	882.25	675.94	1.31
Honey with glucose-fructose syrup	494	599.3	430	1.39
Honey with isosweet	501	1126.4	286.5	3.93

At λ = 425 nm the samples with low *I'/I* ratio (between 0.2 and 1.0), correspond to high water content, determined with refractometer according to a reference table provided in the official method [16]. The samples of dew honey, sunflower and the one with added sweetening glucose-fructose syrup have a water content of respectively 18.1%, 18.2% and 19%. This fact may be the reason for the lower intensity of the fluorescence maximum, caused by the presence of lower content of proteins, peptides and free amino acids [25]. All the rest samples of bee honey have water content between 16.4% and 17.4%.

Observed data about intensity ratio do not give enough information for distinguishing natural honey samples from those with added sweeteners. In searching new possibilities the first derivatives of fluorescence spectra have been determined. The first derivatives of the fluorescence spectra of the investigated honey samples have been obtained for excitation wavelengths of 425 nm because the differences at this wavelength are the largest. The first derivatives of fluorescence spectra are shown in Fig. 5.



a-linden honey, acacia honey, sunflower honey, thistle honey



b- honey with glucose, honey with isosweet, dew honey

Fig. 5. First derivatives of the fluorescence spectra of natural honeys and honeys adulterated with sweeteners

Although the *I'/I* ratio of the investigated samples differs considerably, the first derivatives of the natural honeys are similar and characterized with two clear peaks, first at 422 nm and second in the range 480-489 nm. First peak may be attributed to the presence of tryptophan and tyrosine in comparatively big quantities in natural honey samples, while the second one is related to the presence of riboflavin [25].

For the samples with glucose and iso-sweet the first peak shifts by -30 nm to about 392 nm, while the second peak does not exhibit an essential shift. Instead, an increase of the underlying area is observed for the adulterated samples. The shift of first peak may be attributed to nicotine amide adenine dinucleotide that is present in big quantities in the sugar syrup and that weakens the fluorescence [24]. For the natural honey samples, with the exception of the thistle honey, the peak is clearly observable with a small underlying area and not additional maxima above 600 nm. For the samples with iso-sweet and glucose noises are observed which are not characteristic for the samples of natural polyfloral or monofloral honey. For the samples with added sweeteners two narrow peaks are observed

at about 427.27 *nm* and 434.27 *nm* that are characteristic only for these samples with sweeteners and do not appear in the fluorescence spectra first derivatives of any other honey samples.

The intensity of the maximum in fluorescence emitting spectrum at different irradiation wavelengths for the samples in Tables 1 & 2 does not allow distinguishing the natural samples from those with artificial sweeteners. Another means for definite separation in two groups – first of the natural polyfloral and monofloral honey samples and the second of those samples with sweeteners and dew honey may be achieved by investigating the ratio of fluorescence maxima at λ = 425 and 375 nm.

The data are presented in Table 5.

Table 5. The ratio of fluorescence maxima at λ = 425 and 375 nm

Honey sample	I_{375},a.u	I_{425},a.u	I_{425}/I_{325}
Acacia	1142.8	3828.8	3.35
Linden	1515.3	3828.8	2.53
Sunflower	711.3	762.2	1.07
Thistle	1559	3601	2.31
Polyfloral forest honey	2599.6	6311.1	2.43
Dew	882.25	3601.9	4.08
Honey with glucose-fructose syrup	599.3	2420.6	4.04
Honey with isosweet	1126.4	5170	4.59

The ratios I_{425}/I_{325} obviously distribute the honey in three main groups (Table 5):

First group is this of natural polyfloral and monofloral honeys from wild plants, whose ratio I_{425}/I_{325} lies between 2 and 3.5.

Second one is for cultivated plants (sunflower) with a ratio close to 1.

Third is the group of honeys with added artificial sweeteners with the highest ratio I_{425}/I_{325} - between 4 and 4.6. Similar results are obtained in [13]. The ratio could be used to monitor the adulteration of honey with sugar syrup.

4. CONCLUSIONS

Fluorescence spectra of all investigated honey samples have peaks at λ = 490*nm* and an additional one for honey with isosweet at λ = 500.5 *nm*.

The ratios I_{425}/I_{325} of peak intensities of fluorescence spectra enable to sort honey samples in three main groups: for natural polyfloral and monofloral honeys from wild plants this ratio is between 2 and 3.5, for cultivated plants (sunflower) with a ratio close to 1 and for honeys with added artificial sweeteners this ratio is highest between 4 and 4.6.

The first derivatives of the fluorescence spectra of the investigated honey samples have been calculated and it was found that it shows specific features for the samples with artificial sweetener. The first derivatives for blossom honey and dew honey shows two peaks at

about 422nm and 480 nm. The first derivatives for samples with sweeteners with the most clearly expressed maximum are these around 383nm, 480 nm and 533nm.

Observed results show that the investigation of fluorescence spectra creates an instrument for distinguishing honey samples with added artificial sweeteners from natural ones except for dew honey.

ACKNOWLEDGEMENTS

This work was inspired by the investigations made in frames of the project ISEKI-food, in which UFT, Plovdiv and Agricultural University, Plovdiv participate since 2006 till the moment. The partial financial support for this work from the NI 13 FF003 research grant of the Plovdiv University is acknowledged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Feas X, Pires J, Iglesias A, EStevinho M. Characterization of artisanal honey produced on the Northwest of Portugal by melissopalynological and physico-chemical data. Food and Chemical and Chemical Toxicology. 2010;48:3462-3470.
2. Christensen J, Norgaard L, Bro R, Engelsen S. Multivariate. Autofluorescence of intact food systems. Chemical reviews. 2006;106:1980-1994.
3. Lachman J, Kolihova D, Miholova D, Kosata J, Titera D, Kult K. Analysis of minority honey components: Possible use for the evaluation of honey quality. Food Chemistry. 2007;101:973-979.
4. Conzalez-Mizet ML, Terrab A, Hernandez D, Fernandez-Recamales MA, Heredia FJ. Multivariate correlation between color and mineral composition of honey and their botanical origin. Food Chemistry. 2005;53:2574-2579.
5. 5.Silva L, Videira R, Monteiro An, Valentao P, Andrade P. Honey from Luso region: Physicochemical characteristics and mineral contents. Microchemical journal. 2009;93:73-77.
6. Juszczak L, Socha R, Roznowski J, Fortuna T, Nalepka K. Physicochemical properties and quality parameters of herbal honeys. Food Chemistry. 2009:113:538-542.
7. Przybylowski P, Wilcrynska A. Honey as an environmental marker. Food Chemistry. 2001;74:289-293.
8. Khalifa Al, Arify Al. Physicochemical characteristics and pollen spectrum of some Saudi honeys. Food Chemistry. 1999;67:21-25.
9. Council Direktle 2001/110 EU of 20 December 2001 relating to honey (2002) Official journal of the European Communities. 2002;10:47-52.
10. White WJ, Riethof ML. Composition of honey of the effect of storage on carbohydrates, acidity and diastase content. Journal of Food Science. 1999;26:63-66.
11. Volwohl G, Salem MS, Nour ME. Chemical and Physical Properties of Egyptian Honeys. Proc. 4 Int. Conf. Apic Trop. Climates, Cairo. 1989;240-244.
12. Karoui R, Dufour J, Bosset O, Baerdemaeker J. The use of front face fluorescence spectroscopy to classify the botanical origin of honey samples produced in Switzerland. Food Chemistry. 2007;101:314–323.

13. Glosh N, Verma Y, Majumder SK, Kupta PK. A fluorescence spectroscopic study of honey and cane sugar syrup. Food Science Technology Research. 2005;11:59-62.
14. Martin GI, Macias EM, Sanches JS, Rivera BG. Detection of honey adulteration with beet sugar using stable isotope methodology. Food Chemistry. 1998;281-286.
15. Kaspar R, Romdhane K, Dufour E, Bosset OI, Bogdanov S, Amado R. Authentication of the Botanical Origin of Honey by Front-Face Fluorescence Spectroscopy. A Preliminary Study. Journal of Agricultural and Food Chemistry. 2006;54: 6858-6866.
16. Association of Official Analytical Chemist. Official Methods of Analysis. Arlington. VA, 1995.
17. Council Direction 2001/110 EU of 20 December 2001 relating to honey. Official Journal of the European Communities. 2002;10:47-52.
18. Chepurnoy IP, Expertise of quality of honey Moscow: Daskov and Co Publishing House, 2002.
19. Cometto PM, Faye PF, Naranjo Di Paola, Rubio RD, Aldao MAJ. Comparison of free amino acids profile in honey in the Argentinian regions. Journal of Agricultural and Food Chemistry Journal of Agricultural and Food Chemistry. 2003;51:5079-5087.
20. Rodriguez-Delgado MA, Malovana, Perez JP, Borgez T, Montelongo Garcia. Separation of phenolic compounds by high-performance chromatography with absorbance and fluoretric detection. Journal of Chromatography. 2001;912:249-257.
21. Pirini A, Conte LS, Francioso O, Lercker G. Capillary gas chromatographic determination of free amino acids in honey as a means of determination between different botanical sources. Journal of High Resolution Chromatography. 1992;15:2633-2637.
22. Amiot M, Aubert J, Gonnet S, Tacchini MM. Les composés phénoliques des miels: etude préliminaire sur l'identifi cation et la quantifi cation par familles. Apidologie. 1989; 20:115-125.
23. Tomás-Barberán F, Martos I, Ferreres F, Radovic BS, Anklam E. HPLC fl avonoid profi les as markers for the botanical origin of European unifl oral honeys. J. Sci. Food. Agric. 2001;81:485-496.
24. Sanz ML. Del Castillo MD, Corzo N, Olano A. Furoylmethyl amino acids and hydroxymethylfurtural as indicators of honey quality. Journal of Agricultural and Food Chemistry. 2003;51:4278-4283
25. Ruoff K, Karoui R, Bogdanov, et all. Authentication of the botanical origin of honey by font-face fluorescence spectroscopy. Journal of Agricultural and Food Chemistry. 2005;53:1343-1347

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=392&id=31&aid=3542