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Characterization of Estonian honeys by botanical origin

Evelin Kivima[a,b*], Andrus Seiman[b], Raili Pall[a], Evelyn Sarapuu[a], Kaie Martverk[a], and Katrin Laos[a,b]

[a] Department of Food Processing, Tallinn University of Technology, Ehitajate tee 5, 12618 Tallinn, Estonia
[b] Competence Center of Food and Fermentation Technologies, Akadeemia tee 15A, 12618 Tallinn, Estonia

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Abstract. This study characterizes Estonian honeys based on their physico-chemical properties and chemical composition. Melissopalynological analysis was carried out to determine the botanical origin of each honey. According to pollen analysis, 39% of the honeys analysed appeared to be unifloral rape (*Brassica napus*), raspberry (*Rubus idaeus*), or heather (*Calluna vulgaris*) honeys.

Fluorescence spectroscopy was used to estimate both the physico-chemical parameters and floral content of each honey sample by comparing these estimates with experimental data measured using standard techniques. The r^2 correlation between estimated values and experimental data was above 0.7 for several parameters, including free acidity with an r^2 of 0.919.

Key words: honey, physico-chemical properties, melissopalynology, front-face fluorescence.

1. INTRODUCTION

Honey is a complex product composed of monosaccharides such as glucose and fructose and other components, including amino acids, proteins, minerals, enzymes, and vitamins (White, 1975). The exact composition of any given honey depends mainly on the plant sources it is derived from, but also on the weather, soil, and other factors; therefore no two honeys are identical (Crane, 1980).

Quality parameters of honey are specified in a European Directive, which brings out the physico-chemical criteria for honey, such as moisture content, electrical conductivity, free acidity, diastase activity, hydroxymethylfurfural (HMF) content, ash content, and sugar content (EU, 2002). These parameters, together with melissopalynological analysis, can be used to authenticate the botanical origin of honey. In recent years the botanical origin of honey has also been determined using front-face fluorescence spectroscopy because the spectra obtained using this method contain a large amount of information regarding the chemical content of honey. Natural fluorophores in honey include aromatic amino acids, nucleic acids, HMF, furosine, and phenolic compounds. The concentrations of these fluorophores can vary to a large degree depending on the geographical and floral origin of the honey (Ruoff et al., 2006).

A library of knowledge of honey types allows one to discern honeys from different regions in Europe and those that originate from other continents (Maurizio, 1975). Pollen analysis of multifloral honeys indicates their botanical composition, as represented by the spectrum of pollen variability, and can also be used to determine if a honey is a blend of different honeys, and if so allows one to approximate the blending ratios (Agashe and Caulton, 2009). Precise identification of the discrimination point between multifloral and unifloral honeys can nevertheless be difficult. However, there are specific physico-chemical properties that can be used to confirm the results of microscopical analysis.

Considering the number of floral sources visited by the bees and small areas of certain plant types during the flowering period, pure unifloral honeys can rarely be obtained in Estonia, with the most common exception being rape honeys. The most widespread plants in Estonia that provide both pollen and nectar are willow,

* Corresponding author, evelin.kivima@gmail.com

dandelion, white clover, raspberry, red clover, willow-herb, fruit trees, and berry bushes, in addition to heather, which is one of the most highly valued honey plants (Tammet, 2007).

Domestic honey is highly appreciated in Estonia, and several quality analyses are performed every year; however, very few scientific studies have analysed the relationships between the botanical origin of honey and its physico-chemical properties. Furthermore, studies that make use of front-face fluorescence spectroscopy to classify honey are scarce. Consequently, the aim of this study is to characterize unifloral and multifloral honeys of Estonian origin and thus contribute to the growing library of characteristics and botanical origins of honeys from around the world.

2. MATERIALS AND METHODS

2.1. Honey samples

Eighteen honey samples were collected directly from beekeepers who operate in different areas of Estonia. These honeys were stored at 18 ± 2 °C in air-tight glass containers until further analysis.

2.2. Melissopalynological analysis

Melissopalynological analysis was carried out according to the non-acetolytic method described by Louveaux et al. (1978). The pollen counts were expressed as percentages after counting 500–600 pollen grains. The identification of the pollen types was based mainly on the reference collection of the Department of Food Processing of Tallinn University of Technology and data provided by Ricciardelli D'Albore (1997). An Olympus CX21 (Japan) binocular light-microscope with 40×15 magnification was used.

2.3. Physico-chemical parameters

Physico-chemical properties, such as moisture content, pH, free acidity, electrical conductivity, diastase activity, and HMF were determined according to the official Estonian methods (EVS, 1997).

The fructose and glucose content were both determined using high-performance liquid chromatography (HPLC) (Waters, USA). The chromatograph was equipped with Alliance Separations Module 2695 (Waters, Milford, MA, USA), Aminex HPX-87H 300 mm × 7.8 mm column (BioRad, Philadelphia, PA, USA), and Refractive Index Detector 2414 (Waters, Milford, MA, USA). Each sample contained 0.4 g of honey dissolved in 50 mL of Milli-Q water, filtered through a 0.2 μm Millipore filter, and diluted in addition 10 times with an HPLC eluent (0.05 M H_2SO_4). The injection volumes of the samples were 20 μL, with a flow rate of 0.6 mL/min (isocratic). The HPLC sample peaks were identified by comparing the retention times obtained from standards. Triplicate injections were performed, and the average peak areas from these technical replicates were used for peak quantification.

Mineral content analysis was carried out using an ion chromatograph system (Waters, Milford, MA, USA) that consisted of Conductivity Detector 432, Isocratic HPLC pump 1515, and IC-Pac 3.9 mm × 150 mm Cation Column 432 (Waters, Ireland). Honey samples of 5 g were dissolved in 50 mL of Milli-Q water, and this solution was filtered through a 0.2 μm Millipore filter. The injection volume of the samples was 20 μL with a flow rate of 1 mL/min. For data analysis we used Breeze software (Waters, Milford, MA, USA).

2.4. Front-face fluorescence spectroscopy

Fluorescence measurements were performed using an Instant Screener® (ISC) Analyzer (LDI Ltd., Tallinn, Estonia). This compact spectro-fluorometer has a 10 mL optical cell and is equipped with a 5 W pulsed Xenon lamp capable of generating excitation emission matrixes (EEM) or spectral fluorescence signatures (SFS). The SFSs were measured in a front-face optical layout (35°) from the surface at excitation wavelengths from 230 to 350 nm and at emission wavelengths from 250 to 565 nm with 5 nm intervals in both directions.

Raw spectral data were rearranged into three-dimensional data arrays, with each dimension corresponding to the sample array, emission data, and excitation data. Data were decomposed and analysed in three dimensions using an algorithm implemented in the N-way toolbox, Matlab (Andersson and Bro, 2000).

2.5. Statistical analysis

Statistical analysis was performed using Matlab (Mathworks, Natick, MA, USA). Principal component analysis (PCA) was carried out in order to visualize data from different honey samples and to identify their similarities and differences. The analysis was made on the basis of physico-chemical properties such as moisture content, pH, free acidity, electrical conductivity, diastase activity, mineral content, and sugar composition (glucose and fructose). In addition, Pearson correlation coefficients were calculated between all measurements.

3. RESULTS AND DISCUSSION

3.1. Melissopalynological analysis

The most numerous pollen types identified in the samples were rape (*Brassica napus*), white clover (*Trifolium repens*), melilot (*Melilotus officinalis*), raspberry (*Rubus idaeus*), and willow (*Salix* spp.) (Table 1).

Table 1. Content of pollen types in honey samples, %. Percentages in boldface refer to unifloral honeys; the plus sign (+) stands for minor pollen (<1%)

Pollen type	Honey samples																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Aceraceae																		
Acer spp.		+			1	4	3	1					3	4	+	3	3	
Betulaceae	2	1	+		+		1	+		+	+		1	1		1	2	
Boraginaceae																		
Echium vulgare	+		+			+					3					2		
Compositae																		
Centaurea cyanus			+					+										
Taraxacum officinale						1		+					3	+	1	1	+	
Cruciferae																		
Brassica napus s.l.	17	11	**60**	**77**	**76**	51	50	43	40	27	9	+	23	17	29	2	16	37
Ericaceae	1										3							
Calluna vulgaris									**16**	**27**	4							
Fabaceae																		
Galega officinalis			2	5			2	+					5	6	17			3
Lathyrus pratensis s.l.									2		3							
Gramineae									1		+							
Hippocastanaceae																		
Aesculus hippocastanum				+		1		+				+		+		+		
Hydrophyllaceae																		
Phacelia tanacetifolia														3			2	
Leguminosae																		
Melilotus officinalis s.l., *Trifolium repens* s.l.	1	4	5	10	2	5	10	4	18	28	3	21	1	7	27	18	27	15
Trifolium pratense s.l.	+		1	5	+	+	5	+	3	4	19	1	+	14	+		+	3
Menyanthaceae																		
Menyanthes trifoliata											1							
Onagraceae																		
Epilobium angustifolium										+								
Ranunculaceae	+								+									
Rhamnaceae																		
Frangula alnus			1		2				2	3	1	**42**	**22**	+	+		1	
Rosaceae																		
Rubus idaeus s.l.	**67**	**79**	17	2	8	7	17	14	6	5	31	33	32	20	23	36	17	40
Filipendula ulmaria			+	+			+		2	1	3		1			+	2	
Salicaceae																		
Salix spp.	6	5	9	+	6	27	7	34	1	1	14	+	5	22	2	15	24	
Umbelliferae	+	+	1	+	+	1	+	+	+	+	1	+		1	+	2	1	

All of these plant species are relatively common in Estonia. Two of the honey samples analysed contained pollen of alder buckthorn (*Frangula alnus*) and also traces of honeydew elements. Three of the honey samples contained heather (*Calluna vulgaris*) pollen.

Usually, honey is considered unifloral when 45% of the relative frequency of all pollen counted is identified as belonging to a single taxon. However, because of the numerous over- or under-represented pollen types, pollen percentages can vary considerably between different unifloral honeys. Therefore, to correctly interpret the botanical origin of a honey, sensory and physico-chemical data should also be taken into account. Because the pollen of rape in honey is over-represented, honey samples with over 60% rape pollen are considered unifloral. In contrast, as the pollen of heather is under-represented, even honeys with 10% pollen identified within this taxon may be considered unifloral (Von Der Ohe et al., 2004). This view is further supported by the work of Salonen et al. (2009). In accordance with Bryant and Jones (2001), we classified honey samples as being unifloral raspberry when 45% of the pollen distribution originated from *Rubus idaeus*. Taking the pollen types into account, 7 of the 18 analysed honey samples (Table 1) are potentially unifloral raspberry (1 and 2), rape (3, 4, and 5), and heather (9 and 10) honey varieties.

3.2. Physico-chemical parameters

The acidity of honey is an important parameter during the extraction and storage of honey, because it influences the texture, stability, and shelf life (Terrab et al., 2004). All honey samples analysed were acidic and found to range between pH 3.38 and 5.12 (Table 2). Two of the honey samples have higher pH values relative to the others (the pH values of samples 12 and 13 were 5.12 and 4.52, respectively). This may be due to a higher content of alder buckthorn pollen and/or the presence of honeydew elements (Table 1).

Moisture content is an important quality parameter that influences the shelf life of honey (Bogdanov et al., 2004). It depends on various factors, including the harvesting season, the degree of maturity reached in the hive, and climate factors (Finola et al., 2007). The moisture content of all 18 honey samples ranged between 16.1% and 18.9%. These percentages are below the upper limits of ≤20% and ≤23% for heather honeys set by the relevant EU directive (EU, 2002).

Diastase is a starch digesting enzyme whose activity is used as an indicator of honey freshness because it becomes denatured during heat treatment; it has reduced activity in heated or old honeys (White, 1975). The diastase numbers (DN) of the 18 honey samples ranged between 16.2 and 32.9, and are thus all higher than the minimum of 8 DN set by European legislation (EU, 2002).

Table 2. Physico-chemical parameters of honey samples

Sample	pH	Moisture, %	Diastase (DN)	HMF, mg/kg	Electrical conductivity, mS/cm	Free acidity, mmol/kg
1	3.41	16.7	28.0	5.8	0.2	29
2	3.55	18.3	25.8	<1	0.1	20
3	3.52	17.5	19.4	3.8	0.2	23
4	3.51	17.3	20.7	<1	0.1	20
5	3.56	17.4	26.9	1.9	0.1	21
6	3.72	17.9	21.3	1.9	0.3	23
7	3.59	16.1	26.0	<1	0.1	19
8	3.68	17.1	16.2	<1	0.2	21
9	3.53	18.2	28.5	3.8	0.4	43
10	3.79	18.9	32.9	2.9	0.6	54
11	3.38	17.0	25.7	3.8	0.3	35
12	5.12	16.8	28.0	<1	0.4	17
13	4.52	17.3	17.6	<1	0.4	14
14	3.48	17.8	23.0	<1	0.2	22
15	3.75	17.0	21.9	<1	0.2	22
16	3.69	16.1	25.1	<1	0.2	22
17	3.80	18.9	29.1	1.9	0.3	25
18	3.53	16.1	22.5	1.9	0.1	16
Mean	3.73	17.4	24.4		0.2	25
SD	0.43	0.86	4.36		0.14	10.00
Range	3.38–5.12	16.1–18.9	16.2–32.9	max. 5.8	0.1–0.6	14–54

The HMF, a compound that is formed by the decomposition of fructose in the presence of an acid, is also an important indicator of honey quality because the amount of HMF increases in honey that is subjected to higher temperatures (Crane, 1980). The amount of HMF found in all honey samples was below 5.8 mg/kg, and well below the limit of 40 mg/kg, stated by European legislation (EU, 2002). This indicates that these honey samples had not been overheated.

Electrical conductivity is a good indicator of the botanical origin of honey and is currently used routinely instead of measuring the ash content (Bogdanov et al., 2000). The electrical conductivity of the 18 honey samples ranged from 0.1 to 0.6 mS/cm, which indicates their floral origin because all were below the limit of 0.8 mS/cm for blossom honeys and mixtures of blossom and honeydew honeys (EU, 2002; Ouchemoukh et al., 2007). All three honeys that contained heather pollen had higher values of electrical conductivity (0.3–0.6 mS/cm), which agrees with a measurement of 0.73 mS/cm for pure heather honey by Persano Oddo and Piro (2004). The honey samples that contained pollen of alder buckthorn also had higher electrical conductivity (0.4 mS/cm), although this could also be due to honeydew. Rape honey samples had the lowest electrical conductivity (0.1–0.2 mS/cm), which is in accordance with values reported by Persano Oddo and Piro (2004).

The free acidity of honey may be explained by the presence of organic acids in equilibrium with their corresponding lactones, or internal esters, and some inorganic ions, such as phosphate (Finola et al., 2007). Free acidity values ranged between 14 and 54 mmol/kg. All honey samples, except for heather honey of sample 10 with a free acidity of 54 mmol/kg, met the relevant EU standard being under 50 mmol/kg (EU, 2002), which indicates the absence of unwanted fermentation. Also Persano Oddo and Piro (2004) state that honey samples that contain heather pollen have high values of free acidity (see honey samples 9, 10, and 11 in Table 2).

Glucose and fructose are the main sugars in honey and their actual proportion depends largely on the source of the nectar (Anklam, 1998). Normally, fructose predominates slightly, with some exceptions being rape and dandelion honeys (Crane, 1980). In our study 72.2% of the honey samples analysed had fructose as the dominating sugar with a mean value of 36.53 g/100g (Table 3). Glucose values were lower with a mean value of 34.79 g/100g. Samples that contained mostly rape pollen had the highest concentration of glucose (see honey samples 3–8 in Table 3).

The fructose/glucose ratio ranged between 0.89 and 1.20, indicating their floral origin because it is known that flower honeys have a fructose/glucose ratio of

Table 3. Fructose and glucose content of the analysed honeys (g/100g) and fructose/glucose ratio (F/G)

Sample	Glucose	Fructose	F/G
1	34.83	38.15	1.10
2	36.16	38.29	1.06
3	37.18	36.30	0.98
4	40.32	35.78	0.89
5	37.86	36.64	0.97
6	35.00	35.10	1.00
7	38.64	36.78	0.95
8	36.96	35.46	0.96
9	32.44	37.77	1.16
10	32.99	39.53	1.20
11	35.99	39.36	1.09
12	28.84	33.61	1.17
13	30.22	33.08	1.09
14	34.19	35.31	1.03
15	34.97	37.16	1.06
16	30.83	35.27	1.14
17	33.40	35.88	1.07
18	35.42	38.02	1.07
Mean	34.79	36.53	1.06
SD	2.99	1.79	0.09
Range	28.84–40.32	33.08–39.53	0.89–1.20

about 1 while in honeydew honeys the ratio ranges between 1.5 and 2.0 (Gleiter et al., 2006). Five of the honey samples analysed had fructose/glucose ratios under 1, an effect caused by their having a higher content of rape pollen (Table 1). According to Persano Oddo and Piro (2004), the fructose/glucose ratio of unifloral rape honey is lower than 1, which agrees with our findings.

The mineral composition of the honey samples is presented in Table 4. Minerals such as sodium, potassium, magnesium, and calcium were identified. The mineral content of honey is generally small and depends on the composition of the nectar of the plants that dominate its makeup (Felsner et al., 2004). Light blossom honey has a lower mineral content than dark honey such as honeydew and heather (Bogdanov et al., 2007). Potassium was quantitatively the most important mineral, whose content ranged from 125.79 to 2854.78 mg/kg, where the lower values are for rape honeys and the higher values are for honey samples that either originated from heather or contained alder buckthorn pollen. The sodium and magnesium contents in the samples were lower with average values of 15.46 mg/kg and 19.37 mg/kg, respectively. The calcium content ranged from 20.37 to 100.33 mg/kg, with heather honeys having the highest content. Generally, the results of this study confirmed that light-coloured honeys have a lower mineral content (rape and raspberry honeys) than darker honeys (alder buckthorn and heather honeys).

Table 4. Mineral content of the analysed honeys, mg/kg

Sample	Na	K	Mg	Ca
1	9.62	292.69	20.85	53.88
2	9.65	237.14	17.73	36.54
3	13.18	459.87	24.87	50.40
4	13.82	126.37	14.65	46.27
5	6.64	253.80	18.53	40.17
6	6.85	578.88	21.34	63.65
7	5.67	262.90	17.24	49.98
8	9.04	517.93	25.23	53.02
9	40.72	1271.23	20.79	76.17
10	62.55	2854.78	24.96	100.33
11	24.22	902.75	20.27	56.82
12	19.44	1235.58	23.46	39.80
13	8.17	1381.53	16.50	20.37
14	8.11	257.64	14.95	42.86
15	11.70	569.73	25.49	55.73
16	13.35	485.75	5.53	39.42
17	10.79	862.22	24.28	56.63
18	4.77	125.79	12.05	29.20
Mean	15.46	704.25	19.37	50.62
SD	14.50	667.78	5.35	17.85
Range	4.77–62.55	125.79–2854.78	5.53–25.49	20.37–100.33

3.3. Front-face fluorescence spectroscopy

3.3.1. Description of fluorescence spectra of honey samples

Fluorescence spectroscopy is a useful tool to fingerprint or classify honey samples because a large number of different substances can affect their spectral signature (Ruoff et al., 2006). To record fluorescence data we applied SFS technology, which was found to provide extra information compared to normal emission or excitation spectra. Examples of unique fingerprints of honey samples of various unifloral origin are presented in Fig. 1. The peaks of the spectra of unifloral heather, rape, and raspberry honey samples vary by shape and height.

In the measured excitation and emission range, a typical SFS signal of measured honey samples contains three fluorescence peaks with varying intensities. The maximum of the most informative peak is located in the area EX:270–290/EM:320–350. This area corresponds to aromatic amino or nucleic acids and mainly includes tryptophane residuals. This peak is very common not only in honey but in various samples of biological origin. Although the tryptophane peak is detected in all honey samples, it serves as a sensitive contribution to the fingerprint of honey samples because its emission spectra change in accordance to its local environment. This peak is higher in honey samples that contain more rape pollen, although this conclusion is not quite straightforward because almost all samples contain rape pollen to some degree.

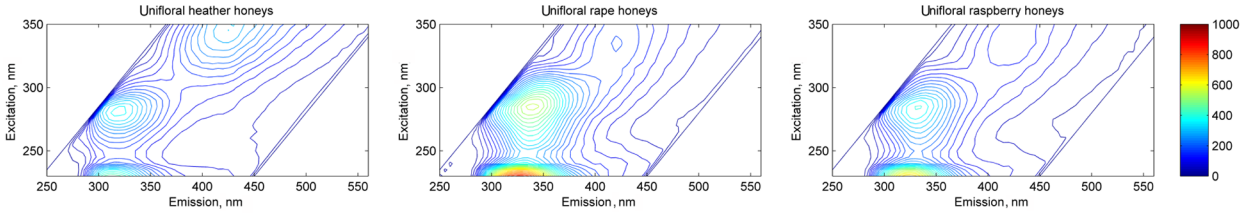

Fig. 1. Three typical peaks in unifloral honey samples.

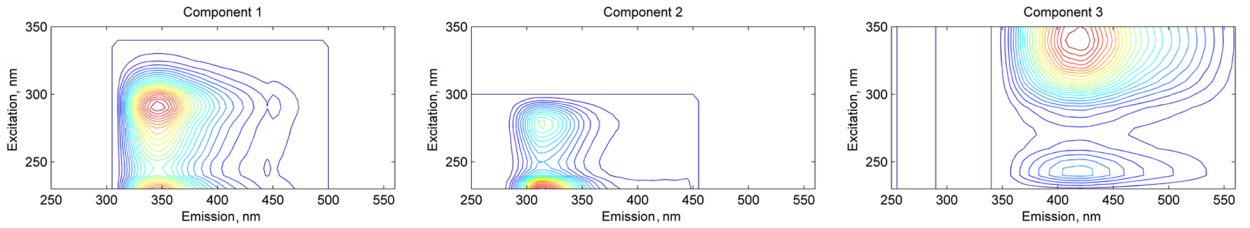

Fig. 2. Reconstructed spectra of three components of the PARAFAC model corresponding to three peaks typically found in honey spectra.

The maxima of the highest peak are located around EX:230/EM:320–335. For samples with lower intensities, the peak maxima are located towards lower emission wavelengths, as observed in honey samples that contain heather pollen (samples 9–11). This peak is located in the area that typically corresponds to secondary peaks of tryptophane.

The third obvious peak in typical SFS spectra of honey samples is located in the area of EX:330–350/EM:380–440 and corresponds to vitamins (FADH/NADH, riboflavin, and vitamin A). Compared to the two peaks described previously, the intensity of the third peak showed the most variation between honey samples. The highest values for the third peak were observed in honey samples that contain heather pollen.

3.3.2. Chemometric analysis of SFS

For chemometric analysis of fluorescence spectra, the PARAFAC algorithm was applied to decompose the SFS data into a number of trilinear components that can be presented as scores that directly relate to the relative concentration of components whose emission and excitation spectra are described with factor loadings. To enable for easier physical interpretation of the results, PARAFAC was applied using non-negativity constraints in all three modes.

The SFS data for honey were first modelled using PARAFAC with 1 to 6 factors. Comparison of core consistency values revealed that three factors should be suitable to model this particular kind of data. This was confirmed using split-half analysis.

Three factors of the model in Fig. 2 correspond roughly to the same three peaks described above, although the separation is not perfect, the peaks overlap, and components often contain traces of information from several of the peaks described. Nonetheless, the first component corresponds to the so-called tryptophane peak but contains traces of the secondary tryptophane peak. In contrast, the second component corresponds to the secondary tryptophane peak but contains traces of the primary peak. The third component corresponds to the vitamin peak, although there are traces that describe the area between two peaks of tryptophane. Therefore, it can be assumed that the third component also contains information regarding changes in the shape of the tryptophane peak.

A plot of the scores that result from the PARAFAC model (Fig. 3) reveals that differences between spectra that correspond to various groups of honey are rather small. Honey samples that contain heather pollen are distinguished from the rest of the group by a high score of the third PARAFAC component and a low score of the first PARAFAC component. This is evidence that these samples contain less tryptophane. The third typical peak corresponds to the concentration of vitamins or other substances corresponding to the third typical peak in honey, as described above. Compared to all other spectra, raspberry honey samples have SFS spectra with lower intensity. Therefore, these samples form a distinct group from the rest of the samples in the plot of the PARAFAC model scores. The rest of the honey samples correspond mainly to unifloral rape, honeys with a high amount of rape, and the rest of the multifloral honeys. All these have rather similar spectra and therefore cannot be classified using the scores plot.

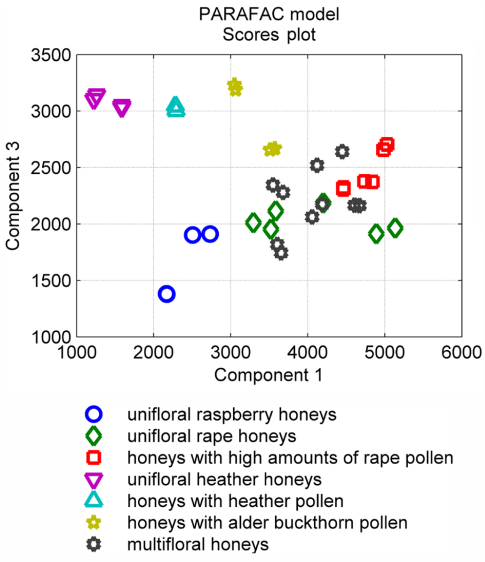

Fig. 3. Scores plot of the PARAFAC model.

Besides PARAFAC modelling, chemometric analysis was applied to determine whether SFS spectra can be used to estimate various physico-chemical parameters of honey samples or their floral content (Table 5). This was done using various multivariate calibration models (PCR, PLS, N-PLS, PARAFAC model scores). The measured spectra were divided randomly into calibration and validation sets (25 and 11 spectra each, splitting ratio roughly 70 : 30).

The calibration set was used to generate calibration models, which later were applied to estimate values of various physico-chemical parameters or the floral content of validation set samples. To compare the results obtained with various calibration techniques, correlation coefficients were calculated between the measured values and model estimations found using the validation set. Because the number of spectra in the validation set was relatively small, the results depend on the selection of the spectra. To prevent against systematic bias, the validation process was repeated ten times using spectra that were randomly divided between calibration and validation sets. Average values from these ten validation steps were used to compare calibration models and evaluate the potential use of the SFS method to estimate the physico-chemical parameters or floral content. The average results from the validation process are reported in Table 5. The PCR and PLS methods provide the most reliable results. The correlation between SFS estimations and real data for free acidity is 0.919. For electric conductivity and contents of minerals and glucose or fructose, the Pearson correlation coefficient is around 0.8. For seven plants in the table the correlation between SFS estimations and experimental data is between 0.717 and 0.829. These results were achieved using available SFS spectra. We expect that with a larger and more diverse calibration dataset better correlation between model predictions and measured values could be achieved.

Table 5. Comparison of tested calibration models for estimating values of physico-chemical parameters and floral content

	Model			
	PCR (12 components)	PLS (12 components)	N-PLS (10 components)	PARAFAC (2 components)
Physico-chemical property[a]				
pH	0.739	0.780	0.457	0.178
Free acidity	0.919	0.853	0.823	0.306
Electrical conductivity	0.859	0.861	0.792	0.675
Glucose content	0.852	0.873	0.314	0.076
Fructose content	0.775	0.822	0.162	0.251
Mineral content	0.776	0.838	0.785	0.713
Floral content[a]				
Acer spp.	0.736	0.717	0.599	0.154
Taraxacum officinale	0.704	0.718	0.770	0.099
Cruciferae (*Brassica napus* s.l.)	0.786	0.763	0.747	0.061
Calluna vulgaris	0.811	0.828	0.731	0.565
Menyanthes trifoliata	0.772	0.762	0.422	0.171
Frangula alnus	0.788	0.800	0.644	0.194
Rosaceae (*Rubus idaeus* s.l.)	0.787	0.829	0.648	0.216

[a] The table contains results where the correlation (r^2) between experimental results and estimations was at least 0.7.

3.4. Statistical analysis

The results of principal component analysis (PCA) are shown in Fig. 4. The first component (PC1) contained 43.0% of the data variance and was positively related to the glucose content, electrical conductivity, and free acidity, and negatively related to fructose content. The second component (PC2) contained 28.7% of the data variance and was positively related to pH, and negatively related to both fructose and mineral content. All honey samples that contain heather pollen (9, 10, and 11) have positive PC1 values, while honey samples with alder buckthorn (12, 13) have highly positive PC2 values. Most multifloral honeys appear in the centre of the graph and have similar physico-chemical properties, whereas unifloral rape honey samples (3, 4, and 5) and honey samples that contain high amounts of rape pollen are slightly separate from the other honeys under the group of multifloral honey cluster having negative PC1 and PC2 values. In addition, one sample of unifloral raspberry honey is located close to the honey that contains heather pollen in small amounts, while the other raspberry honey sample is more closely related to unifloral rape honeys. Because the physico-chemical

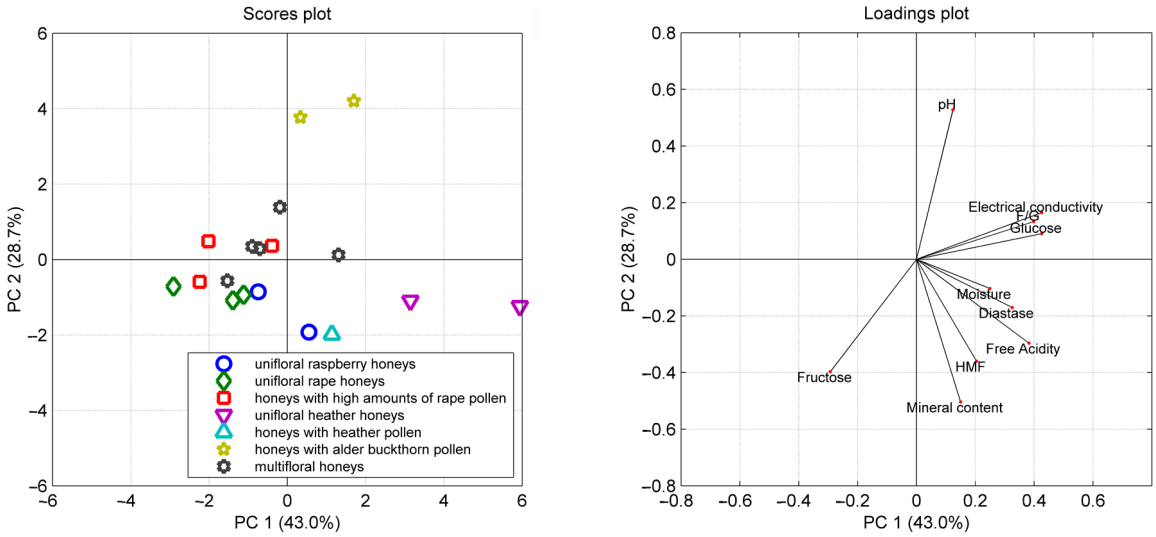

Fig. 4. PCA plot for physico-chemical properties of analysed honeys.

Table 6. Correlation matrix between physico-chemical parameters

Variables	Moisture	pH	Free acidity	El. conductivity	Diastase	HMF	Glucose	Fructose	F/G
pH	−0.045								
Free acidity	0.519	−0.271							
El. conductivity	0.491	0.480	0.651						
Diastase	0.320	0.003	0.622	0.381					
HMF	0.176	−0.386	0.590	0.248	0.383				
Glucose	−0.086	−0.690	−0.120	−0.675	−0.265	0.048			
Fructose	0.160	−0.646	0.650	−0.008	0.501	0.570	0.355		
F/G	0.173	0.376	0.512	0.715	0.587	0.264	−0.844	0.196	
Mineral content	0.502	0.394	0.723	0.949	0.456	0.204	−0.534	0.158	0.671

F/G – fructose/glucose ratio.

parameters of the two raspberry honey samples are similar, except for free acidity, the difference in the scores is related to this latter property.

The Pearson correlation matrix is presented in Table 6. It can be seen that the highest correlation is between electrical conductivity and mineral content ($r^2 = 0.949$), which is not surprising because the electrical conductivity depends on the mineral content and free acidity in honey: the higher their values, the higher the resulting conductivity (Bogdanov, 2002). As a result of the previous strong correlation, a correlation was also found between free acidity and mineral content ($r^2 = 0.723$). Similar correlations were reported by Feás et al. (2010) and Saxena et al. (2010), who reported correlation coefficients of 0.995 and 0.980, respectively. This dependence might be explained by the observation that a higher mineral content in honey corresponds to a higher salinized fraction of the acids present (Finola et al., 2007). A relatively good correlation was found between the fructose/glucose ratio and electrical conductivity ($r^2 = 0.715$).

4. CONCLUSIONS

In conclusion, the results of physico-chemical analysis indicate that all samples of Estonian honeys are of good quality and meet the requirements of the relevant European Directive (2001/110/EC) for all parameters, with one exception where a heather honey was found to have free acidity slightly exceeding the regulated limit. The mineral content was higher in honey samples that contain heather or alder buckthorn pollen. In all honey samples potassium was the most abundant mineral. The honey samples that were classified as blossom honeys with small traces of honeydew elements and various unifloral honeys, such as rape, raspberry, and heather honeys, were identified by their physico-chemical

properties, mineral content, front-face fluorescence spectroscopy, and basic melissopalynological analysis.

PARAFAC analysis of the measured fluorescence spectra revealed a similar grouping between the different samples as was found by PCA analysis of the physico-chemical parameters. Moreover, various calibration models were used to estimate the physico-chemical parameters and floral content according to fluorescence spectra. For several parameters, the results were promising because with a very limited calibration set, the correlation (r^2) between experimental data and estimated values was higher than 0.8 (0.919 for free acidity). It is expected that with a more extensive calibration set better correlation may be obtained. Nevertheless, more extensive research should be conducted with unifloral and multifloral honeys to more precisely characterize them.

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Eesti mee iseloomustamine botaanilise päritolu järgi

Evelin Kivima, Andrus Seiman, Raili Pall, Evelyn Sarapuu, Kaie Martverk ja Katrin Laos

On iseloomustatud Eesti meesorte füüsikalis-keemiliste omaduste põhjal. Uuriti nende happesust, niiskussisaldust, vabade hapete sisaldust, elektrijuhtivust ja diastaasi aktiivsust. Samuti tehti hüdroksümetüülfurfuraali (HMF), glükoosi, fruktoosi ja erinevate mineraalainete (naatrium, kaalium, magneesium, kaltsium) koguste analüüs. Mee botaanilise päritolu määramiseks viidi läbi õietolmuanalüüs, mille kohaselt olid 39% uuritud meesortidest rapsi (*Brassica napus*), vaarika (*Rubus idaeus*) või kanarbiku (*Calluna vulgaris*) monofloorsed meed.

Tulemused näitasid, et Eesti meed on hea kvaliteediga ja vastavad Euroopa direktiivi (2001/110/EC) nõuetele. Mineraalainete sisaldus oli suurem kanarbiku ja paakspuu (*Frangula alnus*) õietolmu sisaldavas mees.

Mee füüsikalis-keemiliste omaduste ja taimse koostise hindamiseks kasutati fluorestsentsspektroskoopiat. Fluorestsentsspektrite järgi arvutatud tulemuste ja eksperimentaalsete mõõtmiste vaheline determinatsioonikordaja r^2 oli mitme parameetri korral suurem kui 0,7.