Hungarian University of Agriculture and Life Sciences

APPLICATION OF CLASSICAL AND CORRELATIVE ANALYTICAL METHODS FOR AUTHENTICATION OF HONEY

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LIST OF ABBREVIATIONS

^{1}H NMR	proton nuclear magnetic resonance spectroscopy:
^{13}C EA/LC IRMS	^{13}C stable isotope mass spectrometry coupled with elemental analysis/liquid chromatography
a_w	water activity
AAE	ascorbic acid equivalent
ACRRS	electronic tongue drift correction method using additive correction relative to reference samples
AF	bastard indigo *(Amorpha fruticosa)* honey
ANOVA	one-way analysis of variance
AS	milkweed *(Asclepias syriaca)* honey
AUS	authenticity study
BBGOIS	basic botanical and geographical origin
BN	rape *(Brassica napus)* honey
CA	*Codex Alimentarius*
CAH	*Codex Alimentarius Hungaricus*
CS	chestnut *(Castanea sativa)* honey
CV	cross-validation
CUPRAC	cupric ion reducing antioxidant capacity
CART	classification and regression trees
detr	detrending
EC	European Council
ELC	electrical conductivity
EN	electronic nose
ET	electronic tongue
EUnonEU	blend of honeys from European Union and non-European Union countries
EURP	EUnonEU acacia honey
EUTI	EUnonEU linden honey
FAO	Food and Agriculture Organization of the United Nations
FRAP	ferric reduction antioxidant power
FS	F40 high-fructose corn syrup from the Kall Ingredients
FT-IR	Fourier-transform infrared spectroscopy
GAE	gallic acid equivalent

GC	gas chromatography
GC-FID	gas chromatography with flame ionization detector
GF	self-made glucose/fructose syrup (40/60)
HA	sunflower *(Helianthus annuus)* honey
HD	honeydew honey
HFCS	high-fructose corn syrup
HMF	hydroxymethylfurfural
HPLC	high-performance liquid chromatography
HTE	heat treatment experiment
IHC	International Honey Commission
LC-HRMS	liquid chromatography–high-resolution mass spectrometry
LDA	linear discriminant analysis
LOD	limit of detection
LOQ	limit of quantification
MSC	multiplicative scatter correction
NIR	near infrared spectroscopy
OISWP	origin identification study extended with pollen analysis
PCA	principal component analysis
PCA-LDA	principal component analysis coupled linear discriminant analysis
PLSR	partial least squares regression
R^2C	determination coefficient of the training set
R^2CV	determination coefficient of the cross-validation set
RPDC	residual prediction deviation of the training
RPDCV	residual prediction deviation of the cross-validation set
RMSEC	root mean square error of the training set
RMSECV	root mean square error of the cross-validation set
RI	rice syrup
RP	acacia *(Robinia pseudoacacia)* honey
RP-HPLC	reversed-phase high-performance liquid chromatography
Sgol	Savitzky-Golay smoothing
SNV	standard normal variate
SSAPS	sugar syrup adulteration preliminary study
SSAWLC	sugar syrup adulteration extended with lower concentrations
TI	linden (*Tilia* spp.) honey

TEQ	trolox equivalent
TPC	total polyphenol content
% v/v	volume/volume percentage
% w/w	weight/weight percentage

1 INTRODUCTION

Honey is a natural sweetener produced by honeybees *(Apis mellifera)* and used by humankind since ancient times as a medical and food product. According to the FAO, the world's honey production is almost 23,000,000 tons. Amongst them, Hungary is one of the most important honey producers and exporters in the world: in 2018 Hungary was the 17th honey producer of the world and the 6th honey producer in Europe. The total honey production was 28,000 tons, from which Hungary exported 21,000 tons putting Hungary in 11th place among honey exporters all around the world (FAO, 2021). In Hungary different types of honeys are produced, from which acacia honey is produced in the highest amount, however, the production of rape and sunflower honey is also significant. Besides these chestnut, milkweed, phacelia, solidago, and linden honeys are also important. Beekeeping has high importance in Hungary and the Hungarian Beekeeping Association collects the beekeepers and produces the National Beekeeping Program. According to their report in 2018, 22447 beekeepers were in Hungary and the average honey production/beehive was about 21.02 kg for one year (Ministry of Agriculture, 2019). From these results we can see that Hungary has a high role in honey production not only at a European but also on a world level, therefore monitoring the quality of honey and checking its authenticity has high importance.

Honey authenticity and its monitoring are in the focus worldwide, which can be explained by its high nutritional and market value. Related to authenticity, the most important questions are botanical and geographical origin identification and adulteration detection. The origin identification of honey is a quite challenging task and is usually performed by the combination of three methods: sensory, physicochemical, and pollen analysis of the honey. This means that several aspects have to be analyzed when deciding the origin of the sample which is not easy. The composition of the honey and its physicochemical and sensory properties depend on its origin. The botanical origin has the highest effect on the composition, but the difficulty is that numerous types of honey exist worldwide. If we only look into Europe, there are more than 100 plant species from which bees produce unifloral honeys. Another important determining factor is the geographical origin of the sample. Regarding this, the climate, the quality of the soil, and the surrounding vegetation have a high role in the formation of the composition of the different honey types. Moreover, besides the origin, there are also important factors that can affect the composition of the honey such as the processing method (filtration, heat treatment, *etc*.) and the storage conditions (holders, temperature, *etc*.). Therefore, all these effects make the origin identification of honey quite challenging because significant differences can be found in composition not only between botanical types but also within unifloral honeys. Due to these

facts, it is impossible to have a reference honey per botanical type, which could be used as an etalon for the different unifloral honeys, and also it is not easy to determine compositional criteria for the different types. Nevertheless, in the lack of these criteria how could we identify the origin of the samples. Moreover, in the last decades, fraudulent activities related to honeys have been increasing. Among the most abundant adulteration types are the mislabeling of the origin (botanical or geographical) or the honey mixture with different sugar syrup materials. According to the report of The Grocer's article, the fraudulent activities of foods have increased during the pandemic, where honey was among the most exposed products (Nott, 2021). Moreover, other intentional/or non-intentional factors can influence the honey quality and composition, such as the heat treatment of the samples. Therefore, we can see that the analysis of authenticity is a very challenging task, and it is always in the focus worldwide. Hence, the origin identification and detection of adulteration and other manipulations are important tasks. In the practice - as it has been mentioned before - for the identification of the origin of the samples different techniques are applied, and it is also valid for the adulteration detection. The problem, on one hand, is that these methods – such as chromatography techniques, isotopic ratio analysis, mass spectrometry, nuclear magnetic resonance analysis, *etc*. – are sometimes expensive, time-consuming, and need a lot of reagents, while on the other hand, they are still not 100% accurate. Another problem in the case of botanical origin identification is that the compositional criteria of the different unifloral honeys are not available. Some countries define limits for some of the physicochemical parameters of different unifloral honeys, however, these are not available for all the honey types (Thrasyvoulou *et al.*, 2018). As an example, in Hungary, these regulations are only available for the acacia and linden honey unifloral types (*Codex Alimentarius Hungaricus*, 2009). However, in the lack of these criteria for the different unifloral samples, it is a hard task to identify the origin of the honey.

Therefore, there is a demand for methods that can be applied for the aforementioned problems. Moreover, there is also a need for the establishment of a reference database of the honeys from different botanical types. This is not available yet, but it would be useful for the characterization of Hungarian honey and a helpful tool in origin identification. A solution for the origin identification and adulteration detection can be the reference database combined with the application of correlative techniques, as the latter can provide fingerprint-like data about the analyzed sample. Among these techniques, we can find spectroscopy-based methods such as Fourier-transform infrared (FT-IR), Raman or near infrared spectroscopy (NIR), and electronic sensory analysis methods (Aykas *et al.*, 2020). NIR technique is based on the interaction between the analyte and the light. From the data obtained it is possible to conclude the composition of the sample because the NIR light causes a vibration at the different overtones and

combination bands. Previously, NIR has been used for different purposes regarding honey analysis such as origin identification and detection of adulteration (Aouadi *et al.*, 2020). However, according to the best of our knowledge, apart from our work, its use has rarely been reported for origin identification or adulteration detection in the case of Hungarian honey. Moreover, it has never been applied before our work for heat treatment detection of Hungarian honey samples (Bodor *et al.*, 2017). As an alternative to the sensory analysis methods, nowadays the use of the artificial senses - such as electronic noses (EN) and tongues (ET) - started to play a high role in the research era of food analysis. According to the literature ET was successfully applied for honey analyses aiming at origin identification and adulteration detection (Aouadi *et al.*, 2020). Moreover, similarly to NIR, the literature available on the application for the heat treatment detection of honey is missing.

These two methods could serve as alternative tools of composition and sensory analysis of the honey samples. However, if these could be applied for the origin identification and adulteration detection - especially if we apply them in combination with the reference database - are still not completely solved scientific questions. With the application of advanced statistical tools and analysis methods, it is possible to analyze these data together and investigate their possibilities in the authentication of honey.

2 AIMS

This work aimed to analyze the applicability of reference (moisture content, pH, electrical conductivity pH, ash content, antioxidant properties – TPC, CUPRAC, FRAP, and color – L*a*b*, of honey, sugars, HMF), melissopalynology, and correlative techniques (electronic tongue and near infrared spectroscopy) for the botanical and geographical origin identification, adulteration, and heat treatment detection of honeys. Based on these, three principal objectives were determined:

1) To apply reference methods, electronic tongue, and near infrared spectroscopy **for the botanical and geographical origin identification**
   a. To give a descriptive characterization of the main honey types of Hungary based on the performed physicochemical determinations
   b. To build botanical and geographical origin classification models for the main botanical types of Hungary using electronic tongue, and near infrared spectroscopy
   c. To investigate the pollen profile of Hungarian honeys from eight main botanical origins
   d. To develop botanical and geographical origin classification models for the main botanical types of Hungarian honeys using near infrared spectroscopy combined with the pollen data
   e. To build classification models of the honeys mixed with sugar syrup using acacia and linden honey and perform independent prediction on the honeys from EUnonEU regions to reveal the suspect of adulteration
2) To apply mainly near infrared spectroscopy and electronic tongue **for the adulteration detection of honey** supported by reference measurements
   a. To provide descriptive analyzes of honeys mixed (adulterated) with sugar syrup using the main physicochemical methods (pH, electrical conductivity, moisture content)
   b. To develop classification models of the different honey types (acacia, linden, rape, sunflower, honeydew) adulterated with rice, F40 (high fructose corn syrup), and glucose-fructose syrup to see the discrimination efficiency of the electronic tongue and near infrared spectroscopy
   c. To develop predictive models to regress on the added sugar syrup concentration of the aforementioned honey types adulterated with the syrups
3) To apply electronic tongue, near infrared spectroscopy, and reference methods **for the detection of low and high-level heat treatment of honey**
   a. To provide descriptive analyzes of the honeys (acacia, bastard indigo, sunflower) after the application of the heat treatment (40°C, 60°C, 80°C, 100°C for 60, 120, 180, and 240

minutes) using reference methods such as determination of color, pH, electrical conductivity, and moisture content)

b. Reveal the efficiency of hydroxymethylfurfural analysis in the detection of heat treatment of honey
c. To develop classification models for the discrimination of temperature, time, and heat treatment level using near infrared spectroscopy and electronic tongue

3 LITERATURE REVIEW

In this section, the description of the physicochemical characteristics, national and international legislations are shown. Hereby, I aimed to introduce the most abundant challenges and difficulties of the authentication of honey. At last, indicative examples are given on honey research so far, mostly related to the authenticity of honey.

3.1 Characterization of honey

In this section, the production, physicochemical composition, and properties are introduced. Moreover, I aimed to introduce the most common honey types in Hungary and the international and national legislations.

3.1.1 The production and harvest of honey

Honey is made by the honeybees from the nectar, secretions of the living plants, and excretion materials of the sucking insects living on the trees. During the transportation of the collected nectar or other sweet materials, they add enzymes from their hypopharyngeal glands and transfer them to the bees in the colony in the hive. Then the bees pass it to each other and deliver them to the honeycombs at a moisture content of 30-40%. The reduction of the moisture content is done by their wings that they use as a fan. During this process, the bees add enzymes to the honey. Amongst the enzymes, we can find invertase that transforms sucrose into fructose and glucose. Another important enzyme is the glucose oxidase that oxidizes the glucose to hydrogen peroxide (to reduce bacterial spoilage) and gluconic acid. During the entire process, the moisture content of the honey is reduced due to the fanning and the temperature (35°C) in the beehive, however, during the process, the honeybees also suck the honey up and release it back, which further decrease the water content. When the moisture content of the honey reaches 20% then the bees cap the honeycombs, and by this, they prevent the increase of the moisture (Bogdanov, 2011a).

When the honeycombs are capped (about 80%) the beekeepers can start to harvest. Nowadays, the harvest is done mostly by the decapping of the combs followed by centrifugation. Honeys can be filtered with a higher mesh size not to decrease the pollen content and then honey is placed in big holders. Glass is the most suitable for the storage of honey, therefore usually the producers and beekeepers fill the honey directly in the glass jars. The type of storage, the dish, temperature, and light can highly influence the appearance and other properties of the honey (Bogdanov, 2011a).

3.1.2 Composition of honey

According to the legislation (The European Council, 2001; *Codex Alimentarius Hungaricus*, 2002), honey consists mainly of sugars, and other substances of bees such as enzymes and

organic acids, however, besides these numerous nutritionally active and useful components can be found in the different honey types. The main compounds of honey are going to be detailed in the following subsections.

3.1.2.1 Sugars

In honey, sugars are the main constituents that make up about 95% of the dry matter of the honey. In honey more than 25 different types of sugars have been identified. The main sugars are monosaccharides, which represent 75% of the sugars in honey. Beside the monosaccharides disaccharides and trisaccharides and other oligosaccharides can also be found (Siddiqui, 1970; Doner, 1977; White, 1978; Bogdanov, 2014).

Monosaccharides

The monosaccharides of the honeys are glucose and fructose. The ratio of these can be characteristic to the type of honey, as the sugar composition depends on the botanical origin of them. Based on previous findings the fructose/glucose (F/G) ratio is about 1.12-1.89 in acacia, 0.32-1.24 in rape, 1.36-1.86 in chestnut, 1.07-1.77 in honeydew, 1.04-1.41 in linden, 1.00-1.34 in sunflower (Mateo and Bosch-Reig, 1997; Cotte *et al.*, 2004; Oddo and Piro, 2004; Ruoff, 2006; Juan-Borrás *et al.*, 2014; Anjos *et al.*, 2015a; Guelpa *et al.*, 2017; Kumar *et al.*, 2018; Nayik *et al.*, 2018; Pascual-Maté *et al.*, 2018b; Geană *et al.*, 2020), 1.28 in milkweed honeys (Kasper-Szél *et al.*, 2003), and 1.51 in bastard indigo honeys (Zhu *et al.*, 2020). Besides glucose and fructose, arabinose also has been found in honey produced by stingless bees (de Sousa *et al.*, 2016).

Disaccharides

In honey, several disaccharides were identified. Among these disaccharides, we can find sucrose which was detected in several types of honey. Other important disaccharides are maltose, isomaltose. maltulose, isomaltulose, nigerose, turanose, kojibiose, laminaribose, α,β-Trehalose, gentiobiose, and melibiose (Mateo and Bosch-Reig, 1997; Cotte *et al.*, 2004; Ouchemoukh *et al.*, 2010; Escuredo *et al.*, 2014; Anjos *et al.*, 2015a; Nayik *et al.*, 2016; Boussaid *et al.*, 2018; Kumar *et al.*, 2018; Pascual Maté *et al.*, 2018).

Trisaccharides and other oligosaccharides

Similarly to disaccharides, numerous trisaccharides also have been identified in honeys such as melezitose, maltotriose, 1-ketose, raffinose, and erlose (Mateo and Bosch-Reig, 1997; Cotte *et al.*, 2004; Ruoff, 2006; Ouchemoukh *et al.*, 2010; Escuredo *et al.*, 2014; Anjos *et al.*, 2015a; Nayik *et al.*, 2016; Pascual Maté *et al.*, 2018).

Besides the trisaccharides, maltotetraose also was found in honey samples from different botanical origins such as lavender, honeydew, apple, heather, or chestnut (Pascual Maté *et al.*, 2018).

3.1.2.2 Enzymes

It has been already mentioned before that during the elaboration of the honey the bees add their enzymes to the honey, therefore even after the production, especially in fresh honey we can find enzymes such as diastase, glucose oxidase, invertase, and catalase. These enzymes get damaged during the long-term storage or as a result of heat treatment. Therefore, the enzymes, especially invertase and diastase, can be used as indicators of freshness or authenticity, moreover, they can help in the detection of heat treatment and fraudulent activities (Bogdanov, 2014).

3.1.2.3 Organic Acids

The pH of the honey is usually in the acidic range, which can be associated with its organic acid content of it which is about 0.57% of the honey. The most important organic acid of the honey is the gluconic acid that is formed during the ripening of the product as a result of the action of the glucose oxidase enzyme (Da Silva *et al.*, 2016). Numerous other organic compounds have been identified in honey that can be grouped the following way:

- alpha-hydroxy acids
  - citric, citramalic, glycolic, lactic, malic, mandelic, tartaric acid (Del Nozal *et al.*, 1998; Nozal *et al.*, 2003; Mato *et al.*, 2006; Brugnerotto *et al.*, 2019)
- simple mono- and polycarboxylic acids
  - acetic, formic, fumaric, glutaric, maleic, malonic, oxalic, propionic, succinic acid (Del Nozal *et al.*, 1998; Horváth and Molnár-Perl, 1998; Steeg and Montag, 2000; Brugnerotto *et al.*, 2019)
- sugar-derived acids
  - shikimic, quinic, galacturonic, gluconic acid (Cherchi *et al.*, 1994; Mato *et al.*, 1997, 2006; Del Nozal *et al.*, 1998; Nozal *et al.*, 2003)
- ketoacids (Cherchi *et al.*, 1994; Del Nozal *et al.*, 1998)
  - pyruvic acid
- fatty acids (Horváth and Molnár-Perl, 1998)
  - stearic, margaric acid

3.1.2.4 Proteins and amino acids

Proteins and amino acids are also important constituents of honey, and it has been reported that they can be used as indicators of the geographical origin, moreover, the proline is the most abundant amino acid of honey, is also used as a freshness indicator (Bogdanov, 2014; Pascual-Maté *et al.*, 2018a). Generally, most of the amino acids, have been identified in honey before

such as alanine, arginine, aspartic acid, cysteine, cystine, glucosaminic acid, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, methionine sulphoxide, ornithine, phenylalanine, proline, ß-alanine, serine, threonine, tryptophan, tyrosine, valine (White, 1978; Adnan *et al.*, 2014)

3.1.2.5 Vitamins

Vitamins are also present in honey in lower amounts. According to the literature, vitamin C was found in various honey types, but its amount can easily decrease as a result of the oxidation, storage, filtration, and processing of the product. Vitamins from the vitamin B group have been also detected in different honey types such as thiamine, riboflavin, niacin, panthotenic acid, pyridoxine, folic acid, and biotin. Vitamin C and niacin can be found in the magnitude of milligrams, while others only in micrograms (Da Silva *et al.*, 2016).

3.1.2.6 Minerals

Similar to the amino acids, most of the minerals can be found in honey and they can also be used as an indicator of the geographical origin. The mineral content of honey is usually between 0.02% and 1.03% and shows a strong correlation with the electrical conductivity and ash content (Bogdanov *et al.*, 2008; Bodor *et al.*, 2019a). Among the minerals, potassium can be found in the highest quantity representing about the third of the total mineral content. The minerals in honey can be divided into main and trace elements. Amongst the main elements, we can find potassium, sodium, calcium, magnesium, iron, copper, manganese, chlorine, phosphorus, sulfur, silica, selenium, chromium, and zinc. Aluminum, arsenic, barium, bromine, cadmium, cobalt, fluoride, iodide, lead, lithium, molybdenum, nickel, rubidium, and strontium are counted as trace elements (White, 1978; Bogdanov *et al.*, 2008).

3.1.2.7 Biologically active antioxidants

In honeys, numerous types of antioxidant molecules can be found. Most of them belong to polyphenols. These can be flavonoids and non-flavonoids. Among the flavonoids, several different compounds have been identified, such as quercetin, kaempferol, pinobanksin, chrysin, pinocembrin, luteolin, apigenin, myricetin, galangin, naringenin, acacetin, catechin, tricetin, epicatechin, genistein, ellagic acid, and hesperidin. The latter eight compounds are not that frequent and have been found only in some types of honey, while the others are quite common. The non-flavonoid components are usually phenolics that can be derived from benzoic acid, cinnamic acid, and hydroxy-phenylacetic acid. Benzoic acid, syringic acid, 3-hydroxy benzoic acid, 4-hydroxy benzoic acid, methyl-syringate, methyl-4-hydroxy-benzoate, gallic acid, protocatechuic-acid, vanillic acid, genistic acid, o-anisic acid, and eusdemic acid are the benzoic

acid derivates. The homogentisic acid is derived from hydroxy-phenylacetic acid. Cinnamic acid derivates are trans-cinnamic-acid, p-coumaric acid, ferulic acid, caffeic acid, isoferulic acid, chlorogenic, and rosmarinic acid. Some metabolites of the phenolic acids also have been identified in honeys such as mandelic acid, homoanisic acid, phenylacetic acid, 3-phenyllactic acid, and phenylpropanoic acid (Bodor *et al.*, 2021a).

Besides the composition the physicochemical properties are also key factors of the characterization of the honeys in general and some of the properties can contribute to the origin identification.

3.1.3 Physicochemical properties

3.1.3.1 Moisture content

Water is an important constituent of honey, usually giving 13-23% of the honey. The quantity of water has a significant role in the stability against fermentation and exposure to spoilage. The water activity of the sample has also a significant role in the fight against microbial contamination. The water activity of honey is typically low (a_w<0.6) (White, 1978; Chen, 2019). According to the legislation, in general, the moisture content of the honeys should be a maximum of 20% (**Table 1**).

3.1.3.2 pH and acidity

The pH of honey is also an important characteristic of the products. As has been mentioned in chapter 3.1.2.3, the pH of honey is in the acidic range due to its organic acid content, and it is usually in the range of 3.3-5.5.

3.1.3.3 Electrical conductivity

The electrical conductivity (ELC) of honey is a crucial factor in the determination of the origin and discrimination of blossom honeys (see exceptions in **Table 1.**) from honeydew honeys. The ELC of the blossom honeys is below 0.8 mS/cm, while chestnut and honeydew honeys have ELC higher than 0.8 mS/cm. The ELC of honey comes from the mineral and acid content of the honey that is serving as electrolytes (Bogdanov, 2011b).

3.1.3.4 Density, fluidity, and viscosity

Honey is a liquid with viscous properties, and its viscosity depends on the temperature, water content, however, the granulation/crystallization of the honey also influences the viscosity of the products. Usually, products with higher water content are more fluid and flow faster, but the temperature has also an important role, where for the handling of honey 30°C is optimal. The composition of the honey has an influence on the viscosity of the sample, such as the

fructose/glucose ratio, polysaccharides in honey, and so on (Oroian, *et al.*, 2018). Some special honey types have characteristic viscosity attributes such as the manuka and heather honeys.
The density of honey is usually 1.5x the density of the water and in the case of honey, it is expressed in specific gravity (Bogdanov, 2011b).

3.1.3.5 Optical rotation

The optical rotation of honey is attributed to the sugar composition, as some of the sugars have a negative while others have a positive optical rotation. It is also helpful in the discrimination of the honeydew honeys from blossom honeys because the honeydew honeys are characterized by positive while blossom honeys by negative optical rotation (Oddo and Bogdanov, 2004).

3.1.3.6 Color

The color of honey is a very important factor in the appreciation of honey by consumers. It is a physical/sensory property that is immediately perceived. The color of the honey can be determined in several ways, however, honey is still marketed according to the Pfund value, where the color is determined in the range of water white-dark amber. The darker color of honey has been associated with higher nutritional value; therefore the color has high importance also at the market, price, and acceptance of the product (Oddo and Bogdanov, 2004; Bogdanov, 2011b; Bodor *et al.*, 2021b).

3.1.4 National and international legislation regarding honey

In the EU legislation related to honey is based on the *Codex Alimentarius* (CA) (*Codex Alimentarius Commission*, 2001), by the directive of the European Council (EC) (The European Council, 2001). In Hungary, the honey regulation can be found in the *Codex Alimentarius Hungaricus* (CAH) in line with the international rules (*Codex Alimentarius Hungaricus*, 2002). However, these rules are mainly at the general level for the honeys, and these do not contain compositional criteria for the individual honey types. However, in some countries including Hungary (*Codex Alimentarius Hungaricus*, 2009) there are some legislations at national levels that determine criteria of some unifloral honey types (Thrasyvoulou *et al.*, 2018). The amendments and corrections of the 2001/110/EC can be found in the 2014/63/EU (European Council and European Parliament, 2014). The amendments contain correction of the naming of the honey blends from different countries (description found below in 3.1.4.1), description regarding pollen which is a natural component of honey (not an ingredient), *etc*. As a general rule, the members of the European Union countries have to determine their national regulation which cannot be more permissive than the EU rules, but they can be stricter. The criteria related

to the general composition of the honey based on the EC, CA, and CAH can be found in **Table 1**. From the table, it can be seen that the three regulations are almost the same and that the Hungarian legislation meets the requirement of both CA and EC directives.

In the further part, the Hungarian legislation will be introduced more deeply.

3.1.4.1 *Codex Alimentarius Hungaricus* (CAH 1-3-2001/110 regulation Honey)

According to this, honey is made by the honeybees (*Apis mellifera*) from three different sources: the nectar, the secretions of the living plant part, and the excretions of the sucking insects on the living plants. The bees collect these materials and transform them with the combination of their specific substances (enzymes). This is followed by the deposit to the bee-hexagons and then they dehydrate it and leave it in the honeycomb until the ripening. The legislation discriminates the honey according to various categories (*Codex Alimentarius Hungaricus*, 2002):

1) Origin
   a. blossom honey, which originates from the nectar of the plants
   b. honeydew honey, which produced from the secretions of the living plant parts and the excretions of the sucking insects (*Hemiptera)* on the living plants
2) According to the production or the appearance
   a. comb honey: Honey that is sorted by the bees in the cells of freshly built broodless combs or artificial combs made of beeswax. These are sold as whole combs or sections of combs.
   b. chunk honey or cut comb in honey: honey containing one or more honeycomb pieces
   c. drained honey: Draining process is applied for the collection from the combs
   d. extracted honey: Centrifugation process is applied for the collection from the combs
   e. pressed honey: Pressing process is applied for the collection from the combs with or without applying a moderate heating ≤45°C
   f. filtered honey: Honey that is collected in the way that the organic or inorganic matter is removed by filtration, which results in a significant decrease in pollen.
3) The baker's honey is only suitable for industrial use, or as an ingredient in other foods. It is processed, but this can result in changes such as it can have a foreign taste or odor, can begin to be fermented, or can be overheated.

The compositional criteria claim that honey is composed mainly of sugars (mostly fructose, glucose) and other substances such as enzymes, organic acids, and the solid particles collected by the bees. The color of honey ranges from colorless to dark brown. The consistency of the

honey can be liquid, viscous, partly, or entirely crystallized. The taste and the odor of the sample depend on the botanical origin (*Codex Alimentarius Hungaricus*, 2002).

No food or other foreign ingredient (including food additives) can be added to the honey that is intended to be sold on the market or for human consumption. The honey also has to be free from exogenous organic or inorganic materials. Honey should not have any foreign taste or odor (except baker's honey) and it cannot be fermented. Its acidity cannot be artificially modified, or it cannot be heated in the way to destroy the natural enzymes or to significantly inactivate them (*Codex Alimentarius Hungaricus*, 2002). Except for filtered honey, no pollen or other natural substance can be removed only if it is the consequence of the elimination of the foreign organic or inorganic material. The quality criteria can be found in **Table 1** (*Codex Alimentarius Hungaricus*, 2002).

Labeling rules:

The "honey" term can be only applied for the products that meet the criteria described in paragraph 3.1.4 and can be traded only under this term. The different terms described above under 1), 2), 3) can be applied only to the products defined therein and can be used in trade to designate them. These terms can be substituted with the term "honey" except the "filtered honey", "comb honey", a slice of comb honey, "chunk honey or cut comb in honey", or "baker's honey". However, in the case of the baker's honey the term "intended for cooking or baking only") should appear close to the name of the product (*Codex Alimentarius Hungaricus*, 2002).

Except for the filtered and baker's honey, the name of the product can be supplemented by information referring to:

- botanical origin (only if the product comes entirely or mainly from the indicated origin and meets the criteria of the sensory, melissopalynological, and physicochemical characteristics of the source.)
- regional, topographical, or territorial origin - if the product comes entirely from stated origin.
- specific quality criteria

When using baker's honey as an ingredient of a food product in the name of the product the term „honey" can be used, however, among the ingredients, the term „baker's honey" should be applied. On the label of the product, the origin must be stated with the name of the country. If honey comes from a mixture of different countries the following expressions can be used:

- "blend of EU honeys" or "blend of non-EU honeys" or "blend of EU and non-EU honeys"

Besides these general rules, in some countries such as Hungary, there are honey types in which specific rules are applied. These specific rules of Hungary are going to be introduced in the following section.

Table 1 Regulation of honeys according to the European Council and *Codex Alimentarius* Commission and *Codex Alimentarius Hungaricus*

Parameter	European Council Directive (2001/110/EC)		*Codex Alimentarius* (Revised 2001-CODEX STAN 12-1981)		*Codex Alimentarius Hungaricus* (1-3-2001/110 regulation Honey)	
	General	Exceptions	General	Exceptions	General	Exception
Moisture %	≤20%	Heather (*Calluna* spp.) and bakers honey ≤23% Bakers honey from heather (*Calluna* spp.) ≤25%	≤20%	Heather (*Calluna* spp.) honey ≤20%	≤20%	Heather (*Calluna* spp.) and bakers honey ≤ 23% Bakers honey from heather (*Calluna* spp.) ≤25%
Fructose + glucose g/100g	≥60g/100g	Honeydew and blossom-honeydew blend honeys ≥45g/100g	≥60g/100g	Honeydew and blossom-honeydew blend honeys ≥45g/100g	≥60g/100g	Honeydew and blossom-honeydew blend honeys ≥45g/100g
Sucrose g/100g	≤5g/100g	Lavender *(Lavandula spp.)* Borago *(Borago officinalis)* ≤15g/100g; Acacia *(Robinia pseudoacacia),* alfalfa *(Medicago sativa*), citrus (Citrus spp.), French honeysuckle *(Hedysarum),* leatherwood *(Eucryphia lucida, Eucryphia milliganii),* Menzies Banksia (*Banksia menziesii),* red gum *(Eucalyptus camadulensis)* ≤10g/100g	≤5g/100g	Lavender *(Lavandula spp.)* Borago *(Borago officinalis)* ≤15g/100g; Acacia *(Robinia pseudoacacia),* alfalfa *(Medicago sativa*), citrus (Citrus spp.), French honeysuckle *(Hedysarum),* leatherwood *(Eucryphia lucida, Eucryphia milliganii),* Menzies Banksia (*Banksia menziesii),* red gum *(Eucalyptus camadulensis)* ≤10g/100g	≤5g/100g	Lavender *(Lavandula spp.)* Borago *(Borago officinalis)* ≤15g/100g; Acacia *(Robinia pseudoacacia),* alfalfa *(Medicago sativa*), citrus (Citrus spp.), French honeysuckle *(Hedysarum),* leatherwood *(Eucryphia lucida, Eucry- phia milliganii),* Menzies Banksia (*Banksia menziesii),* red gum *(Eucalyptus camadulensis)* ≤10g/100g
Water-insoluble solids g/100g	≤0.1 g/100g	Pressed honey ≤0.5g/100g	≤0.1 g/100g	Pressed honey ≤0.5g/100g	≤0.1 g/100g	Pressed honey ≤0.5g/100g
Electrical conductivity	honey not listed in	Honeydew, chestnut, and blends of these except with	honey not listed in	Honeydew, chestnut, and blends of these except with	honey not listed in	Honeydew, chestnut, and blends of these except with

Parameter	European Council Directive (2001/110/EC)		*Codex Alimentarius* (Revised 2001- CODEX STAN 12-1981)		*Codex Alimentarius Hungaricus* (1-3-2001/110 regulation Honey)	
	General	**Exceptions**	**General**	**Exceptions**	**General**	**Exception**
mS/cm	the exceptions and the blend of these ≤0.8 mS/cm	types listed below: Bell heather *(Erica)*, eucalyptus, linden (*Tilia* spp.), ling heather *(Calluna vulgaris)*, manuka and jelly bush *(Leptospermum*), strawberry tree *(Arbutus unedo)*, tea tree *(Melaleuca spp)* ≥.0.8 mS/cm	the exceptions and the blend of these ≤0.8 mS/cm	types listed below: Bell heather *(Erica)*, eucalyptus, linden (*Tilia* spp.), ling heather *(Calluna vulgaris)*, manuka and jelly bush *(Leptospermum*), strawberry tree *(Arbutus unedo)*, tea tree *(Melaleuca spp)* ≥.0.8 mS/cm	the exceptions and the blend of these ≤0.8 mS/cm	types listed below: Bell heather *(Erica)*, eucalyptus, linden (*Tilia* spp.), ling heather *(Calluna vulgaris)*, manuka and jelly bush *(Leptospermum*), strawberry tree *(Arbutus unedo)*, tea tree *(Melaleuca spp)* ≥.0.8 mS/cm
Free acidity	≤50 milli-equivalent s acid/ 1000g	Baker's honey ≤80 milli-equivalents acid / 1000g	≤50 milli-equivalent s acid/ 1000g	No exception	≤50 milli-equivalent s acid/ 1000g	Baker's honey ≤80 milli-equivalents acid / 1000g
Diastase activity (Schade units)	≥8	Except for baker's honey; Naturally lower enzyme-containing honey (*Citrus* spp.) and HMF not more than 15 mg/kg - ≥3	≥8	Naturally lower enzyme-containing honeys and HMF not more than 15 mg/kg - ≥3	≥8	Except for baker's honey; Naturally lower enzyme-containing honeys (*Citrus* spp.) and HMF not more than 15 mg/kg - ≥3
HMF mg/kg	≤40 mg/kg	Except for baker's honey Honey from tropical region, or blends of these honey ≤80 mg/kg	≤40 mg/kg	Honey from tropical regions, or blends of these honeys ≤80 mg/kg	≤40 mg/kg	Except for baker's honey low enzyme-containing honey, where the diastase activity is at least 3 Schade units ≤15 mg/kg Honeys from tropical regions, or blends of these honeys ≤80 mg/kg

3.1.4.2 *Codex Alimentarius Hungaricus* (2-100/2009 – Honey with distinctive quality indication)

In Hungary legislation deals with honeys with distinctive quality indication. This legislation aims to provide help to trade stakeholders with a description of some traditional Hungarian honey types. These marks can only be applied to the honeys describe here, and cannot be applied for honeydew, filtered, or baker's honeys. In addition to the CAH 1-3-2001/110 regulation on honey, some additional criteria could be applied for acacia, multiflora, and linden honeys. This additional information can be found in **Table 2**.

Table 2. Expected criteria of special honeys based on the CAH 2-100/2009 directive (*Codex Alimentarius Hungaricus*, 2009)

Quality parameter	Acacia	Linden	Multiflora honey
Physicochemical characteristics			
Moisture content *% w/w*	≤18.5		≤18.5
Sucrose *% w/w*	≤6		≤3
HMF content m/kg	≤20		≤25
fructose/glucose ratio	1.5-1.8		
proline content mg/kg	≥ 200		≥ 200
diastase activity (Schade units)	≥10		≥10
Sensory and melissopalynological requirements			
Appearance (color, clarity)	mirror-like, from water white to bright yellow, with a greenish fluorescence	mirror-like, light amber	mirror-like, from colorless to dark brown, characteristic to the dominant unifloral type
Odor	like acacia flower	like linden flower	variable, characteristic to the dominant unifloral type
Taste	sweet, characteristic, weak aroma	sweet, mildly bitter aftertaste, characteristically succulent	sweet, characteristic to the more intense unifloral honey types
Consistency, texture	no or slowly crystallize	moderately crystallize with small grains	varied according to the dominant unfloral honey, slowly or quickly crystallize
Pollen ratio %	above 15 *Robinia* sp. (if under 15, but above 5, the fructose/glucose ratio should be above 1.55)	30 (*Tilia* spp.)	depending on the harvest period
Pollen density	≥200	≥200	≥200

The directive also contains important rules regarding the storage and process conditions.

Packaging, storage, and transport

Storage under the sun is not allowed and honey should be stored closed. During the storage, the temperature must be between 5°C and 40°C. The package should be capped the way to be able to be destroyed when it is opened. During the collection period, the feeding of bees is forbidden. Honey cannot originate from honeybees under medical treatment. During the collection of the honey from the beehives, no chemical material can be applied. During the process, the core temperature of the honey cannot exceed 40°C (*Codex Alimentarius Hungaricus*, 2009)

3.1.5 Most common Hungarian honey types and their characteristics

In Hungary, the most common honey types are acacia, chestnut, sunflower, honeydew, rape, milkweed, and linden. The most important characteristics related to the physicochemical composition, sensory characteristics, and pollen characteristics can be seen in **Table 3**.

Table 3. Physicochemical, sensory, and pollen characteristics of the most abundant unifloral honeys in Hungary

Honey	Physicochemical characteristics	Characteristic pollen	Sensory characteristics
Acacia	F/G ratio is high >1.55 low ELC, enzymes, glucose, proline high in fructose and sucrose slow crystallization	*Robinia pseudoacacia* occasionally underrepresented in honey (7-60%) Must be at least 15% in Hungarian honey or 5% if fructose/glucose >1.55 directive	**Appearance**: mostly liquid, light color **Odor**: weak intensity, warm or fresh fruit aroma **taste**: weak intensity, floral, fresh fruit, warm, aftertaste duration is short
Chestnut	low G/W ratio, high F/G ratio high ELC must be >0.8 mS/cm, pH slow crystallization	*Castanea sativa* pollen overrepresented >86%	**Appearance:** usually fluid, dark to very dark **Odor:** strong intensity, woody, warm chemical **Taste:** strong intensity, woody, spoiled, warm chemical, long aftertaste persistence, astringent
Honeydew	low fructose, glucose, and F+G (min 45g/100g), G/W high pH, ELC , must be >0.8 mS/cm, slow crystallization (however melezitose can contribute to faster)	No characteristic pollen. Usually, honeydew elements are counted such as hyphae, spores, unicellular algae higher amount of nectarless plants	**Appearance:** dark to very dark color **Odor:** medium intensity, woody, warm **Taste:** medium intensity, woody, and warm aroma, medium sweet, taste persistence is mild, can be astringent
Linden	average values for most of the parameters sucrose can be higher than 5g/100g, ELC can be higher moderate crystallization	*Tilia* spp. (*Tilia cordata*, *Tilia platyphyllos*) occasionally underrepresented, 1-56% Must be at least 30% in Hungarian honey	**Appearance:** Color: light- medium-dark, bright (yellow) tone, can be crystallized **Odor:** strong intensity, chemical, woody, fresh **Taste:** strong intensity, chemical, woody, fresh, long aftertaste persistence, astringent
Milkweed		no characteristic pollen	**Appearance:** similar to acacia, **Odor:** sweet odor **Taste:** characteristic aroma
Rape	high glucose content, F+G, and G/W low values of F/G (<1.0), ELC, proline quick crystallization	*Brassica napus* pollen is overrepresented >60%	**Appearance:** light color, crystallized, **Odor:** medium intensity, spoiled or vegetal **Taste:** fresh (fruity) aroma, short aftertaste refreshing like fondant
Sunflower	high F+G, and low F/G ratio, G/W is elevated slightly high proline, and acidity quick crystallization	*Helianthus annuus*, occasionally underrepresented	**Appearance:** bright yellow, medium-dark, crystallized **Odor:** weak intensity, floral, warm, vegetal **Taste:** weak-medium, floral, warm, vegetal, fruity

The table is based on the literature: (Oddo and Piro, 2004; Amtmann, 2009; *Codex Alimentarius Hungaricus*, 2009; Hungarian Standars Institution, 2017)

ELC: electrical conductivity, F+G: fructose+glucose, F/G: fructose/glucose ratio, G/W: glucose/water ratio

3.2 Importance of botanical and geographical origin of honey

In this section the importance of the botanical and geographical origin of honey is introduced, moreover, studies on the origin identification are reported.

3.2.1 Botanical origin of honey

As it was described before honeys are made from different sources such as nectar, juices of the plants, and the excretions of the sucking insects, therefore honeys according to their botanical source are very different. Unifloral honeys - that are honeys that are collected mainly or wholly from the declared plant source – are usually more valuable than the multiflora types of the honeys blends. However, these labels must be proven. The honey can only be labeled as unifloral honey from the plant if it has the characteristics of that type of honey based on the physicochemical, sensory, and melissopalynological analysis. Also, the problem is that these rules for the botanical honey types are not detailed in the law, as has been shown in paragraph 3.1.4.1 about the legislation of the honey. Defining the unifloral honeys is not an easy task, as because of the variability of the honeys there are no reference honey for the individual botanical types, moreover during the honey production bees collect their materials from different species plants. Furthermore, no analytical technique is available for the determination of the exact amount of nectar from one plant, and the pollen concentration does not completely reflect the nectar amount in the honey. In the melissopalynological analysis one important factor is the amount of the characteristic pollen, which designates the botanical origin (for example for sunflower honey the characteristic pollen is sunflower (*Helianthus annuus*) pollen). Sensory parameters are also not completely reliable because if bees collect from plants that have stronger aroma can change the characteristic sensory properties of the honey (Bogdanov *et al.*, 2004). In general, the origin identification of honey is done based on the determination of the physicochemical, melissopalynological, and sensory properties of the honey. The botanical origin of the honey has a high influence on the composition and sensory properties of the honey, therefore characteristic parameters can be found for some individual honey types. The International Honey Commission (IHC) aimed to provide information on some unifloral honey types for their reference parameters (Bogdanov *et al.*, 2004; Oddo and Piro, 2004; Oddo and Bogdanov, 2004). Chemical analysis and determination of the physicochemical properties such as sugar composition, amino acids, volatile compounds, phenolic compounds can provide information on the botanical origin identification of the honey, especially for the unifloral honey types. However, these analyses do not provide sufficient information for the origin identification as other factors also influence the composition of the honey (Kaškoniene and Venskutonis, 2010).

Such factors can be the process, storage conditions, and geographical origin. The geographical origin of the honey can also have a high influence on the composition, therefore even within botanical types, we can find significant differences according to the composition and other properties of the honey. In the determination of the geographical origin the pollen analysis can have great importance because it can reflect the vegetation of the stated country. Besides the pollen analysis physicochemical analysis such as the amino acid or mineral analysis of the samples can help in the geographical origin identification (Anklam, 1998; Bogdanov, 2014; Uršulin-Trstenjak *et al.*, 2017; Pascual-Maté *et al.*, 2018a).

In the next subsection, some studies related to the origin identification of honey samples from the botanical and geographical origin points of view are introduced.

3.2.2 Studies for origin identification of honey

Physicochemical properties such as water content, pH, ash content, electrical conductivity sugars, proline content, phenolic composition, and mineral content has been applied for the botanical and geographical origin identification using multivariate analyses such as principal component analysis, linear discriminant analysis, and canonical discriminant analysis (**Table 4**). The identification accuracy of the botanical origin was quite high using the physicochemical parameters (Ampuero *et al.*, 2004; Oroian and Ropciuc, 2017; Popek *et al.*, 2017; Pauliuc *et al.*, 2020a). Moreover, using the data of minerals also provided good classification accuracy for the botanical origin and contributes to the separation of geographical origin (Louppis *et al.*, 2017; Uršulin-Trstenjak *et al.*, 2017; Sajtos *et al.*, 2019). The phenolics all alone were not as successful as the physicochemical properties according to Oroian and Ropciuc (2017). Besides these, studies report that the occurrence of terpenoids such as carotenoids and volatile compounds can also contribute to the origin identification of honey (Jerković and Kuś, 2014).

Artificial sensory instruments were also applied for the origin identification of honey in different countries (**Table 4**). Electronic nose achieved a good classification accuracy of >96% of honeys from different botanical origins (Ampuero *et al.*, 2004), while Ballabio *et al.*, (2018). could achieve only 41% of classification. Electronic tongues based on different principles such as impedimetric, voltammetric and potentiometric achieved different 70.8-90% (Elamine *et al.*, 2019; Oroian and Ropciuc, 2019; Pauliuc *et al.*, 2020b).

Spectroscopic techniques operating above the visible range, such as infrared spectroscopy, Raman spectroscopy, Fourier-transform infrared spectroscopy have also been successfully applied in the origin identification and for the prediction of the physicochemical parameters of honey (Kędzierska-Matysek *et al.*, 2018; F. Anguebes-Franseschi *et al.*, 2019; Aykas *et al.*, 2020; Anjos *et al.*, 2021). Near infrared spectroscopy was applied for the origin identification of honey with the correct classification of 79-88.20% (**Table 4**).

Table 4. Summary table of the applied indicative examples of physicochemical methods, artificial senses, and NIR for origin identification

Aim of the analysis	Honey/origins	Used methods	Results	Reference
Physicochemical characteristics, phenolics				
Botanical origin classification with Classification and regression trees (C&RT)	acacia, rape, honeydew, linden, heather	pH, total acidity, ash, reducing sugars, total sugars, sucrose, moisture, electrical conductivity, viscosity, diastase activity, HMF, proline	98.61% classification accuracy	(Popek *et al.*, 2017)
Botanical origin pattern analysis using Principal component analysis (PCA)	thyme, mint, rape, raspberry, sunflower	moisture, pH, free acidity, HMF content, electrical conductivity, color, total flavonoid content, sugars, polyphenols, and organic acids	good separation of rape, sunflower, and thyme	(Pauliuc *et al.*, 2020a)
Botanical origin identification with linear discriminant analysis (LDA)	acacia, linden, honeydew and sunflower, multiflora	moisture content, electrical conductivity, pH, ash content. color (CIE L*a*b*), phenolic composition, water activity, free acidity	LDA of phenolic composition: 58.0% LDA of physicochemical and phenolics: 81.82%	(Oroian and Ropciuc, 2017)
botanical origin identification using LDA	thyme, citrus *Abies*, *Pinus*	minerals, moisture, pH, electrical conductivity, total acidity, lactone, ash, free acidity, color (CIE L*a*b*)	physicochemical parameters: 92.9% minerals: 96.1%	(Louppis *et al.*, 2017)
botanical origin identification using canonical discriminant analysis (CDA)	acacia, forest, chestnut, linden, and sunflower honeys	mineral composition	100% classification accuracy	(Sajtos *et al.*, 2019)
geographical origin classification using PCA and cluster analysis	acacia	water, free acids, electrical conductivity, minerals	regions could be separated	(Uršulin-Trstenjak *et al.*, 2017)

Artificial sensory analysis and near infrared spectroscopy				
botanical origin – PCA, discriminant factor analysis (DFA)	acacia, dandelion, fir, rape, chestnut, linden	electronic nose equipped with MS detector using SPME sampling	98% classification accuracy	(Ampuero *et al.*, 2004)
Botanical origin identification using LDA	acacia, linden, rape, buckwheat, and honeydew	electronic nose equipped with semiconductor sensors	temperature influences, 35°C was the most promising	(Dymerski *et al.*, 2014),
botanical origin discrimination pattern using PCA and dendrogram	lavender, bupleurum honeys	impedimetric electronic tongue	good separation and clustering	(Elamine *et al.*, 2019)
botanical origin identification using LDA	acacia, sunflower, linden, multiflora, honeydew	voltametric electronic tongue	90% classification accuracy	(Oroian and Ropciuc, 2019)
botanical origin identification using LDA	raspberry, thyme, rape, mint, sunflower	voltametric electronic tongue	85.4% classification accuracy	(Pauliuc *et al.*, 2020b)
botanical origin identification using artificial neural network (ANN)	rosemary, citrus, honeydew, multiflora	potentiometric electronic tongue	~94% classification accuracy	(Escriche *et al.*, 2012)
botanical origin identification using discriminant factor analysis	chestnut, eucalyptus, sulla, orange blossom	potentiometric electronic tongue electronic nose (18 MOS sensors)	ET: 70.8% classification accuracy fusion: 87.5% classification accuracy	(Di Rosa *et al.*, 2018)
geographical origin using PCA	multiflora honey	voltametric electronic tongue	good separation	(Sobrino-Gregorio *et al.*, 2020)
botanical origin classification using partial least squares regression discriminant analysis (PLS-DA)	citrus, chestnut, sunflower, honeydew, multiflora, acacia, rhododendron, linden	electronic nose NIR	EN: 41% classification accuracy NIR: 79% classification accuracy	(Ballabio *et al.*, 2018)
botanical origin identification using PLS-DA	vitex, acacia, jujube	electronic nose electronic tongue NIR	EN: 96.67 % ET: 88.20 % NIR: 88.20% correct classification	(Gan *et al.*, 2016)
botanical origin identification using CDA	acacia, multiflora, linden, chestnut	NIR	NIR: >79% classification accuracy	(Bisutti *et al.*, 2019)

Based on the aforementioned studies we can conclude that all the types of measurements can be useful in honey authentication, however, none of the methods can provide 100% accuracy, because of the variability of the honey and the numerous factors that could influence the composition and other properties of the honey.

3.3 Adulteration of honey

Adulteration of honey has become more frequent in the last decades. Honey is one of the most adulterated food products in the world. Numerous fraudulent or misleading activities have been reported related to honey products. In this paragraph, the most common types of adulteration techniques of honey are introduced.

One of the most common adulteration types of honey is mislabeling. In this case, the botanical or the geographical origin is falsified. It can happen unintentionally, as not all the honeys are tested, especially in the case of small beekeepers, however, in most of the cases, it is intentional. The most serious level of this type is when pollen is added to ultrafiltered honey (and the filtration is not labeled) and it is sold as a unifloral honey. Another type is when filtered honey is mixed with honey of good quality. Moreover, in some cases, the adulterating persons also add natural substances to the honey such as enzymes and pollen to mimic normal honey (Zábrodská and Vorlová, 2014; European Commission, 2018).

However, as it was mentioned before, it is hard to identify the origin of the honey as the legislation do not determine regulatory limits for the pollen content of the individual honey types (Zábrodská and Vorlová, 2014; European Commission, 2018).

Adulteration with sulfite-ammonia caramel has also been reported before. This is a food colorant registered as E150d and it was used for the darkening of the honey (Zábrodská and Vorlová, 2014; Elflein, 2019).

Harvesting immature honey is also one type of adulteration that usually happens when the honey producers collect the honey before the bees close the honeycombs, which could lead to a higher moisture content which is about to lead to fermentation of the product. However, in some cases and some countries because of the climate, it is hard to harvest honey with less than 20% moisture content (Zábrodská and Vorlová, 2014; European Commission, 2018).

The most common and frequent type of adulteration of honey is the adulteration with exogenous sugars and sugar syrups. Direct adulteration is the dilution of honey with cheaper syrups, while in the case of indirect adulteration bees are fed by these sugars in the collection period. Both are hard to detect and can lead to an increase of honey volume but decrease in the quality of the product. The sugars can be classified into two groups the C4 and C3 sugars based on the carbon metabolism of the plant source. The plants that belong to the C3 group fix the CO_2

through the Calvin cycle. Wheat, rice, and beet belong to this group. C4 plants use the Hatch-Slack cycle to fix the CO_2, corn and sugarcane belong to this group. From these plants, numerous types of sugar syrups are produced that can be added to the honey as an adulterant. Usually, the detection of C3 sugars is more difficult than the C4 sugars (Zábrodská and Vorlová, 2014; European Commission, 2018; Elflein, 2019).

3.3.1 Adulteration detection studies

Adulteration detection of honey is also an interesting task as honeys are remarkably diverse, moreover, there are numerous ways of adulteration. In the paragraph above origin identification studies were presented, while in this section the focus will be on studies related to the detection of sugar syrup adulteration. According to the report of the European Commission, a standardized method is needed to detect the adulteration of honey (European Commission, 2018). According to the presentation of Lutz Elflein on the 5th International Symposium on Bee Products, there are numerous ways to detect the adulteration of the honey using sugar syrup, however, none of them is 100% accurate (Elflein, 2019):

Stable Isotope Mass Spectrometry coupled with Elemental Analysis/Liquid Chromatography (δ^{13}C EA/LC-IRMS):

Between 2015 and 2017 the EC used it to reveal honey adulteration on the European market. They found that it has good detection accuracy in the case of the common adulteration methods. The limit of detection for C4 sugars was found to be 3-5%, while for C3 sugars it was only 10-30%. The study showed that this method has a difficulty to detect the very sophisticated adulteration methods such as sugar syrups that are produced to have similar physicochemical properties to honey (especially regarding the sugar ratio).

^{1}H NMR – nuclear magnetic resonance spectroscopy:

It was introduced for honey analysis in 2013, and a database is needed for its accurate work. It was found to be not so sensitive to the adulteration detection of honey as the limit of detection was above 15%, however, it can be useful in the origin identification. Another drawback is that to be comparable the laboratories have to use the same compartments. The Chinese honey-tailored syrups could not be detected below 40%.

LC-HRMS - liquid chromatography–high-resolution mass spectrometry - screening

LC-HRMS is a new method, and it is very sensitive. The benefit of this technique is that it makes possible the detection of both known and unknown adulterants. The limit of detection of the routine measurements is around 5%.

Elflein (2019) also reported that in the past numerous techniques were applied to detect the adulteration of the honey, but the aforementioned methods are the most promising.

These techniques besides others, such as HPLC (high-performance liquid chromatography) or GC (gas chromatography) have been applied in numerous research articles to detect the adulteration of honey, but these techniques are often time-consuming, expensive, and destructive (Downey *et al.*, 2003; Zhu *et al.*, 2010; Tura and Seboka, 2019). Despite their efficiency, none of the methods above can detect all the syrup types with the same accuracy, therefore, there is still a need for easy-to-use or rapid methods that can detect the adulterants. The correlative techniques can be also a solution and their capability has been tested recently. In the following, some examples of the use of electronic tongue and NIR spectroscopy are introduced for the sugar syrup detection in honey.

Honey adulteration using inverted sugar and malt wort syrup in 5-50% was analyzed using an electronic tongue. The tongue was equipped with Ag/AgCl reference electrode, working electrodes such as Ag and Au, and a glassy carbon electrode and cyclic voltammetry method were used. The authors also determined the electrical conductivity, pH, color (L*a*b*), hue, and chroma of the samples. Authors found that the adulteration significantly influenced the physicochemical composition of the samples and based on the PCA based on the results of ET and physicochemical parameters, the 5% and 10% adulterated samples were close to the authentic samples (Ropciuc *et al.*, 2017). Bougrini *et al.,* (2016) performed an adulteration detection study using voltammetric electronic tongue where authentic honeys were mixed with glucose syrup and sucrose syrup in the concentrations of 2 %, 5%, 10% and 50% perfect. Authors reported that all the adulterated samples were classified with 100% accuracy. Honey from the different botanical origin and adulterated with sugarcane syrup (40%) were analyzed in a Malaysian study. In the study researchers applied electronic nose and chalcogenide-based electronic tongue for the classification of the samples. The electronic nose provided better classification accuracies than the electronic tongue, however the fusion of the two methods resulted in 100% correct classification for all the sample types (Zakaria *et al.*, 2011). In a Spanish study heather, orange blossom and sunflower honeys were mixed with barley, brown rice, and corn syrup at 2.5%, 5%, 10%, 20% and 40%. The samples were analyzed using a pulse voltammetric electronic tongue. Pattern recognition was performed by the PCA, and partial least squares regression models were built for the prediction of the added syrup concentration. The PCA models showed clear separation pattern of the samples, where honeys mixed with syrup separated from the others and a nice separation tendency was observed according to the adulteration level, too. PLSR provided a good correlation between the predicted and the added syrup concentration of higher then 0.9 R^2, except for the heather honey adulterated with rice syrup and orange blossom honey mixed with corn syrup. Prediction errors were also calculated, these were in all the cases 5.5% (Sobrino-Gregorio *et al.*, 2018).

Similarly, the correlative electronic nose and tongue, spectroscopy-based techniques such as Raman, FT-IR and near infrared spectroscopy were also used for the adulteration detection of the samples. Raman spectroscopy was applied for the detection of acacia, linden, honeydew, sunflower, and multiflora honey adulteration with 5%, 10%, 20%, 30%, 40%, and 50% with fructose, glucose, inverted sugar, and hydrolyzed-inulin syrup. The correct classification of the authentic sample was 83.93% after cross-validation (Oroian *et al.*, 2018). Fourier-transform infrared spectrometer was used to detect pure Mexican honey with corn syrup and cane sugar syrup in 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90%. PCA results showed clear separation of the adulterated samples from the pure honey (Rios-Corripio *et al.*, 2011). In another study FT-NIR spectrophotometer was used for the detection of glucose/fructose solution adulteration of honey. The method was able to differentiate the pure and adulterated samples with 95% classification accuracy (Zhu *et al.*, 2010) Chinese researchers mixed jujube, acacia, and vitex honey with corn and rice syrup at 5%, 10%, 20% and 40%. Electronic nose provided the worst results for the detection of the adulteration, misclassification was found during the calibration and validation data set. Electronic tongue provided better results where the calibration and prediction abilities were 98.43% and 100%, respectively. Near infrared spectroscopy showed correct classification of all the samples with 100% accuracy (Gan *et al.*, 2016). In another study honey was mixed with high-fructose-content sugar syrup and near infrared spectrometry was used to detect the adulterations. They found that the water structure of the honey samples changed as a result of syrup addition, thus higher absorbance was found in the adulterated samples in the region of the less H-bonded water of 1320-1420 nm (Bázár *et al.*, 2016). Honey was analyzed by NIR in a Chinese study where XDS NIR spectrometer was applied to detect the adulteration of authentic honey with beet, corn, maltose, HFCS, and rice syrup in the range of 10-60%. NIR provided 85.71% classification accuracy (Huang *et al.*, 2020). Benchtop NIR was also used to detect adulteration of honey using rice, invert sugar and brown cane syrups in the concentration range of 5-40%. The results of this study showed 0.98 R^2 higher than between the added and the predicted syrup concentration (Aliaño-González *et al.*, 2019).

3.4 Heat treatment of honey

Heat treatment is also an important aspect of the honey quality. Heat treatment of the honey is usually applied during the process of the products. The aim of this process is to reduce the viscosity of the sample to make the handling and portioning easier, moreover, it also aims to decrease the moisture content to prevent the activity of the yeast (Turkmen *et al.*, 2006; Samira, 2016). The main reason of the heat treatment is to prevent the crystallization of the honey, which is a natural process, but it is not preferred by the consumers. The crystallization rate of the honey

is highly influenced by the fructose/glucose ratio of the sample: samples with higher fructose content crystallize more slowly, and honeys having high amount of glucose (< 280-300 g/kg) crystallize quickly. Besides the F/G ratio other factors also can affect the crystallization: melezitose content (especially in honeydew honey) can lead to more rapid crystallization of the honey. The glucose/moisture ratio, especially when it is >2.1, can also accelerate the crystallization (especially in the case of the sunflower honeys) (Tosi *et al.*, 2008). The speed of the crystallization has an effect on the size of the crystals, while the slower crystallization leads to bigger and rougher crystal structure, the quick crystallization that is associated with fine, sometimes even cream-like crystal structure (Bogdanov, 1993). The liquefaction of the honey can be performed by different methods such as using special honey heating devices, drying chamber, water bath, microwave heating, *etc.* Moreover, sometimes pasteurization of the honey is applied, aiming to decrease the chance of contamination by yeast and bacteria. In the case of pasteurization, the temperature is relatively high (>50°C), but the time of treatment is short, that prevents honey from the loss of its nutritional value (Escriche *et al.*, 2008). However, not only pasteurization is applied to honey, but also long-term heat treatments, because the liquefaction is a time-consuming process. According to Bogdanov (1993), liquefaction of 20 kg honey at 40°C needs about 24 hours, while this is only 16 hours at 50°C. Beekeepers and producers can store honey in bigger barrels – even above 100 kg – that amount needs much more time to get liquefied. For the liquefaction at least 40°C should be applied, but long term heating even at this temperature can lead to the degradation of the enzymes and the formation of hydroxymethylfurfural (HMF). This latter can be formed even during long-term storage, especially if honey is stored in metal dishes. The problem is that at temperatures higher than 50°C the quality of the honey is worsened and the formation of the HMF is quicker. According to the legislation to detect the freshness and heat treatment of honey, the enzyme activity and HMF content have to be monitored. However, enzyme activity naturally decreases with the storage time, therefore it is mostly suitable for the fresh honeys (Tosi *et al.*, 2004). Numerous studies showed the decreasing effect on the enzyme activity and accelerated formation of HMF as a result of heat treatment. However, most of the studies showed that heat treatment ≤60°C did not result in significant increase in the HMF content of the honey, but other parameters can change even below this level (Visser *et al.*, 1988; Dimins *et al.*, 2006; Tosi *et al.*, 2008; Turhan *et al.*, 2008; Cozmuta *et al.*, 2011; Chua *et al.*, 2014; Al-Diab and Jarkas, 2015; Samira, 2016; Bodor *et al.*, 2017). Another problem is that during the heat treatment of higher temperature the useful components can degrade. Changes can happen in the vitamin content (Chua *et al.*, 2014), antioxidant capacity and also in the sensory (Inan *et al.*, 2012) parameters, such as aroma and color of the honey (Turkmen *et al.*, 2006). It has to be also highlighted that the changes in the

composition depend on the type of honey and its physicochemical parameters. Honey having lower pH values are more exposed to the formation of HMF than honeys with higher pH (Kesić *et al.*, 2017).

3.4.1 Heat treatment studies

In a Hungarian study long-time storage of the honey at high temperatures (75°C and 90°C) was monitored. Samples were taken through five hours every hour. Authors determined the L*a*b* color parameters of the samples and they found that the honeys become darker (lower L* values) and they tended to be more yellow and red (higher b* and a* values) (Csóka *et al.*, 2014). In another Hungarian study heat treatment at 40, 50, 60, 80, 100°C was applied for 5, 10, 15 and 20 minutes in water bath. Authors found that the treatment did not influence the moisture content of the samples, however the pH and electrical conductivity showed changes. The HMF content also increased as a result of the heating, moreover the total flavonoid and polyphenol content showed decreasing tendency (Czipa, 2010). In a Turkish study increase in the DPPH antioxidant capacity was found when honeys were heated at 50, 60, 70 °C for 12 days. This can be due to the formation of new antioxidants as a result of the Maillard reaction (Turkmen *et al.*, 2006). During this reaction, the amount of natural antioxidant (that are mostly polyphenols in the case of honey) compounds decrease, but meanwhile new antioxidants –melanoidins- are formed, that can lead to the increase of the global antioxidant capacity. In our previous preliminary study this phenomenon was also proven where honeys were heated at 40°C, 50°C and 60°C for 30, 60 and 120 minutes. In that study we found that based on the two-way ANOVA the time interval and its interaction with the temperature had a significant effect on the ABTS antioxidant capacity of the samples. In this case significant increase was obtained for honey treated for 60 minutes, but decrease was detected in the case of the samples heated for 120 minutes. In the same study (Bodor *et al.*, 2017). Results also showed the significant effect of temperature and its interaction with the time interval in the case of the HMF content, and significant changes were also observed in the case of the color L*a*b* parameters. As novel methods, that have not been introduced before for the detection of heat treatment of honey, electronic tongue and near infrared spectroscopy was applied. Electronic tongue results showed that the samples kept at room temperature could be discriminated from the treated samples, with the exception of the acacia honey. NIR results showed similar results to the ET. In the case of the linden, sunflower and multiflora honey even changes as a result of treatment at 40°C were detected using the novel methods, while HMF content analysis was not sensitive enough to detect this low level heat treatments (Bodor *et al.*, 2017). Since then, Italian researchers also applied NIR for the detection of heat treatment at 39°C for 30 minutes and at 55°C for 24 minutes Results showed that this low

level heat treatment (39°C, 30 min) did not have an effect on the spectra of the analyzed honey, however the overheating at 55°C could be discriminated from the control and the low level heat treatment also (Segato *et al.*, 2019). The applicability of FT-IR spectroscopy was also investigated for the detection of heat treatment of acacia, eucalyptus, and orange blossom honeys. Honey samples were heated at 40°C for 3.5, 5.5, 7.5 and 24 hours, and at 70°C for 15, 30, 60, 90, 120 minutes. The results provided better classification of the samples heated at 70°C than those at 40°C. Authors concluded that the FT-IR can be a good quick alternative method for monitoring honey quality (Antonova *et al.*, 2021).

These studies show that the correlative techniques also can have a good accuracy in the detection and the prediction of the sugar syrup in honey. However, in Hungary there is a lack of data regarding analysis of honey from the point of view of adulteration detection, especially regarding the application of correlative techniques. The electronic tongue and NIR has not been used before in the Hungarian literature for the origin identification and adulteration or heat treatment detection, moreover before our studies heat treatment detection has never been tested using the ET and NIR.

4 MATERIALS AND METHODS

In this section the materials and methods and the used sample preparations are going to be introduced. My thesis is separated to three main parts therefore, the materials and methods are subsectioned according to these. A summary table of the experiments and the used methods in them is found in **Table 5.**

Table 5. Summary table of the applied samples and methods in the experiments of the thesis

Method name	**Botanical and geographical origin identification studies**			**Sugar syrup adulteration experiments**		**Heat treatment study**
	BBGOIS	**OISWP**	**AUS**	**SSAPS**	**SSAWLC**	**HTE**
Moisture	✓	✓	✓	✓	✓	✓
pH	✓	✓	✓	✓	✓	✓
Electrical conductivity	✓	✓	✓	✓	✓	✓
Ash	✓		✓			
Sugars[#]	☑		✓			
Total polyphenol content	✓	✓	✓			
CUPRAC	✓	✓	✓			
FRAP	✓	✓	✓			
HMF						✓
Pollen analysis	☑	✓	☑ (authentic)	☑ (authentic)	☑ (authentic)	☑ (authentic)
L*a*b* (Konica Minolta)	✓	✓	✓			
L*a*b* (ColorLite)						✓
Classical sensory analysis			✓			
Electronic tongue	☑		✓		☑ (on sunflower)	✓
NIR (benchtop)		✓		✓	✓	
NIR (handheld)						✓

☑ denotes the experiments where not all the samples were measured with the respective technique

[#]glucose, fructose, sucrose

The first main part contains the botanical and geographical origin identification studies: namely the basic botanical and geographical origin identification study (BBGOIS), origin

identification study extended with pollen analysis (OISWP) and the authenticity study (AUS). The second main part focuses on the sugar syrup adulteration experiments, this has two subparts: the sugar syrup adulteration preliminary study (SSAPS), and the sugar syrup adulteration study extended with lower concentrations (SSAWLC). The last part is of the heat treatment experiment (HTE). The samples and the methods are going to be described in this chapter.

4.1 Materials

4.1.1 Honey samples of the botanical and geographical origin identification

The samples measured along the thesis were stored at room temperature in a relatively dark place. Samples of one study section (with the exception of the origin identification part) were measured within a short time as the experiments were not planned for longer periods (1-5 days).

4.1.1.1 Samples for the basic botanical and geographical origin identification study

In the origin identification study numerous honey types such as 28 acacia (*Robinia pseudoacacia*), 15 linden (*Tilia* spp.), 15 rape *(Brassica napus)*, 11 sweet chestnut (later chestnut - *Castanea sativa)*, 11 milkweed (*Asclepias syriaca*), 17 sunflower (*Helianthus annuus*), 8 bastard indigo (*Amorpha fruticosa*), and 11 multiflora honeys were analyzed. In addition, some rare honey types were also investigated such as two raspberries, one ramsons (*Allium ursinum*), one buckwheat (*Fagopyrum esculentum*), raspberry *(Rubus idaeus*), one shortpod mustard (*Sinapis incana*), one oleaster (*Elaeagnus angustifolia*), one milk thistle (*Silybum marianum*), and one sage *(Salvia pratensis*). The honeys originated from different parts of Hungary. The details about the origin and collection year can be found in **Appendix Table 1**. The samples were collected directly from the beekeepers. The main regions of Hungary were Alföld (Great Plain), Kisalföld (Small Plain), Északi-középhegység (Northern Mountains), Nyugat-magyarországi-peremvidék (Western Hungary), Dunántúli-középhegység (Transdanubian Mountains), and Dunántúli-dombság (Transdanubian Hills) (Dávid, 2013)

4.1.1.2 Samples for origin identification study extended with pollen analysis

In this study 87 samples were analyzed: 19 acacia (*Robinia pseudoacacia*), 11 linden (*Tilia* spp.), 10 rape *(Brassica napus)*, 10 chestnut *(Castanea sativa)*, 10 milkweed (*Asclepias syriaca*), 10 sunflower (*Helianthus annuus*), and 7 bastard indigo (*Amorpha fruticosa*). All the samples were collected directly from beekeepers from different regions of Hungary. The main regions of Hungary were the same as in 4.1.1.1.

4.1.1.3 Samples for the authenticity study

In this part honeys from different botanical and geographical origin were used, where among the samples 12 acacia (*Robinia pseudoacacia*) and 9 linden (*Tilia spp.)* authentic honeys were examined, collected from producers. 10 acacia and three linden honeys from retail were also used in this study. These honeys were labelled as blends of the European Union and non-European union honeys (later EUnonEU and RPEU for acacia and TIEU for linden) in accordance with the EC legislations (The European Council, 2001). In addition three-three mixtures of sugar syrup and acacia or linden honey were prepared in a way to have 10:90, 20:80, 50:50 sugar syrup:honey ratios *(% w/w*). These honeys were labelled as RP10%, RP20%, RP50% for acacia and TI10%, TI20% and TI50% for linden, where the numbers denote the concentration of the syrup.

4.1.2 Sugar syrup adulteration studies

4.1.2.1 Sugar syrup adulteration preliminary study

During the preliminary study linden (*Tilia* spp.) honey was used. Rice syrup – RI and beet syrup – BE - were mixed with the honey samples in % *w/w* 0.5%, 1%, 2%, 2.5%, 5%, 7.5%, 10%, 15%, 20%, 30%, 40%, and 50% ratios of the syrup. After mixing, the samples were placed in water bath for 1 hour at 37°C to ensure the homogenization. All the samples were prepared in three replicates in three independent plastic containers (60 ml) with screw cap. In total 81 samples were prepared in this experiment.

4.1.2.2 Sugar syrup adulteration study extended with the lower concentrations

During the extended study only lower concentrations were chosen based on the experience of the preliminary study and also on the literature. In the literature usually higher concentrations are applied, therefore this study is filling this gap (Lang *et al.*, 2015; Gan *et al.*, 2016; Shafiee *et al.*, 2016; Longin *et al.*, 2019; Yang *et al.*, 2020), however we know that in the practice higher concentrations are applied. The aim here was to find the lowest detectable concentration.

In the extended experiment acacia, linden, honeydew, rape, and sunflower honey was used. This experiment was done in two sets. At first the acacia and linden experiments have been performed to see if instrument can be used at these low levels. Then the experiment with the sunflower, honeydew and rape honey was done. The samples were mixed in *% w/w* 3%, 5% and 10% with the syrups.

The acacia (RP) and linden (TI) honeys were mixed with rice – (Bio Reis Syrup, dm-drogerie markt GmbH & Co. KG, Karlsruhe, Germany) and K-Sweet F40 – FS – (high-fructose content corn syrup, Kall Ingredients, Tiszapüspöki, Hungary). The sunflower (HA), honeydew (HD) and

rape (BN) honeys were mixed with the rice, F40 and a self-made glucose/fructose syrup (GF). The GF syrup was prepared with the ratio of 60:40 fructose:glucose: 240 g of analytical grade fructose and 160 g glucose were weighed in a beaker and 100 ml of distilled water was added. The mixture was put in water bath at 60°C and heated until all the crystals dissolved. The 60°C was only applied for the preparation of the self-made GF syrup. All the samples were prepared in three replicates (R1, R2, R3). The coding of the samples was the following: BNCO000R1, where the first two letters denote the honey type (BN, HA, HD, RP, TI), the second two letters are for the syrup (CO- control, SY-syrup, RI, FS, GF) the next three numbers denote the concentration of syrup (000, 003, 005, 010, 100) and the last digits denote the replicates (R1, R2, R3). In total 141 samples were examined in this experiment.

4.1.3 Heat treatment experiment

Acacia (RP-*Robinia pseudoacacia*), bastard indigo (AF-*Amorpha fruticosa*) and sunflower (HA-*Helianthus annuus*) honey types were collected directly from beekeepers, making sure that honeys have not been heat treated (control samples) before. The samples were collected the way to include honeys from slow crystallizing (acacia), moderate crystallizing (bastard indigo) and quick crystallizing types (sunflower.). The bastard indigo and sunflower honeys were crystallized. Three bottles of 1 kg honey (R1, R2, R3) were used from each type, and the honeys were from the same barrel. Honeys were weighed (50 g each) into 51-51 portions (17 from each bottle of honey) of 100 ml glass sample holders with plastic cap allowing tight closure of the bottle. There was no heating dew condensation observed during the cool down.

Venticell 111 drying chamber (MMM Medcenter Einrichtungen GmbH., München Germany) was used for the heat treatment of the honeys at 40°C, 60°C, 80°C and 100°C for 60, 120, 180 and 240 minutes, resulting in 17 levels of heat treatment (time temperature combinations e,g.: 60°C 120 minutes) including control (not heated) and 51 samples per type of honey type (altogether 153 samples). The coding of the sample is the following: HA060180R1 where letters are for the type of honey, the first three numbers are for temperature in °C (040, 060, 080, 100), the following three numbers are for the heating time in minutes (060, 120, 180, 240) and the last R1, R2, and R3 are meaning the repeats (from the different bottles). Before the measurements, the honey samples were cooled down to room temperature (25°C). The experimental heat treatment levels were chosen to cover a wide range from the highest legally allowed (40°C) to some of the extreme, but in practice occasionally applied temperatures (up to 100 °C) based on personal experience and literature.

4.2 Methods

In the methods section the used methods are going to be introduced. For the characterization of the honeys reference methods were used (the ones that are usually applied in the literature and practice to describe the characteristics of the samples) such as sugars, pH, electrical conductivity, ash content, moisture content, and HMF. Moreover, the determination of the antioxidant capacity and color and melissopalynological analysis of the honeys were performed. As novel correlative techniques electronic tongue and near infrared spectroscopy were used for the authentication of the samples.

4.2.1 Reference methods

The reference methods such as moisture content, pH, ash content and electrical conductivity were applied according to the method book of the International Honey Commission (Bogdanov, 2009).

4.2.1.1 Moisture content

Samples were liquefied to no to contain crystals (if needed), then spread on the Abbe-type refractometer (Carl Zeiss, Jena, Germany) at 20°C. All the samples were measured by two readings of the refraction index and the moisture values were taken from the conversion table of the IHC (Bogdanov, 2009).

4.2.1.2 pH

The determination of pH was adapted from the aforementioned method book, but the amounts were modified according to the following (owing to some samples would not have enough quantity as written in the IHC method book): 1.33 g of sample was weighed on an analytical scale, then 10 ml of freshly distilled (carbon dioxide free) water was pipetted on it. The solution was homogenized and three readings were performed per sample after calibration of the device. Mettler Toledo SevenMulti with pH probe (Mettler Toledo, Columbus, Ohio, USA) was used for the determination of the pH.

4.2.1.3 Electrical conductivity

After the determination of the moisture content the electrical conductivity solutions were prepared to have 20% dry matter content solutions (Bogdanov, 2009). All the samples were determined with three consecutive readings using a conductometer probe of the Mettler Toledo SevenMulti conductometer (Mettler Toledo, Columbus, Ohio, USA).

4.2.1.4 Ash content

For the determination of the ash content 5-10 g of sample was weighed in an ash dish (previously weighed) and 1-3 drops of olive oil was added. The samples were ashed using laboratory Teclu-burner until they became completely grey. Then the furnace was preheated to 600 °C and the samples were placed in the furnace for four hours. After the four hours, samples were replaced to a desiccator and weighed (Bogdanov, 2009). All the samples were measured using two replicates from two independent weightings.

4.2.1.5 Sugar composition

Sugar composition (sucrose, fructose, glucose) was determined using RP-HPLC (Waters,Milford, Massachusetts, USA). The device was equipped with a refraction index detector and Kromasil 100-5 NH2 MZ column (250 mm × 4.6 mm, particle size: 5 µm). Throughout the analysis the flow rate was 1.5 ml/minute, and the whole analysis was performed at 25 °C. As mobile phase solution of water:acetonitrile 28/72 *% v/v* was used. For the calibration sucrose, fructose, and glucose standards were prepared at three different concentration levels. All the samples were analyzed in two replicates, where 1 g of honey was dissolved in distilled water and filled up to volume in a 100 ml volumetric flask. After homogenization, the sample solution was filtered through Chromafil XTRA RC45/24 filter using syringe. For the analysis 10 µl of solution was injected. Before the analysis, the crystals were dissolved using water bath at 40°C.

4.2.1.6 Antioxidant properties

For the three antioxidant properties the same sample preparation was applied. 1.0 g of honey was weighed in and the weighed amount was recorded. Sample was dissolved in a small amount of distilled water and 10 times w/v solution was prepared in a 10 ml volumetric flask. All the parameters were determined using a Thermo Helios Alpha (Thermo Fischer Scientific Inc., Waltham, Massachusetts, United States) UV-VIS spectrophotometer (±0.001 units of absorbance, 1 cm of light path). All the samples were analyzed in 5 replicates.

Total polyphenol content (TPC):

Folin-Ciocalteu method (Singleton and Rossi, 1965) was applied to determine the total polyphenol content of the honey samples. 1 ml of sample solution was pipetted to a test tube and 7.5 ml of distilled water was added using dispenser. This step was followed by the addition of the 0.5 ml of Folin-Ciocalteu reagent, then after 3 minutes 1 ml of Na_2CO_3 solution was pipetted. Samples were homogenized using a vortex and then put in dark place for 30 minutes. After the

incubation time against distilled water blank the absorbance was recorded at 750 nm. As calibration standard gallic acid was used.

Ferric reduction antioxidant power (FRAP):

FRAP value of the honey samples was determined according to the method of Benzie & Strain (1996). In the beginning of the analysis the reagents were prepared: 0.54 g of $FeCl_3$ was weighed in a volumetric flask of 100 ml, then the flask was filled up to volume using distilled water. In the following 0.3123 g of TPTZ powder (2,4,6-tripyridyl-S-triazine) was weighed in a 100 ml volumetric flask and filled up to volume using 40 mM HCl solution. These two reagent solutions were poured into 1000 ml volumetric flask and 500 ml of acetate buffer at 3.6 pH was added.

For the sample measurement the following solution was prepared: 0.5 ml of honey solution was pipetted into a test tube, then 7.5 ml of FRAP solution was added using dispenser. The test tubes were placed in a water bath at 37°C for one hour, then the absorbance of the samples was recorded at 653 nm against distilled water blank. For the calibration ascorbic acid was used.

Cupric ion reducing antioxidant power (CUPRAC):

Cupric ion reducing antioxidant power was analyzed according to the method of Apak *et al.* (2008). As a first step the reagents were prepared such as $CuCl_2$ (10^{-2} M) solution, NH_4-acetate buffer at pH 7, and neocuproine solution (0.156 g of neocuproine powder dissolved in ethanol and filled up to volume in 100 ml volumetric flask). 1 ml of the buffer, $CuCl_2$, and neocuproine solution were mixed with 0.2 ml of honey solution and 0.9 ml of distilled water. These mixtures were homogenized using a vortex, then put into dark for 30 minutes. The absorbance of the solutions against distilled water blank were recorded at 450 nm. For the calibration Trolox was used and the CUPRAC antioxidant power was expressed in trolox equivalent (TEQ).

4.2.1.7 Hydroxymethylfurfural

HMF content of the samples was determined using the Winkler method based on the guide of the IHC (Bogdanov, 2009).

The chemical reagents were prepared as the first step: 0.500 g barbituric acid was weighed into a volumetric flask of 100 ml, then 70 ml of distilled water was added and warmed in the water bath (60 °C) to dissolve the reagent and then the flask was filled up to volume with distilled water. For the other reagent 10.0 g of p-toluidine was weighed in and 50 ml of isopropanol was added and placed in water bath at 60 °C. Then, 10 ml of acetic acid was added and the solution was poured to 100 ml volumetric flask and filled up to volume using isopropanol.

Sample preparation: 2.0 g of honey was weighed into a 25 ml beaker, then 4 ml of distilled water was added. The honey was homogenized with the water and 200 µl of Carrez I (potassium-hexacyanoferrate) and 200 µl of Carrez II (zinc-acetate) solution was pipetted. Samples were then filtered and filled up to volume in 10 ml volumetric flask.

Measurement: 1 ml of the honey solution was pipetted out into 4-4 quartz cuvettes (1x1x4 cm), then 2.5 ml of p-toluidine solution was added. After waiting of 2 minutes 0.5 ml of barbituric acid solution was added to three of the cuvettes, and to the blank 0.5 ml of distilled water was pipetted. Each of the samples had their own blank solution. The sample solutions were mixed and after three minutes all the samples were scanned using Thermo Helios Alpha (Thermo Fischer Scientific Inc., Waltham, Massachusetts, United States) UV-VIS spectrophotometer (±0.001 units of absorbance, 1 cm light path) at 550 nm. The HMF content of the samples was calculated based on the following equation 1:

equation 1) $$\frac{192 * \text{Absorbance} * 10}{\text{Weigh of the sample}}$$

4.2.1.8 Color determination

Color determination of the honey samples for the origin identification study were analyzed using Konica Minolta 410 colorimeter (Konica Minolta Inc. Chiyoda City, Tokyo, Japan) in five replicates per sample. For the calibration of the device the white tile provided by the producer was used.

Color determination for the heat treatment study was done using ColorLite sph850 (ColorLite GmbH, Germany). For the calibration of the device distilled water was used.

During both analyses the L* (lightness, higher the value, lighter the sample), a* (greenish hue: negative values, reddish hue: positive values), and b* (blueish hue: negative values, yellowish hue: positive values) were determined.

4.2.2 Melissopalynology

Acetolysis method was applied for the preparation of the honey samples (Erdtman, 1960). The analyses consisted of the steps of **Figure 1**. Honeys were well mixed before the analysis to homogenize the pollen concentration everywhere. After every centrifugation step the supernatant was decanted. After the last centrifugation step the decanted material (pollen) was put into Eppendorf tubes and they were placed for sedimentation for 1 hour. For the slide preparation one drop of the pollen solution was added, then gelatinized glycerin was put into it and these were heated together until the sample was half dried. Then a cover slide was placed on it. The counting of the pollen was done up to 300 pollen grains starting from the middle line of the slide.

During the counting 400x magnitude of a light microscope (Carl Zeiss AG, Aalen, Germany) was applied. Identification of the pollen was done using the reference collection of the Department of Palynology and Climate Dynamics of the University of Göttingen and the book of the pollen of Central Europe (Beug, 2015).

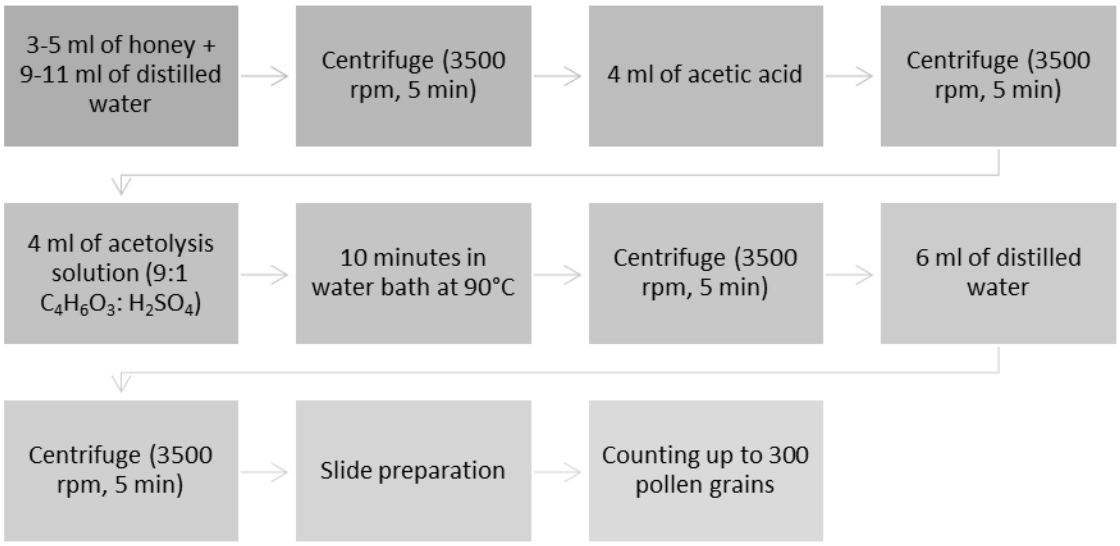

Figure 1. Steps of the melissopalynology analysis

4.2.3 Sensory analysis of the acacia and linden samples

Sensory analysis of the honey samples has been performed in a sensory laboratory. The analysis and the laboratory were designed to fulfill the requirements of International Organization for Standardization (ISO) standards (ISO, 1994, 2003, 2007). The sensory panel consisted of 12 members; all the samples were analyzed in two sessions. The linden and acacia samples were analyzed separately. The two unifloral types were also analyzed related to different sensory descriptors chosen from the aroma wheel of honey after evaluating them (Piana *et al.*, 2004).

4.2.4 Rapid correlative techniques

4.2.4.1 Near infrared spectroscopy (NIR)

In my thesis a benchtop and a handheld device were used. The benchtop device was the MetriNIR analyzer (MetriNIR Research, Development and Service Co., Budapest, Hungary), that operates in the spectral range of 740-1700 nm, with 2 nm spectral step and equipped with a dispersion, dual beam InGaAs detector. The handheld instrument is the NIR-S-G1 (InnoSpectra Co., Hsinchu, Taiwan) that allows spectral acquisition in the range of 900-1700 nm, with 3 nm wavelength step suited with DLP microarray InGaAs detector. The spectrometers were turned on

30-60 minutes prior the experiments, which based on preliminary experiments enough for the stabilization of the device.

During all the measurements transflectance setup was applied. The samples were filled in the cuvette ensuring no bubbles present in the layer. During the MetriNIR measurements the layer thickness was 0.5 mm and the cuvette was temperature controlled at 25°C using circulation around the sample in the coat of the cuvette. The handheld measurements were performed in a cuvette without temperature control with the layer thickness of 0.4 mm. In every experiment the samples were measured in a randomized order.

In the case of the **origin identification study extended with pollen analysis (OISWP)** the spectral acquisition of the NIR data was performed with the MetriNIR instrument. All the samples were measured three times (R1, R2, R3) using three consecutive scans, resulting in nine spectra per sample.

The measurements of the **adulteration experiments (SSAPS, SSAWLC)** were performed also using the MetriNIR instrument. During the **preliminary study (SSAPS)** all the samples were measured with five consecutive scans resulting in 15 spectra per adulteration level. The **extended study on the lower concentrations (SSAWLC)** was performed similarly as in the case of the linden and acacia honey. Every sample was measured using five consecutive scans resulting in 15 spectra per adulteration level. The spectral recording of the rape sunflower, and honeydew honey was performed using three consecutive scans per sample resulting in nine spectra per adulteration level.

The **heat treatment study (HTE)** was performed with the handheld instrument. Every sample was filled in the cuvette three times, recording five consecutive scans per fillings resulting in 45 scans per heat treatment level.

4.2.4.2 Electronic tongue

For honey analysis an Alpha Astree (AlphaM.O.S., Toulouse, France) potentiometric electronic tongue equipped with an Ag/AgCl reference electrode and seven chemical modified field effect transistor (CHEMFET) electrodes (ZZ, JB, JE, GA, HA, CA, BB) was applied. The potential change between the reference and the working electrodes was recorded. Before all the analysis a conditioning was applied using 0.01N HCl solution and cleaning (5 minutes conditioning, 10 seconds cleaning for approximately 1 hour) according to the manufacturer's advice. Then a conditioning for calibration phase and calibration was performed. The detailed calibration solutions are going to be described below for the three different studies. As a general setup all the samples were recorded through 120 seconds with 1 second intervals. Then, after

every sample cleaning of the sensors was performed for 20 seconds. Before data processing the last 10 seconds of each signal recording were averaged and used for the data analysis.

The **basic botanical and geographical origin identification study (BBOGIS)** was performed on one part of the samples (the samples used in the experiment can be found in **Appendix Table 1** labelled with "E"). The calibration before the experiment was performed using an independent sample purchased from the local store labelled as multiflora honey. Every sample was measured three different types on three different days. On each day 10 samples were measured plus two reference samples that were used every measurement day. Later during the data analysis these samples were used for the drift correction as reference. Throughout the measurements nine signal recording were done per sample resulting in 27 repeats per sample. The samples were measured through 24 days.

The measurements of the **authenticity study (AUS)** were performed with the same calibration method that was applied in the basic origin identification experiment. The samples were measured using 9 replicates. Same samples were used as the references for the drift correction.

The **sugar syrup adulteration extended study with lower concentrations (SSAWLC)** experiment was performed on the sunflower honeys from the extended sugar syrup adulteration study with low concentrations. For the calibration sample the mixture of all the samples was used. All the samples were analyzed with 12 signal recordings per sample resulting in 36 repeats per adulteration level.

The **heat treatment experiment (HTE)** was performed on two days per honey types. For the calibration, the mixture of the measurable samples of the days was used. As reference the samples of the lowest (control) and the highest (4 hours, 240 minutes at 100 °C) levels were prepared independently. All the samples were measured with 12 signal recordings resulting in 36 replicates per heat treatment level.

4.2.5 Statistical analysis

4.2.5.1 Reference parameters

Descriptive statistics

Descriptive statistics were applied in the case of all the experiments for the reference methods and the sensory profile analysis. The average and the standard deviation, minimum and maximum of the samples were calculated using the Microsoft Excel 365 software (Microsoft Corporation, Redmond, Washington, USA).

One-way analysis of variances (ANOVA)

ANOVA analysis was performed for the reference methods in the case of **basic the botanical and geographical origin identification studies (BBOGIS)**. ANOVA models were built to check if there are significant differences ($p<0.05$) among the different botanical origins.

In the case of the **authenticity study (AUS)** the significant differences were checked among the authentic acacia, authentic linden, EUnonEU acacia and linden and the self-made sugar syrup blended samples.

In the case of the **heat treatment experiment (HTE)** ANOVA was used to check if there is a significant difference between the control and the heat treatment levels in the HMF content.

In the case of all the experiments first the assumptions of the ANOVA test were computed: the normality was evaluated using Shapiro-Wilk and Kolmogorov-Smirnov test and the homogeneity of the variances was analyzed with Levene-test. In the case of the homogeneity of variances assumed Tukey-test was applied for the pair-wise comparison, if it was not assumed then Games-Howell pair-wise comparison was performed as it is not sensitive to the inhomogeneity (Tabachnick and Fidell, 2013).

Two-way analysis of variances

In the case of the heat treatment experiment the effect of the temperature, time and their interaction were evaluated using two-way ANOVA at $p<0.05$ significance level. If the effect of the interaction was significant, the differences within temperature levels among time intervals and within time levels among temperatures were tested using the same assumption tests mentioned in the previous ANOVA analysis section.

4.2.5.2 Pollen analysis of the origin identification study extended with pollen analysis

Pollen diagram

Pollen data was evaluated with the TILIA software where the TILIAGRAPH (Grimm, 1991) was used for the visualization of the results on the pollen spectra. The diagram was created from the taxa that presented in honey in higher than 2%.

Related to the fact that the honeydew and milkweed honeys does not contain characteristic pollen of the plant for the further analysis the data of the acacia, linden, bastard indigo, sunflower, rape, and chestnut honeys was used.

Cluster analysis

Hierarchical cluster analysis was used for the grouping of the samples of the six botanical types based on the Edwards' Cavalli-Sforza's Chord distance using the CONISS function of the TILIA software (Grimm, 1987).

Principal component analysis

Principal component analysis was used for the visualization of the pattern of the dataset, where the biplot (with the 10 most contributing loadings) was applied to show contribution of the different variables (taxa) for the separation of the samples.

Principal component analysis - Linear discriminant (PCA-LDA) analysis

PCA-Linear discriminant analysis was applied for the classification of the dataset according to the botanical groups. The model was validated using threefold cross-validation.

Data fusion and models

The data fusion was performed using low level data fusion approach. During the fusion, the pretreated (pretreatment having the best classification accuracy of validation) NIR spectra was concatenated with the pollen spectra (after the exclusion of the pollen <2%) and with the reference (average of the pH, moisture, and ELC per sample) parameters (first level preprocessing). After the fusion, the scaling and mean centering of the data was performed (second level preprocessing) (Campos and Reis, 2020). During the scaling, the mean centered data points were divided by the standard deviation of the variable. PCA was used for the pattern visualization and PCA-linear discriminant analysis was used to classify the samples according to their botanical and geographical regions. In the case of the geographical origin the main regions of Hungary were class variables (described in 4.1.1.2). The models were validated using threefold cross-validation leaving 1/3 of the dataset for validation, while the training was built on the 2/3 of the dataset. This was performed three times (in each round leaving out another set for the validation) and the average of the three models were given. Moreover, - as PCA-LDA was performed - PC number optimization was computed, where the model providing the best classification accuracy of the validation set and at the same time the lowest difference between training and validation set was chosen for the data interpretation.

4.2.5.3 Data analysis of the NIR data

Pretreatment optimization of the NIR data

For all the analysis types a pretreatment optimization was performed, where in total 41 different pretreatment types were tried. Choosing procedure of the pretreatment is written in more details for the different statistical methods in the following. Amongst pretreatments Savitzky-Golay smoothing with different derivation and window size were used to reduce the noise in the spectra. Moreover, multiplicative scatter correction (MSC), standard normal variation (SNV), detrending (deTr), and their combinations were applied. The list of the applied combinations can be found in **Appendix Table 2**.

Principal component analysis of the NIR data

Principal component analysis models were built using the raw spectra of the selected sample groups. This method was used for the visualization of the pattern of the sample set and the outlier detection in the case of all the experiments.

Principal component analysis-Linear discriminant analysis (PCA-LDA) of the NIR data

In the case of all the PCA-LDA models in all of the experiments the models were built trying all the pretreatment combinations and models having the best average validation accuracy were chosen, moreover the used principal component numbers used for the model was also optimized, choosing the model with the highest validation average accuracy.

During the **origin identification study extended with pollen analysis (OISWP)** the classification models were built to classify the six chosen groups (acacia, linden, sunflower, chestnut, bastard indigo, rape) according to their botanical origin and the geographical origin using the regions. The models were validated using threefold-cross validation, which was performed the same way as mentioned above at the data fusion (see in 4.2.5.2).

Classification models were built separately for the different botanical groups in the case of the **adulteration experiments (SSAPS, SSAWLC)**. Moreover, within the botanical groups, models were built using data of all the syrup types (with all their respective samples) and then separately for the data of the different syrups using three-times cross-validation (see details in 4.2.5.2). PC number optimization was also performed in this case (see details in 4.2.5.2).

Linear discriminant analysis was also used in the **heat treatment experiment (HTE)**, where models were built for the classification of the applied temperature level (control, 40°C, 60°C, 80°C, 100°C), the applied time interval (control, 60, 120, 180, 240 minutes) and also for the heat treatment level (temperature time combinations) from the combination of the time and temperatures resulting in 17 levels including the control. The models were validated using three-fold cross validation (see details in 4.2.5.2). The number of the components were optimized (see details in 4.2.5.2).

Partial least squares regression modelling of the NIR data

Partial least squares regression models of the **adulteration study (SSAPS, SSAWLC)** were calculated for the prediction of the added sugar syrup concentration (%). All the models were evaluated with the different pretreatments and the best models were chosen based on the model parameters detailed below. All the models were validated using the active-class validation, leaving out one group (in this case one repeat e.g. HACO000R1) through each iteration (Pollner and Kovacs, 2016). During the evaluation, the determination coefficient was calculated during the training (R^2C) and validation (R^2CV), so as the root mean square error (RMSEC – training, RMSECV – validation) and the residual prediction deviation (RPDC – training, RPDCV –

validation). This latter was computed with the deviation of the measured values with the error (RMSE). The benefit of this is that it considers the prediction error and the variation of the dataset, which provides more detailed information about the robustness and validity of the model (Luedeling, 2021). RPD value is better if the value is higher, however according to the literature there is no agreement on above which value it is good. Therefore, similarly to Muncan *et al.* (2021) RPD values above 1.5 were considered satisfactory. During building the models an outlier detection was performed using the PLSR-specific boxplot based outlier-detection algorithm (Pollner and Kovacs, 2016). This outlier detection works the following way: first the model is built using all the observations (pretreated) then from the predicted values based on boxplots – where the grouping variable is the measured value (e.g. added concentration %) – the outliers are eliminated and the model is rebuilt using the outlier detected dataset.

4.2.5.4 Data analysis of the electronic tongue data

Before the analysis by the electronic tongue the sensory data were drift-corrected using the "additive correction relative to reference samples" (ACRRS). This method is applied when samples are measured throughout different days. In our case in general two samples are the same each day which for the correction can be applied. The drift of the samples (other than the reference) is corrected using the shift of the sensor signals of the reference samples among the different days (Kovacs *et al.*, 2020).

Principal component analysis of the ET data

Principal component analysis was used for the pattern recognition and for the detection of the outliers. Models were built using the results of all the sensors colored by the respective groups, such as botanical groups, concentration, or heat-treatment levels.

Linear Discriminant analysis of the ET data

In the case of the **basic botanical and geographical origin identification study (BBGOIS)** models were firstly built for the classification of the main botanical groups (acacia, rape, bastard indigo, sweet chestnut, linden, sunflower, honeydew, milkweed, and multiflora). As a second phase to be able to make comparison with the pollen extended study the six groups (acacia, rape, bastard indigo, sweet chestnut, linden, sunflower) were used for classification model of botanical origin discrimination. After this step all the six groups were used for the classification model of the geographical regions. However, due to the higher effect of the botanical origin, the models were built separately for all the honey types. In this case the geographical origin classifications were built for the counties to have, with the exception of the bastard indigo where the districts (*járás* see in **Appendix Table 1**) were the class variables (there were only two counties in their case). All the models were built using threefold cross-validation.

In the case of the **authenticity study (AUS)** that is part of the origin identification part, the models were built separately for the two types of honey (acacia and linden) because based on the PCA it was obvious that they are completely different based on the results of e-tongue. The models were built for the classification of the authentic acacia or linden honeys and the mixture of the honeys with the sugar syrups in 10%, 20% and 50%. The models were validated using threefold cross-validation. Because of the raised doubts about the authenticity of the EUnonEU honeys, the ET data of the EUnonEU honeys used for independent prediction into the built classification models in order to see if they are classified as authentic sample or not. The independent predictions were also done with threefold validation. Three models were built during the threefold cross validation (see details in 4.2.5.2), then the independent dataset was predicted in each model, at last the prediction results were averaged for the independent CV and shown for data interpretation.

In the case of the **sugar syrup adulteration experiments (SSAPS, SSAWLC)** the models were built for the classification of the different syrup mixed sunflower honeys. Four models were built, one that contained the control and all the syrup mixtures (10 levels) and three models for the three different syrup mixtures (F40, rice, GF) having 4-4 groups per models. All the models were validated with threefold cross-validation (see details in 4.2.5.2).

In the case of the **heat treatment experiment (HTE),** models were built for the heat treatment levels, temperature groups and time intervals, separately for the three types of honey (acacia, bastard indigo, sunflower). The models were validated using threefold cross-validation after the pretreatment and outlier detection.

Partial least squares regression of the ET data

PLSR models were built for the prediction of the sensory parameters in the case of the **authenticity experiment (AUS)** of the botanical origin identification main part. The taste parameters that showed significant difference between the self-made honey syrup mixtures and the reference sample were predicted using the drift-corrected electronic tongue data separately for the linden and the acacia honey. The models were validation using leave-one-out cross-validation.

PLSR models were also built for the prediction of the added syrup concentration in the case of the **sugar syrup adulteration extended study with lower concentrations (SSAWLC)** for the sunflower honeys. Four models were built: one having all the syrup, and three models for the three different syrup adulterants. The model parameters mentioned in 4.2.5.3 (Partial least squares regression modelling of the NIR data) above were all calculated. The models were validation using leave-one-sample-out validation.

5 RESULTS AND DISCUSSION

5.1 Results of the origin identification study

In this paragraph the results of the studies related to the botanical and geographical origin identification will be presented.

5.1.1 Results of the basic botanical and geographical origin identification study

5.1.1.1 Descriptive analysis of the Hungarian unifloral, honeydew and multiflora honeys

In this section the descriptive sheets of the unifloral honeys collected from different regions of Hungary are going to be presented.

Acacia honey

The characteristics of the acacia honeys can be found in **Table 6**. For acacia honeys the average characteristic pollen % is around 22.77±10.76 %, which shows that in general they met the requirements of the Hungarian acacia honey provisions, however in some cases values lower than 15% were found. In another Hungarian study the *Robinia pseudoacacia* pollen content of acacia honeys were above 45.27%, however in this work only three honeys were evaluated and all the honeys were from 2018, which could be the reason of the higher characteristic pollen content (Bodó *et al.*, 2021). Further details are given in the 5.1.2.2 section. The moisture content and electrical conductivity also met the requirements of the EU and Hungarian legislations, where the average moisture content was 17.82±0.94% and the electrical conductivity ranged between 94.7-229.0 µS/cm. The ash content of the samples was low, in the range of 0.0001-0.0655%. The honeys were characterized by low total polyphenol content and antioxidant capacity. The samples contained more fructose than glucose, the average glucose fructose ratio, which is an important characteristic of the acacia honeys, was around 1.62.

Our results regarding the physiochemical parameters are in line with the European descriptive sheets (Oddo and Piro, 2004) and a Hungarian study analyzing acacia honeys (Czipa, 2010; Czipa *et al.*, 2019). Interestingly, in the other Hungarian study higher TPC content was found for the acacia honeys (16.5±3.0 mgGAE/100g) and in the doctoral study of Czipa (2010) 42.3±8.4 mgGAE/100g comparing with our results. This can be explained by the difference in the collection periods (especially in the case of the doctoral work) and the probably different geographical origin of the samples. However, in Slovenian, German, Italian and African acacia honeys similar values were found to ours of 44.8±14.8 mgGAE/kg, 46±2 mgGAE/kg, 20.9-36.8 mgGAE/kg and 55.2±2.8 mgGAE/kg, respectively (Maurya *et al.*, 2014). The samples were light and their color was in the greenish and yellowish range of based on the L*, a* and b*

parameters. The color parameters are similar to a research reported by Slovenian researchers (Bertoncelj *et al.*, 2007, 2011).

Table 6. Characteristics of the Hungarian acacia honeys analyzed throughout the thesis

Parameter	Unit	Legislation criteria	Number of honey	Mean	Standard deviation	Min	Max
Characteristic pollen	**%**	above 15*	19	22.77	10.76	7.00	43.00
Moisture	**%**	≤18.5*	28	17.82	0.94	16.20	19.60
pH		NA	28	3.96	0.21	3.45	4.30
Electrical conductivity	**µS/cm**	≤800	28	150.3	29.7	94.7	229.0
Ash	**%**	NA	12	0.0344	0.0208	0.0001	0.0655
Total polyphenol content	**mgGAE100/g**	NA	27	5.39	2.43	2.24	15.46
FRAP	**mgAAE/100g**	NA	25	5.14	2.66	1.69	12.92
CUPRAC	**µmol TEQ/g**	NA	27	13.45	5.85	4.18	32.12
Glucose	**g/kg**	NA	11	259.7	22.6	228.5	299.5
Fructose	**g/kg**	NA	11	417.2	12.2	387.7	429.4
Saccharose	**g/kg**	60*	9	3.67	10.26	0.00	30.96
Fructose/glucose	**g/kg**	1.5-1.8*	11	1.62	0.16	1.37	1.86
Fructose+glucose	**g/kg**	≥600	11	676.9	22.3	649.8	708.6
L*		NA	13	58.48	2.67	51.84	61.48
a*		NA	13	-1.68	0.73	-2.74	0.21
b*		NA	13	13.27	6.61	8.39	29.07

*According to (*Codex Alimentarius Hungaricus*, 2009); NA: not available

Rape honeys

Rape honeys had similar composition to the acacia honeys **(Table 7**). The Brassicaceae medium pollen was in average of 65.33±25.03%, but it is well known that the rape pollen can be overrepresented in the honeys (Hungarian Standars Institution, 2017). Hungarian researchers found higher *Brassica napus* pollen content (>90%) in rape honey collected between 2014 and 2015 (Bodó *et al.*, 2020). In most of the cases the honeys met the requirement of the moisture content and in all of the samples the electrical conductivity was below the 0.8 mS/cm. Compared to acacia, the average electrical conductivity was higher, 231.2±62.0 µS/cm, just like the ash content, which ranged from 0.0589 to 0.0894%. The rape honeys contained also more total polyphenols (7.64±2.09 mgGAE/100g) than the acacia honeys and their FRAP (13.73±5.32 mgAAE/100g) values were also higher, so as the CUPRAC values (23.95±9.03 µmol TEQ/g). These honeys also were among the lighter honeys and most of them had in average greenish tone and more yellowish tone.

The pH of the honeys was similar to the pH in the descriptive sheets (pH = 4.1±0.2), however in the aforementioned Hungarian PhD. thesis (Czipa, 2010) lower values were found (pH=3.5±0.2). The electrical conductivity was also slightly below 200 µS/cm in other studies (Oddo and Piro, 2004; Czipa, 2010). In an international study also similarly to ours, pH was 4.19

and the ELC was of 200 µS/cm. The total polyphenol content was lower in our study compared to the Hungarian doctoral study (Czipa, 2010). Similarly, lower TPC was found in a Romanian study with 13.3±14.10 mgGAE/100g. Czech honey had similarly lower values, less than 9 mgGAE/100g. Regarding the color, in the Romanian study lower L* was found (41.4±3.48), that can be due to the age of the honey (honeys get darker with the ageing) or the geographical origin of the sample (Maurya *et al.*, 2014; Pauliuc *et al.*, 2020a).

Table 7. Characteristics of the Hungarian rape honeys analyzed throughout the thesis

Parameter	Unit	Legislation criteria	Number of honey	Mean	Standard deviation	Min	Max
Characteristic pollen	**%**	NA	10	65.33	25.03	15.33	91.00
Moisture	**%**	≤20	15	18.50	1.20	16.60	21.20
pH		NA	15	4.01	0.14	3.73	4.25
Electrical conductivity	**µS/cm**	≤800	15	231.2	62.0	115.7	400.0
Ash	**%**	NA	6	0.0780	0.0123	0.0589	0.0894
Total polyphenol content	**mgGAE/100g**	NA	15	7.64	2.09	4.88	11.27
FRAP	**mgAAE /100g**	NA	15	13.73	5.32	4.69	22.31
CUPRAC	**µmol TEQ/g**	NA	14	23.95	9.03	14.18	49.38
L*		NA	6	54.60	2.00	52.09	57.84
a*		NA	6	-1.24	1.25	-2.40	0.95
b*		NA	6	23.17	3.10	19.32	27.06

NA: not available

Bastard indigo honeys

Bastard indigo honey is not a well-studied honey type of Hungary. The bastard indigo honeys are mainly collected in the region of Tisza as the plants grow there in higher number. The characteristic *Amorpha fruticosa* pollen was in the range of 13.00-94.33% in the Hungarian honeys in average of 51.19±29.57% (**Table 8**). Similar results were found in a Hungarian research analyzing three bastard indigo honeys and found 74.77% of the characteristic pollen (Bodó *et al.*, 2021). The moisture content of all the bastard indigo honeys was below 20%, that fits the requirements of the legislation. The pH of the samples was in average 3.81±0.33. The electrical conductivity was 301.8±158.6 µS/cm, that is higher than for the rape and acacia honeys. The ash content was also higher than that of the acacia honeys, in average of 0.0614±0.0396%. Regarding the antioxidant parameters, the bastard indigo honeys were characterized by higher FRAP and CUPRAC and TPC values than the acacia and rape honeys. In a Hungarian research similarly higher total polyphenol content was found for the bastard indigo honeys compared to acacia (Bodó *et al.*, 2021). The bastard indigo honeys also have lighter tone based on the L* values, and are greenish and yellowish, similarly to the rape honeys.

As no study is available on Hungarian bastard indigo honeys related to the physicochemical composition (moisture, pH, electrical conductivity), and there is only a few information available in the international literature, the results are compared to a Chinese study. The electrical conductivity of our bastard indigo honeys was higher than in the in the Chinese study (0.2 mS/cm). The pH was slightly lower in our case and the TPC of the Chinese honeys was lower than in our case (270.1 ± 27.15 mgGAE/kg). Their samples were darker (L*= 29.67±2.92) and much more reddish (a*=109.03±11.26), but less yellowish (b*= 4.37±2.18) (Zhu *et al.*, 2020).

Table 8. Characteristics of the Hungarian bastard indigo honeys analyzed throughout the thesis

Parameter	Unit	Legislation criteria	Number of honey	Mean	Standard deviation	Min	Max
Characteristic pollen	**%**	NA	7	51.19	29.57	13.00	94.33
Moisture	**%**	≤20	8	17.24	1.10	15.90	19.40
pH		NA	8	3.81	0.33	3.42	4.34
Electrical conductivity	**µS/cm**	≤800	8	301.8	158.6	129.7	650.0
Ash	**%**	NA	4	0.0614	0.0396	0.0297	0.1120
Total polyphenol content	**mgGAE/100g**	NA	7	9.63	4.52	5.80	18.29
FRAP	**mgASS/100g**	NA	8	19.37	19.23	2.78	59.25
CUPRAC	**µmol TEQ/g**	NA	7	35.25	13.38	14.63	54.44
L*		NA	4	57.10	1.90	54.48	58.89
a*		NA	4	-1.18	0.69	-1.56	-0.14
b*		NA	4	22.33	9.89	15.22	36.84

NA: not available

Milkweed honeys

The characteristics of the Hungarian milkweed honeys can be found in **Table 9**. The moisture content of the samples was under the limit of the legislation, these honeys were more acidic than the other honey types, with pH of 3.71±0.15. The electrical conductivity (253.2±92.9 µS/cm) was higher than the values obtained for the acacia and rape honeys. The ash content ranged between 0.0743-0.4255%. Regarding the antioxidant parameters, the average total polyphenol content was 7.98±1.56 mgGAE/100g, higher than the TPC obtained for the acacia and rape honeys, but less than for the other honey types. The FRAP (12.08±6.99 mgAAE/100g) and CURPAC (19.69±8.64 µmol TEQ/g) values were generally low. The lightness was similar to the other honey types of (55.13±2.82), the a* was higher (-0.47±2.23) than in the case of the acacia, rape, and bastard indigo honeys, but based on the b*, samples were as yellowish (23.69+4.81) as the bastard indigo and rape honeys.

In a Hungarian study the milkweed honeys found to be similarly acidic with pH of 3.63, in another Hungarian research the pH was 3.3, which is a bit lower than in our study (Kasper-Szél *et al.*, 2003). The other physicochemical parameters were also similar to the ones found in our

research. Similarly to the acacia, the TPC was much higher than in our research (102.4±10.8 mgGAE/100g) (Czipa, 2010).

Table 9. Characteristics of the Hungarian milkweed honeys analyzed throughout the thesis

Parameter	Unit	Legislation criteria	Number of honey	Mean	Standard deviation	Min	Max
Characteristic pollen	**%**	NA	NA	NA	NA	NA	NA
Moisture	**%**	≤20	11	17.99	1.51	16.20	20.00
pH		NA	11	3.71	0.15	3.42	4.01
Electrical conductivity	**µS/cm**	≤800	11	253.2	92.9	109.7	457.0
Ash	**%**	NA	4	0.1653	0.1736	0.0743	0.4255
Total polyphenol content	**mgGAE/100g**	NA	11	7.98	1.56	4.92	10.39
FRAP	**mgAAE/100g**	NA	11	12.08	6.99	2.96	22.45
CUPRAC	**µmol TEQ/g**	NA	11	19.69	8.94	6.98	36.54
L*		NA	4	55.13	2.82	50.95	57.07
a*		NA	4	-0.47	2.23	-1.67	2.87
b*		NA	4	23.69	4.81	17.99	29.75

NA: not available

Sunflower honeys

Sunflower honeys showed higher physicochemical (**Table 10**) values in general compared to the aforementioned honey types. The characteristic sunflower pollen was in average of 35.27±23.78%. Hungarian researchers also found *Helianthus annuus* pollen content (based on three honey) in sunflower honeys of 47.44% (Bodó *et al.*, 2021). The sunflower pollen is a type of pollen that can be occasionally underrepresented, that's why sunflower honeys can have lower amounts of sunflower pollen. The moisture content was under 20% in most of the cases. The pH was also below 4, similarly to the other aforementioned honey types, however the electrical conductivity was higher, in average of 440.1±144.0 µS/cm. The antioxidant capacity parameters also showed higher values compared to the acacia, rape, milkweed, and bastard indigo honeys. The L* of the sunflower honey was a bit lower than the aforementioned honey types, moreover, in general these honeys were less greenish (a*=-0.40+2.49), similarly to the milkweed honeys, but much more yellowish (b*=38.45+2.35). This parameter can be attributed to the color materials of the plant itself (carotenoids).

Compared to the Hungarian and international literature available, it can be stated that our pH results are the closest to the European descriptive sheets where the pH is 3.8±0.2, in the Hungarian study slightly lower results were found with (3.7±0.3). The electrical conductivity was higher in general compared to both because the ELC of their sunflower honeys was below 400 µS/cm. The total polyphenol content was much lower than in the other Hungarian studies (108.3±8.7 mgGAE/100g and in another 23.3±0.83 mgGAE/100g) , and also lower than in a

Romanian research (21.1±7.18 mgGAE/100g) (Oddo and Piro, 2004; Czipa, 2010; Pauliuc *et al.*, 2020a; Bodó *et al.*, 2021). The color was found to be lower in the case of the Romanian, Spanish and Czech honeys with L*<50 (Juan-Borrás *et al.*, 2014; Pauliuc *et al.*, 2020a). Similar ash content was found in a Portuguese study, where the sunflower honeys contained 0.18±0.00% ash (Anjos *et al.*, 2015b).

Table 10. Characteristics of the Hungarian sunflower honeys analyzed throughout the thesis

Parameter	Unit	Legislation criteria	Number of honey	Mean	Standard deviation	Min	Max
Characteristic pollen	**%**	NA	10	35.27	23.78	3.99	83.67
Moisture	**%**	≤20	17	18.62	2.16	15.70	23.73
pH		NA	17	3.84	0.35	3.44	4.90
Electrical conductivity	**µS/cm**	≤800	17	440.1	144.0	3.7	676.7
Ash	**%**	NA	6	0.1577	0.0520	0.1029	0.2381
Total polyphenol content	**mgGAE/100g**	NA	17	10.40	3.18	4.77	18.20
FRAP	**mgAAE/100g**	NA	17	30.10	18.93	8.02	73.77
CUPRAC	**µmol TEQ/g**	NA	16	56.28	29.83	19.74	120.7
L*		NA	7	53.16	1.59	51.28	55.10
a*		NA	7	-0.40	2.49	-3.67	2.36
b*		NA	7	38.45	2.35	34.55	42.06

NA: not available

Linden honeys

The characteristics of the Hungarian linden honeys can be found in **Table 11**. The average characteristic pollen content was >30%, however, some of the samples contained less than this value. Similar results were obtained by Bodó *et al.*, (2021), where the *Tilia* spp. pollen content was above 45% (based on the results of three honeys). Moisture content was also below 20% in most of the honeys, and higher pH value was found comparing with the rest BN, AF, RP, and HA honeys. The electrical conductivity and ash content was higher than in the other groups (544.6±157.2 µS/cm and 0.1114±0.0837%, respectively with the exception of milkweed and sunflower for the ash content). The total polyphenol content was 10.62 ±1.82 mgGAE/100g, and the antioxidant capacities were 25.23±11.43 mgAAE/100g and 39.73±10.20 µmolTEQ/g. In this case the fructose was also in a higher amount than the glucose, but the F/G ratio was only 1.30. No sucrose was found in these honeys. The honeys were darker than the acacia, sunflower, rape, and bastard indigo honeys, but they were also greenish and they were in the yellowish region (less yellowish than the sunflower honeys).

Compared to the international and Hungarian studies showed that the pH of our linden honeys was similar to the other Hungarian study where the pH was 4.2 and also fit in the range of the European descriptive sheets. Higher pH was found in a Slovenian honey (4.88±0.44) and electrical conductivity was above 0.6 mS/cm in all of the studies (Oddo and Piro, 2004; Czipa,

2010; Bertoncelj *et al.*, 2011; Pauliuc *et al.*, 2020a). The total polyphenol content was similar to the Slovenian study (83.7±14.3 mgGAE/kg), a Polish study (153.1±5.5 mgGAE/kg), and to Czech study (85.52-98.42 mgGAE/kg) (Maurya *et al.*, 2014). Similarly to all the other honey types in the other Hungarian study, higher TPC was found (85.1±16.3 mgGAE/100g). Slovenian linden honeys found to be a bit lighter with L*= 61.17±3.01, their samples were also more greenish a*=-2.83±0.95 but similarly yellowish b*=31.74±7.15 (Bertoncelj *et al.*, 2011).

Table 11. Characteristic properties of the Hungarian linden honeys analyzed throughout the thesis

Parameter	Unit	Legislation criteria	Number of honey	Mean	Standard deviation	Min	Max
Characteristic pollen	**%**	30*	11	30.14	19.74	5.33	66.45
Moisture	**%**	≤20	15	17.74	1.39	15.60	20.75
pH		NA	15	4.18	0.32	3.79	4.85
Electrical conductivity	**µS/cm**	≤800	15	544.6	157.2	266.3	760.3
Ash	**%**	NA	7	0.1114	0.0837	0.0024	0.2316
Total polyphenol content	**mgGAE/100g**	NA	15	10.62	1.82	7.33	13.55
FRAP	**mgAAE/100g**	NA	15	25.23	11.43	13.45	57.43
CUPRAC	**µmol TEQ/g**	NA	15	39.73	10.20	20.38	58.41
Glucose	**g/kg**	NA	7	303.0	15.8	279.3	322.8
Fructose	**g/kg**	NA	7	392.2	33.2	361.4	457.6
Saccharose	**g/kg**	≤50	6	0.00	0.00	0.00	0.00
Fructose/glucose	**g/kg**	NA	7	1.30	0.16	1.13	1.57
Fructose+glucose	**g/kg**	≥600	7	695.18	26.16	668.14	748.53
L*		NA	6	52.78	1.83	49.98	55.09
a*		NA	6	-0.56	1.11	-1.84	1.48
b*		NA	6	28.03	5.66	20.29	35.80

*According to (*Codex Alimentarius Hungaricus*, 2009); NA: not available

Sweet chestnut honeys

The descriptive properties of the sweet chestnut honeys can be seen in **Table 12**. The characteristic pollen of chestnut honeys was in average 77.43±11.19%, that supports the fact that the chestnut pollen is an overrepresented pollen type and the results of another Hungarian research (Bodó *et al.*, 2021). The moisture content was below the limit of 20% in the case of all the honeys, and in general this honey had the highest pH (4.40±0.22), electrical conductivity (695.3±131.6 µS/cm) and ash content (0.3212±0.0385%). In some cases the honeys did not meet the requirements of the general honey legislations, as they had electrical conductivities lower than 0.8 mS/cm. In general, this honey type had one of the highest antioxidant parameters (TPC 12.43±3.70 mgGAE/100g, CUPRAC µmol TEQ/g, 42.87±10.67 and FRAP 34.72±15.60 mgAAE/100g). This honey type was the darkest and showed more pronounced reddish tone.

Our results showed similar pH to the other Hungarian study where the pH was 4.1±0.4 and the electrical conductivity was found to be 584±112 µS/cm. However, compared to the European

descriptive sheets, our results showed lower electrical conductivity (1138±270 µs/cm) and also lower pH (5.3±0-+.5). Regarding the TPC, our results are similar to the results of the Slovenian study of 191.7± 6.8 mgGAE/kg and 199.9±4.1 mgGAE/kg, but lower than found in the other Hungarian study (140.5±32.5 mgGAE/100g) (Bertoncelj *et al.*, 2007, 2011; Czipa, 2010).The color was also similar to the studies of the Slovenian researchers where the L*= was 46.18±3.94 and L*=48.11±4.27. The a* were also similar 7.66-9.15, but their honeys were more yellowish b*=38.91-41.28 (Bertoncelj *et al.*, 2007, 2011). Spanish researchers found similar results to ours (L*= 51.1±22.0, a*= 5.31±5.65 and b*= 15.5±11.3) (Bentabol Manzanares *et al.*, 2017).

Table 12. Characteristic properties of the Hungarian sweet chestnut honeys analyzed throughout the thesis

Parameter	Unit	Legislation criteria	Number of honey	Mean	Standard deviation	Min	Max
Characteristic pollen	**%**	NA	10	77.43	11.19	60.00	93.00
Moisture	**%**	≤20	11	16.97	1.25	14.60	18.75
pH		NA	11	4.40	0.22	4.06	4.84
Electrical conductivity	**µS/cm**	≥800	11	695.3	131.6	531.0	947.7
Ash	**%**	NA	5	0.3212	0.0385	0.2717	0.3617
Total polyphenol content	**mgGAE/100g**	NA	11	12.43	3.70	5.90	20.49
FRAP	**mgAAE/100g**	NA	11	34.72	15.60	15.60	71.31
CUPRAC	**µmol TEQ/g**	NA	10	42.87	10.67	24.22	63.63
L*		NA	5	48.20	5.85	37.94	52.37
a*		NA	5	5.57	6.01	0.41	14.86
b*		NA	5	27.64	7.01	15.46	33.16

Honeydew honeys

The characteristics of the honeydew honeys collected from Hungary are found in **Table 13**. The moisture content was below 20% in most of the samples, in average 17.43±1.55% of water was present in the honeydew honeys. The pH was similar to the pH of the linden and chestnut honeys, above pH 4. The electrical conductivity ranged between 293.3 and 891.8 µS/cm. This shows that most of the honeys are not eligible for the legislation limits of the 0.8 mS/cm. This can be because of the unintended mislabeling, due to the confusion between forest honeys produced from nectar and those from honeydew. Many times, for both types the denomination on the label is "erdei" (forest honey), that is sometimes the term used for the "harmat" (honeydew) honeys as well. The ash content was 0.3955±0.3341% in average but we can also see that there were honeys having an ash content higher than 1%. The total polyphenol content was similar to the chestnut honeys (14.21±4.29 mgGAE/kg). This honey type was characterized by a higher CUPRAC value (55.19+18.87 µmol TEQ/g) and similarly high FRAP capacity was found. These honeys were the darkest with L* of 42.51±6.56, and the most reddish with a* = 8.15±3.20. The yellowness was similar to the milkweed, bastard indigo, and rape honeys.

The results of the honeydew honeys showed that the pH was similar to the other Hungarian study (pH=4.2±0.1), but lower electrical conductivity and TPC was found in our case. Czech samples also showed higher total phenolics (199.0±242.5 mgGAE/kg) (Maurya *et al.*, 2014). Spanish researchers found electrical conductivity >1000 µS/cm (Shantal Rodríguez Flores *et al.*, 2015; Escuredo *et al.*, 2019).

Table 13. Characteristic properties of the Hungarian honeydew honeys analyzed throughout the thesis

Parameter	Unit	Legislation criteria	Number of honey	Mean	Standard deviation	Min	Max
Characteristic pollen	**%**	NA	NA	NA	NA	NA	NA
Moisture	**%**	≤20	13	17.43	1.55	15.45	21.20
pH		NA	13	4.14	0.24	3.69	4.49
Electrical conductivity	**µS/cm**	≥800	13	524.2	172.0	293.3	891.7
Ash	**%**	NA	8	0.3955	0.3341	0.1134	1.1616
Total polyphenol content	**mgGAE/100g**	NA	13	14.21	4.29	6.44	20.78
FRAP	**mgAAE/100g**	NA	12	47.92	18.12	26.88	81.06
CUPRAC	**µmol TEQ/g**	NA	13	55.19	18.87	35.05	106.4
L*		NA	8	42.51	6.56	31.55	48.32
a*		NA	8	8.15	3.20	3.71	12.90
b*		NA	8	21.80	10.20	4.58	32.58

NA: not available

Multiflora honeys

The properties of the multiflora honeys can be found in the **Table 14**. As these are multiflora honeys, there is no characteristic pollen for this honey type. The moisture content of most of multiflora honeys fulfilled the requirements. The pH in average was 4.00±0.24, while the electrical conductivity ranged between 219.3 and 556.3 µS/cm. The ash content (0.1253±0.0494%) was similar the average values found for the sunflower, linden, and milkweed honeys. The total polyphenol content was 11.66±4.32 mgGAE/100g, higher than in the case of the acacia, bastard indigo, linden, rape, and the sunflower honeys. The antioxidant capacities were also higher than most of the sample types (except honeydew, and chestnut) in the case of FRAP (33.30±24.63 mg AAE/100g), and the CUPRAC (40.24±23.39 µmol TEQ/g) was also higher than the CUPRAC of the acacia, rape, bastard indigo, linden, and milkweed honeys. This shows that the multiflora honeys are of a good quality, despite their cheaper price compared to the unifloral honeys. The multiflora honeys were the third darkest samples with L* of 51.65±6.70. In general, they were in the reddish range, but the values ranged between -4.22 and 13.90. The multiflora honeys were characterized by higher b* than the acacia, rape, milkweed, and bastard indigo honeys.

Table 14. Characteristic properties of the Hungarian multiflora honeys analyzed throughout the thesis

Parameter	Unit	Legislation criteria	Number of honey	Mean	Standard deviation	Min	Max
Characteristic pollen	**%**	NA	NA	NA	NA	NA	NA
Moisture	**%**	≤18.5*	10	18.31	0.98	16.20	19.60
pH		NA	10	4.00	0.24	3.61	4.26
Electrical conductivity	**µS/cm**	≤800	10	350.0	113.3	219.3	556.3
Ash	**%**	NA	10	0.1253	0.0494	0.0609	0.2013
Total polyphenol content	**mgGAE/100g**	NA	11	11.66	4.32	6.32	19.10
FRAP	**mgAAE/100g**	NA	12	33.30	24.63	10.42	92.06
CUPRAC	**µmol TEQ/g**	NA	12	40.24	23.39	18.33	98.91
L*		NA	11	51.65	6.70	38.00	57.14
a*		NA	11	0.94	5.78	-4.22	13.90
b*		NA	11	25.32	8.45	14.56	41.24

*According to (*Codex Alimentarius Hungaricus*, 2009); NA: not available

5.1.1.2 Results of the rare honeys collected from Hungary

The rare honeys that were collected during the research also fulfilled the requirement of the legislations as the moisture content was lower than 20% (**Table 15**). However, the milk thistle honey had 21.20% of moisture content. The highest pH was obtained for the buckwheat honey followed by sage, raspberry, shortpod mustard, ramsons, milk thistle and oleaster. The electrical conductivity was the lowest in the case of the oleaster (255.0 µS/cm), that was similar to the electrical conductivity of rape honeys. Extremely high electrical conductivity was obtained for the sage honey (1451.0 µS/cm). The other honey types had conductivities lower than 800 µS/cm, which is the requirement for the nectar honeys. Similarly, higher ash content was found for the sage (0.6068%) and the milk thistle 0.9175% honeys, and all the others showed slightly higher than 0.09%. The oleaster honey showed similar TPC content to the acacia and rape honeys of 6.67 mgGAE/100g, all the others had TPC somewhat higher than 10 mgGAE/100g, which is similar to the values obtained for the chestnut, linden, and multiflora honeys. The sage showed quite high (34.12 mgGAE/100g) total polyphenol content. The FRAP (110.91 mg AAE/100g) and CUPRAC (157.46 µmol TEQ/g) values were also the highest in this type of honey, but the shortpod mustard and buckwheat also showed high FRAP and CUPRAC values. All the other honey types were similar to the other honeys that are more common. Regarding the color attributes, the oleaster was the lightest (L* = 57.91), the darker honeys were the sage (L*=35.62) and buckwheat honey with L* of 43.05. All the other honey types had L* values higher than 50. The a* values were the highest in the case of the sage honey a*=14.96, that is also higher than all the other honey types, similarly the buckwheat where the a* was 9.44. The raspberry, shortpod

mustard, and milk thistle are slightly reddish, the oleaster and ramsons honeys had greenish tone. The most yellowish honey was the shortpod mustard b*=35.55, followed by the raspberry (b* = 29.95±1.2), ramsons b*=29.29. The buckwheat honey had b*=23.98, that is similar to the b* of the bastard indigo, rape, milkweed, honeydew, and multiflora honeys.

Table 15. Physicochemical characteristics of the rare honey types analyzed throughout the thesis

Parameter	Buckwheat	Shortpod mustard	Sage	Milk Thistle	Oleaster	Ramsons	Raspberry n=2
Moisture %	15.20	17.80	17.40	21.20	19.80	18.60	15.53±0.46
pH	4.86	3.92	4.51	3.78	3.64	3.78	4.04±0.29
Electrical conductivity µS/cm	379.7	403.7	1451.0	388.3	255.0	307.0	385.3±219.7
Ash %	0.1326	0.1329	0.6068	0.9175	0.0954	0.1126	0.16±0.17
TPC mgGAE/100g	12.92	12.40	34.12	11.73	6.67	11.98	10.25±3.89
FRAP mg AAE/100g	50.31	64.30	110.9	33.79	12.55	9.66	32.80±22.62
CUPRAC µmol TEQ/g	38.04	89.89	157.5	36.39	23.14	41.71	31.94±15.16
L*	43.05	51.33	35.62	50.27	57.91	53.47	50.06±0.06
a*	9.44	1.92	14.96	5.22	-2.44	-1.00	3.21±0.48
b*	23.98	35.55	11.74	33.71	14.15	29.29	29.95±1.20

n=1 with the exception of raspberry

5.1.1.3 Comparative analysis of the main botanical groups

ANOVA analysis followed by pairwise comparison showed that there was no significant difference among the tested main botanical groups of honeys in the case of the moisture content at $p<0.05$ level (**Appendix Table 3**). Similar results were found in a Romanian study in the case of the moisture content of the linden, sunflower, and acacia honeys (Oroian and Ropciuc, 2017).

In the case of pH, the lowest value was obtained for the bastard indigo (3.81±0.33) honey which was significantly lower than value of the chestnut (4.40±0.22) and linden honeys (4.18±0.32). There was no significant difference between the rape (4.01±0.14), acacia (3.96±0.21), and sunflower (3.84±0.35) honeys, moreover the rape honey did not differ significantly compared from the linden honey. The chestnut honeys had significantly higher pH values compared to all the groups. Romanian researchers also did not find significant difference for pH of linden, sunflower, and acacia honey (Oroian and Ropciuc, 2017), however in another study of theirs the pH of sunflower and rape honeys found to be significantly different which was not found in our results (Pauliuc *et al.*, 2020a).

The electrical conductivity (ELC) of the acacia honey (150.3±29.7 µS/cm) was significantly lower than for all the groups, with the exception of the bastard indigo (301.8±158.6 µS/cm). The bastard indigo honey also showed significantly lower value compared with the linden

(544.6±157.2 µS/cm) and chestnut (695.3±131.6 µS/cm). The sunflower honey (440.1±144.0 µS/cm) and rape honey (231.2±62.0 µS/cm) also had lower ELC than the chestnut honey. Romanian researchers also found significantly different ELC for sunflower and rape (Pauliuc *et al.*, 2020a). Moreover, Slovenian researchers also detected significant difference among acacia and linden and acacia and chestnut honeys, however, in their research the linden and chestnut honeys also showed significantly different electrical conductivity (Bertoncelj *et al.*, 2011). Furthermore, our results are in line with another Romanian research where acacia, linden and sunflower honeys had significantly different ELC (Oroian and Ropciuc, 2017).

The ash content was also the lowest in the case of the acacia honey (0.0344±0.0208%), but no significant difference was found between the acacia and bastard indigo (0.0614±0.0396%), and acacia and linden honey (0.1114±0.0837%). The chestnut honey (0.3212±0.0385%) showed the highest ash content, significantly higher than for all the other honeys.

The total polyphenol content of the honeys was significantly different in the case of the six groups of honeys (**Appendix Table 4**). Acacia honey had the lowest TPC of 5.39±2.43mgGAE/100g, significantly lower than all the other unifloral types. Moreover, chestnut honey (12.43±3.70 mgGAE/100g) showed significantly higher TPC compared to the rape honeys (7.64±2.09 mgGAE/100g). Similarly to our results, no significant difference was found in the case of rape and sunflower honeys in a Romanian study. Moreover, Slovenian researchers also found that acacia honey has significantly lower TPC than chestnut and linden honeys, however in their case the linden also had significantly fewer amount of polyphenols than chestnut honey (Bertoncelj *et al.*, 2007, 2011)

The CUPRAC antioxidant capacity showed slightly different results where the sunflower (56.28±29.83 µmol TEQ/g) honey had the highest CUPRAC, however it was only significantly higher than the values obtained for the acacia (13.45±5.85 µmol TEQ/g) and rape (23.95±9.03 µmol TEQ/g) honeys. The chestnut (42.87±10.67 µmol TEQ/g) and linden (39.73±10.20 µmol TEQ/g) honeys also had significantly higher CUPRAC compared to the acacia and rape, while the bastard indigo (35.25±13.38 µmol TEQ/g) did not show significantly different value from the others except acacia (**Appendix Table 4**).

The acacia honey had also the lowest FRAP antioxidant capacity of 5.14±2.66 mg AAE/100g, significantly lower than the FRAP of all the other groups except bastard indigo (19.37±19.23 mg AAE/100g). AF honeys showed no significant difference compared to the others. Sunflower, linden, and chestnut honeys had significantly higher FRAP value than rape honey (**Appendix Table 4**).

As it was mentioned previously, the lightest honeys were acacia honeys (58.48±2.67), showing significantly higher L* value then chestnut (48.20±5.85), linden (52.78±1.83), rape (54.60±2.00)

and sunflower honeys (53.16±1.59) (**Appendix Table 5**). The darkest was the chestnut honey, having significantly lower L* than the rape (54.60±2.00), acacia (58.48±2.67), and bastard indigo honeys (57.10±1.90). Our results are in line with a Romanian study, where acacia honeys where significantly lighter than linden and sunflower honeys, but no significant difference was between the last two groups (Oroian and Ropciuc, 2017). Another study on Romanian honeys also showed no significant difference between rape and sunflower honeys (Pauliuc *et al.*, 2020a). Similarly, Slovenian researchers found significant difference between the color of chestnut and acacia honeys, however, based on their results, no significant difference was between the acacia and linden and linden and chestnut honeys (Bertoncelj *et al.*, 2007), but in another study they found similar differences as in our case regarding these honey types (Bertoncelj *et al.*, 2011).

The a* parameter (**Appendix Table 5**) showed different trends compared to the L*. The chestnut honey (5.57±6.01) showed the highest a* value, significantly higher than all the groups with the exception of the sunflower (-0.40±2.49), that did not differ significantly from the rest of the groups. Our results are similar to two Slovenian studies, where the a* of linden and acacia honey was similar, but the a* of chestnut was significantly higher (Bertoncelj *et al.*, 2007, 2011). Romanian researchers found no significant difference either between linden and sunflower honeys regarding their a*, but in their case the acacia showed significantly lower a* compared to these groups (Oroian and Ropciuc, 2017).

Based on the b*(**Appendix Table 5**), the less yellowish type was the acacia (13.27±6.61), with significantly lower b* than the chestnut (27.64±7.01), linden (28.03±5.66) and sunflower (38.45±2.35) honeys. The sunflower honey did not differ in the yellowish tone from the linden honeys but showed significantly higher b* than the other groups. Slovenian researchers also found significant difference between b* of chestnut and acacia, but they also found that the chestnut honey was significantly more yellowish compared to linden (Bertoncelj *et al.*, 2007). Romanian researchers found the same trend for the acacia linden and sunflower honeys (Oroian and Ropciuc, 2017).

5.1.1.4 Results of the electronic tongue measurement

The electronic tongue models built for the classification of botanical and geographical origin are introduced in this section.

Botanical origin identification models

Results of the botanical origin identification model can be seen on **Figure 2**. The figure shows that the group of honeydew honey overlaps almost with all the other types, which is due to the high variation (in the composition) of this honey type. It can be seen that some of the sample points overlapping with the group of chestnut honey belong to the higher quality honeydew

samples that are characterized by high electrical conductivity and belonged to sample 71, that was originally labelled as pine (honeydew) honey. Moreover, based on the figure, it can also be seen that groups of acacia, bastard indigo, rape, and milkweed honeys are close to each other, that may be due to their similar physicochemical composition. The sunflower honeys are separated from the acacia, rape, chestnut, and bastard indigo honeys, moreover, the linden honeys overlap with the honeydew, chestnut, multiflora, and sunflower honey groups. Multiflora groups overlap with almost all the other types, except for the chestnut, which shows overlapping only with the linden and honeydew honeys. This is also because linden, honeydew and chestnut honeys are usually characterized by higher amount of nutrients (minerals, polyphenols, vitamins) and have similar physicochemical properties. The origin identification model built for the classification of the main unifloral and the multiflora honeys provided average recognition and prediction abilities of 57.07% and 57.09%, respectively after the threefold cross validation. Based on the confusion table (**Appendix Table 6**), the acacia honey is classified with the highest accuracy of 93.42%, followed by chestnut (86.56%), sunflower (80.45%), and bastard indigo (64.2%). All the other honey types had ≤60% accuracy, which cannot be considered satisfactorily good.

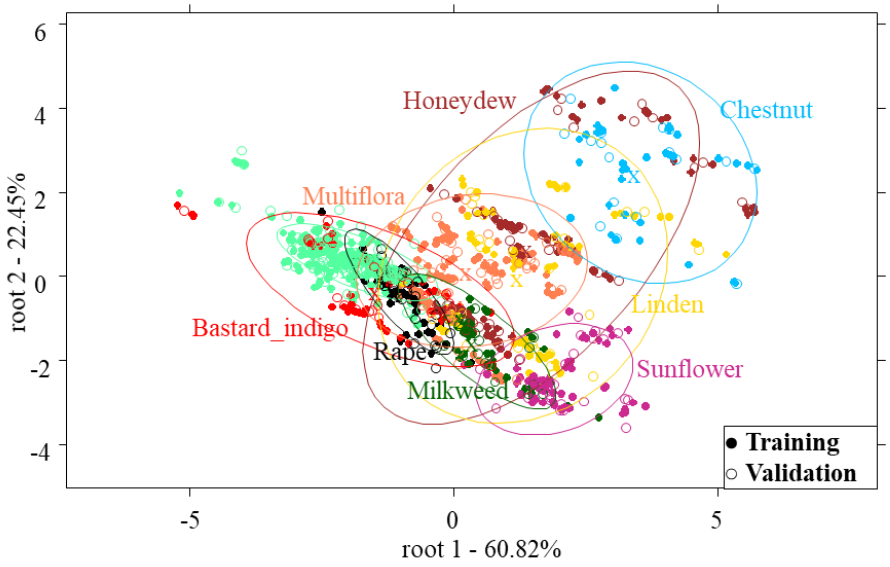

Figure 2. LDA score plot of the electronic tongue data for the classification of the main unifloral and multiflora honeys of Hungary

Another model was built for the classification of the six main groups (acacia, linden, sunflower, rape, chestnut, and bastard indigo). The honeydew, multiflora and milkweed honeys were excluded from these models to have sample sets in line with the extended pollen study models.

The model of the six chosen groups showed a fair separation of the chestnut group from all the others (**Figure 3**). However, linden honeys showed overlapping with the chestnut and sunflower honeys, like in the previous model. The rape, bastard indigo and acacia honeys showed

overlapping with each other along the first two roots. The LDA classification model provided the average classification accuracies of 70.91% and 70.51% during the training and cross validation (**Table 16**). Chestnut honey was classified correctly. The acacia showed correct classification in 93.75% showing misclassification belonging to the bastard indigo (5.92%) and rape (0.33%). The bastard indigo honey was misclassified also belonging to the acacia (32.83%) and rape (1.5%). The rape honey showed a strong misclassification, partly belonging to the acacia (70.15%), so we could not separate these two groups from each other. The sunflower honey was classified correctly in 86.21%, misclassification was found for samples belonging to the linden group (13.79%). The linden honey was classified correctly in 50%, misclassified samples belonging to all the other groups in the range of 22.98-1.72%, except for bastard indigo. In a Croatian study the acacia honeys were also separated from the chestnut and honeydew honeys, which is in line with our findings (Major *et al.*, 2011). Italian researchers found similar results using the same type of electronic tongue, where correct classification was found (70.8%) for the classification of chestnut, orange blossom, eucalyptus and sulla honey (Di Rosa *et al.*, 2018). In a Portuguese study potentiometric electronic tongue achieved 100% correct classification of honeys, however, in their study only a few samples were analyzed, and they applied LOO cross validation (Sousa *et al.*, 2014). Chinese researchers obtained better results for the geographical origin classification by using artificial neural network method with >90% accuracy (Wei *et al.*, 2009).

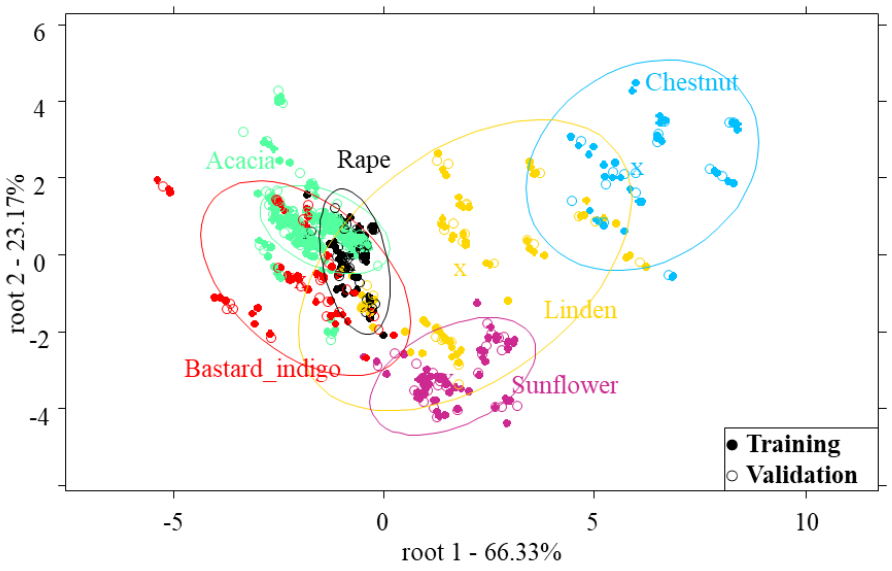

Figure 3. LDA score plot of the electronic tongue data for the classification of the main unifloral groups

Table 16. LDA confusion table of the main unifloral honeys using the data of electronic tongue

		Acacia	Bastard indigo	Chestnut	Linden	Rape	Sunflower
Average training 70.91%	**Acacia**	**93.75**	32.84	0	1.72	70.15	0
	Bastard indigo	5.92	**65.66**	0	0	0	0
	Chestnut	0	0	**100**	10.92	0	0
	Linden	0	0	0	**50.01**	0	13.79
	Rape	0.33	1.50	0	14.36	**29.85**	0
	Sunflower	0	0	0	22.99	0	**86.21**
		Acacia	**Bastard indigo**	**Chestnut**	**Linden**	**Rape**	**Sunflower**
Average validation 70.51%	**Acacia**	**93.75**	32.83	0	2.31	70.83	0
	Bastard indigo	5.92	**65.70**	0	0	0	0
	Chestnut	0	0	**100**	10.34	0	0
	Linden	0	0	0	**49.41**	0	14.93
	Rape	0.33	1.48	0	13.79	**29.17**	0
	Sunflower	0	0	0	24.14	0	**85.07**

Columns represent the actual class membership (%) and the rows represent the predicted class membership (%)

Geographical origin classification models

The results of the geographical origin classification using the data of six aforementioned groups built for the classification of the main geographical regions of Hungary provided average training and validation accuracies of ~59%. The best classification accuracy of >97% was obtained for the samples originating from the Alföld region (**Table 17**). The samples from the Dunántúli-dombság showed misclassification, partly belonging to the Nyugat-magyarországi-peremvidék. This can be due to the fact that these regions are close to each other, and both can be found in the Transdanubian part of Hungary. However, the Nyugat-magyarországi-peremvidék showed overlapping with the Alföld region, misclassification being 9.99% after validation. The Északi-középhegység group was almost completely misclassified as belonging to the Alföld group (>98%) in the validation set. This can also be because of the closeness of the two regions. However, in general these low accuracies can be due to the fact that the effect of the botanical origin is higher than those of geographical origin, therefore, if the samples are from the same plant, but different geographical regions, this can lead to misclassifications because of this effect.

Table 17. LDA confusion table of the region classification model using data of electronic tongue of the six main types

		Alföld	Dunántúli-dombság	Északi-középhegység	Nyugat-magyarországi-peremvidék
Average training 59.46%	**Alföld**	**97.56**	0	96.24	9.28
	Dunántúli-dombság	0.38	**59.38**	0	4.29
	Északi-középhegység	0.38	0	**3.76**	9.28
	Nyugat-magyarországi-peremvidék	1.69	40.62	0	**77.15**
		Alföld	**Dunántúli-dombság**	**Északi-középhegység**	**Nyugat-magyarországi-peremvidék**
Average validation 59.40%	**Alföld**	**97.53**	0	98.13	9.99
	Dunántúli-dombság	0.38	**62.48**	0	4.29
	Északi-középhegység	0.38	0	**1.87**	9.99
	Nyugat-magyarországi-peremvidék	1.71	37.52	0	**75.74**

Columns represent the actual class membership (%) and the rows represent the predicted class membership (%)

Because of this higher effect of the botanical origin, the models will be demonstrated separately for the different botanical types in the following. Classification models were built for the discrimination of the counties (*megye*) and districts (*járás*) (in the case of bastard indigo). The models for sunflower, bastard indigo and linden provided 100% correct classification for all the groups (**Figure 4 c, d, e)**. The model of the chestnut honey showed correct classification of the groups during the training, and 98.17% during validation, where misclassification was found for the Győr-Moson-Sopron county as partly belonging to Zala county in 5.5% (**Figure 4 f**). In the case of the rape honey (**Figure 4 b**) the average training and cross-validation accuracies were 97.24%, 90.75%, respectively. During the validation, the Békés group was misclassified as belonging to Heves in 11.12% and to Jász-Nagykun-Szolnok in 5.5%. Jász-Nagykun Szolnok was classified correctly in 94.45%, where misclassification was found to Békés, while Heves also showed minor misclassification to Jász-Nagykun-Szolnok in 5.58%. The model of acacia (**Figure 4 a**) was a somewhat less accurate, where the average training and validation were 71.85% and 70.68%. Despite the fact that almost all the counties are from the Alföld region, this accuracy is quite good. Pest was classified correctly with 100% accuracy, while Heves also provided a good classification of 90.74%. In the case of all the counties the misclassified samples usually belonged to the surrounding counties. Geographical origin classification was

performed with a high accuracy by Chinese researchers in the case of acacia honey (Wei *et al.*, 2009).

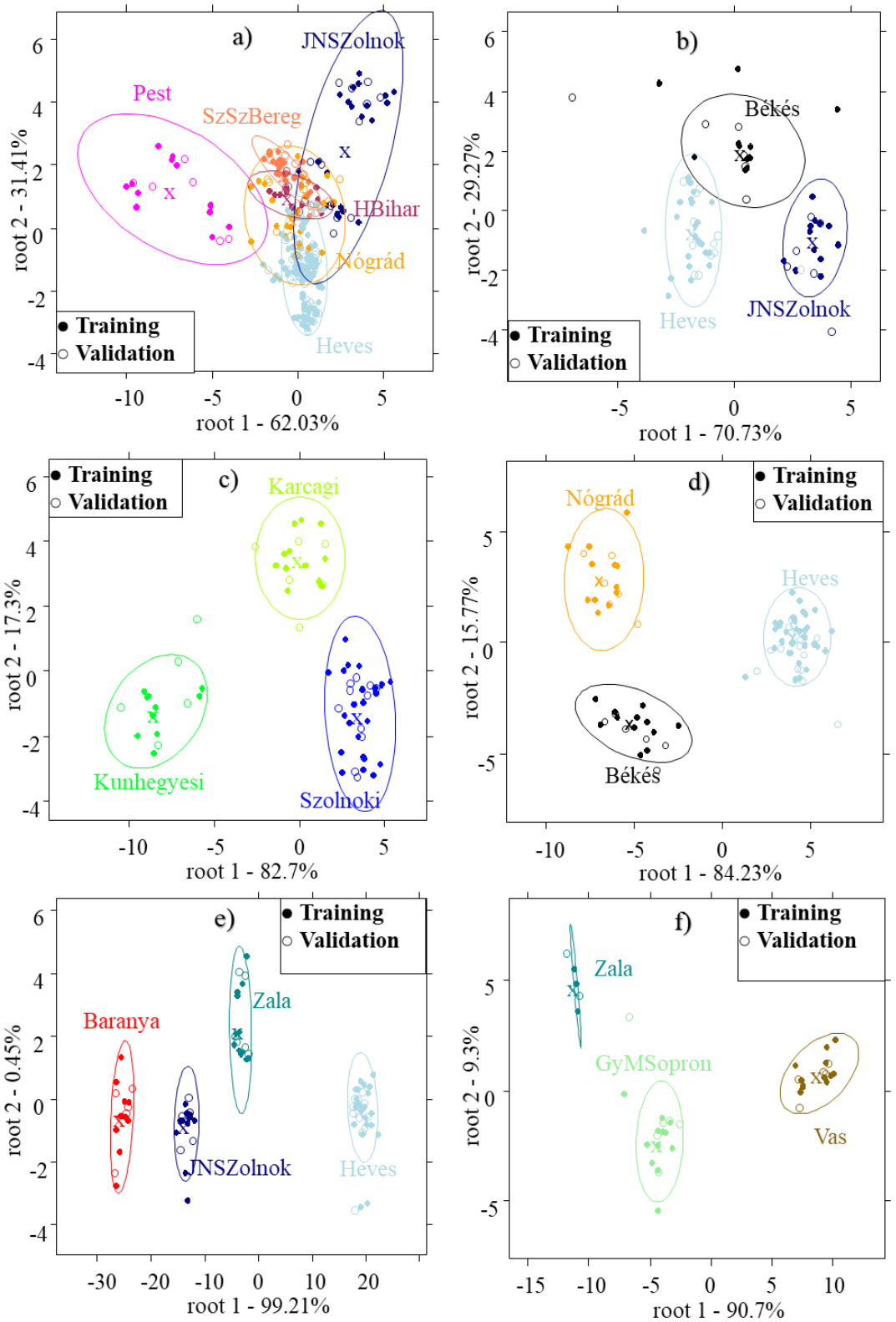

Figure 4. LDA score plots of the geographical origin identification of the individual botanical types a) acacia, b) rape, c) bastard indigo, d) sunflower, e) linden, f) chestnut
GyMSopron: Győr-Moson-Sopron, JNSZolnok: Jász-Nagykun-Szolnok, SzSzBereg: Szabolcs-Szatmár-Bereg, Hbihar: Hajdú-Bihar

5.1.2 Results of the origin identification study extended with pollen analysis

5.1.2.1 Results of the reference methods

The reference methods showed that the moisture content of all the sample groups were below 20%, that is the limit of the EC. Based on the **Figure 5 a)** we can see that the average moisture content of the different sample groups was in the range of 16.83-18.14, where the lowest moisture content was obtained for the chestnut and the highest for the group of the milkweed honeys. The pH of the samples, as shown in **Figure 5 b)** was also similar in the case of the different groups, where the lowest pH was obtained for the milkweed honey samples and the highest for chestnut honey. This is in accordance with the literature, that states that chestnut honey can have higher pH value compared to the others. The pH values of the sunflower, acacia, rape, and linden honeys are also in accordance with the results of the descriptive sheets of the main unifloral honeys in Europe (Oddo and Piro, 2004). These descriptive sheets do not contain the data of the bastard indigo honey and milkweed honeys, however our results for bastard indigo honey are similar to a Chinese study (Zhu *et al.*, 2020). Milkweed honeys were analyzed by Hungarian researchers, who also have found similarly lower pH values (Kasper-Szél *et al.*, 2003). The pH of the honeydew honeys based on the descriptive sheets are higher than the ones in our study, moreover it can be seen that the electrical conductivity of these honeys samples **(Figure 5 c)** is also lower than it should be according to the legislation (0.8 mS/cm), as in our case 566.13±198.00 µS/cm was found. This can be due to the fact that sometimes the labeling of these samples is confusing: two types of denominations are used in Hungary for the "honeydew" honeys: the "*mézharmat*" and the "*erdei méz*", but this latter sometimes means that the sample was collected in a forest region, but from blossoms mostly, not from honeydew, however, this is not always stated. Moreover, it can also be seen that the average electrical conductivity of the chestnut honey (715.10±116.36 µS/cm) is also below the limit of the legislation (0.8 mS/cm). All the other honey types meet the requirements of the legislation and are in the range of the data of the descriptive sheets. The bastard indigo honey has higher electrical conductivity (305.32±159.12 µS/cm) compared to a Chinese study (200 µS/cm), which can be because of the geographical origin and the difference in the surrounding flora (Zhu *et al.*, 2020).

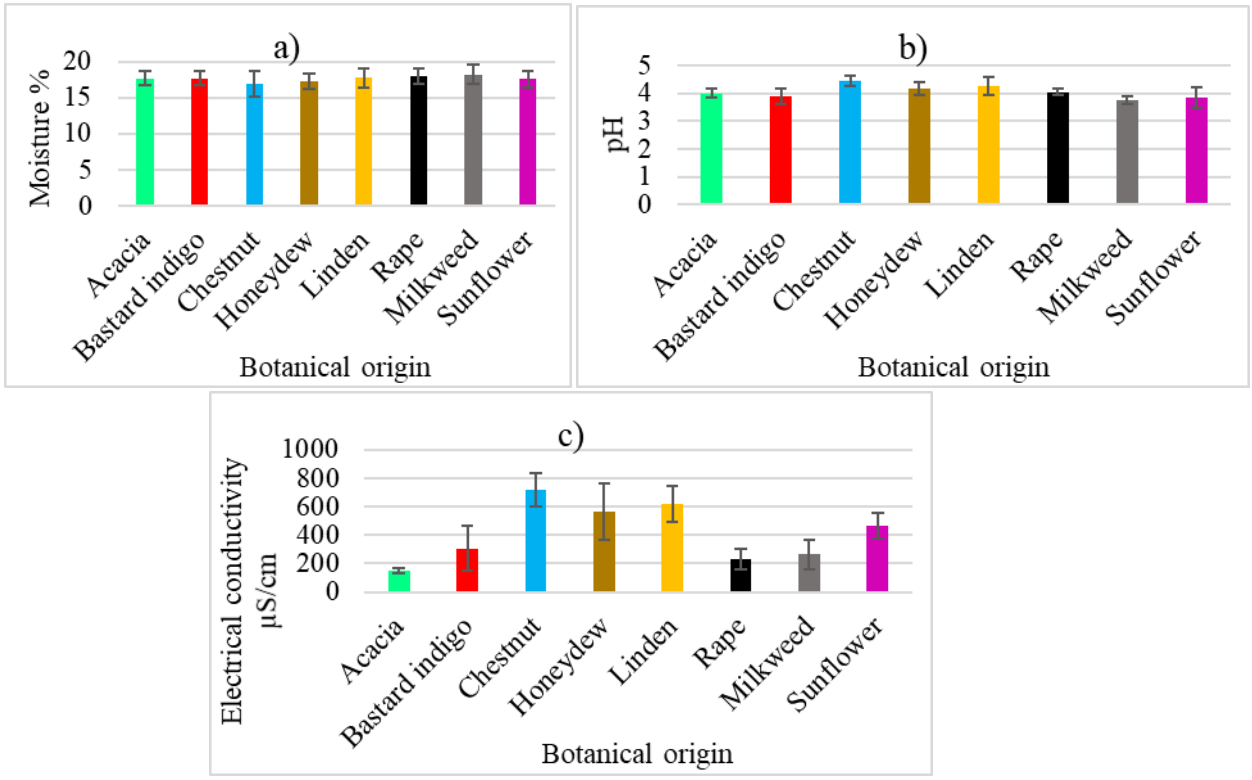

Figure 5. Reference parameters of the honeys of the pollen analysis extended experiment a) moisture content, b) pH, c) electrical conductivity by botanical groups

5.1.2.2 Results of the melissopalynological analysis

Melissopalynological analysis of the 87 honey samples from the eight botanical origins showed 107 identified taxa in the samples. Pollen diversity of the samples was different in the case of eight honey types, where the milkweed (AS) and honeydew (HD) honeys were more diverse with about 19-40 taxa per sample.

The interesting fact about these honeys is that in the case of the honeydew there is no characteristic pollen, while in the case of milkweed honey the milkweed (*Asclepias syriaca)* does not present in honey (Hungarian Standars Institution, 2017), therefore the identification of these honeys based on the pollen is quite challenging. In the case of honeydew honey, some honeydew elements are present that can be counted (Oddo and Piro, 2004; Hungarian Standars Institution, 2017). In the following, these two types were excluded from the botanical modelling based on the pollen spectra.

In the case of the honeydew honey (HD) one of the samples had predominant pollen (present in >45%), where the *Solanum nigrum* type (black nightshade) presented in 66.33%. The rest of the honeys contained only secondary pollen (16-45%) where *Phacelia tanacetifolia* (phacelia), and Apiaceae (carrot, celery of parsley family*), Amorpha fruticosa* (bastard indigo), *Castanea sativa* (sweet chestnut), and Asteraceae such as *Helianthus annuus* (sunflower) or *Senecio* type

presented (ragworts or groundsels) the most frequently. The rest of the pollens presented in less than 15%, that can be seen on **(Figure 6**).

In the case of the milkweed (AS) honeys also only one of the samples had predominant pollen, which was *Phacelia tanacetifolia* (phacelia) in 51.31%. The others had only secondary pollen where the most common types were the *Tilia* (linden), Papaveraceae (poppy family), *Allium vineale* type (wild garlic), Brassicaceae medium (crucifers), *Castanea sativa* (sweet chestnut), *Sorbus* type (whitebeam), and *Trifolium* type (clovers).

Acacia (RP) samples are mainly characterized by the presence of the *Robinia pseducoacacia* (acacia) pollen **(Figure 6**), that ranged between 7-43% in the samples. Hungarian researchers reported similar quantities in their research about pollen analysis of Hungarian acacia honeys (Institute of Apiculture and Bee Biology, 2016). According to the honey legislations of Hungary the acacia honey samples should contain 15% of *Robinia* pollen, but in our finding some of the samples did not meet these requirements. In acacia honeys the *Verbascum* (mullein), *Filipendula ulmaria* (sweet meadow), Fabaceae (legumes), Rosaceae (rose family), *Sorbus* type (whitebeam), Poaceae (grasses), *Cornus sanguinea* (bloody dogwood), and *Pinus* (pines) presented the most frequently. Moreover, Brassicaceae (crucifers), *Frangula alnus* (glossy buckthorn) and Apiaceae (carrot family) also were found in acacia honeys. Their pollen diversity was also higher ranging between 16-30 pollen taxa.

Bastard indigo (AF) belongs to the legume family and its presence in Hungary is the most prevalent around the Tisza River. In the bastard indigo honeys the amount of the *Amorpha fruticosa* - characteristic pollen for the type **(Figure 6**) – was identified in 13-94%. Neither Hungarian, nor European legislation refers to the amount of pollen needed for classification of this type of honey. Moreover, no published data were available on the detailed pollen composition of Hungarian bastard indigo honey before our study (Bodor *et al.*, 2021c). However, Hungarian researchers reported about the mineral composition, characteristic pollen (*Amorpha fruticosa*), and antioxidant properties of this honey type. The researchers analyzed only three honeys collected in 2018, but besides this, according to the best of our knowledge, no study was performed on this honey type (Escuredo *et al.*, 2019). In some of the honeys the amount of the *Amorpha fruticosa* pollen was lower than in the case of the Chinese study, where it ranged between 58-73% (Zhu *et al.*, 2020). The pollen diversity of this type covered a wide range of 5-31 taxa. In bastard indigo honey besides the characteristic pollen Trifolium (clover), *Elaegnus angustifolia* (oleaster), and *Cornus mas* (cornelian cherry). Similarly to acacia, Brassicaceae were also identified in this honey such as *Frangula alnus* and Rosaceae **(Figure 6**).

Chestnut honeys (CS) showed a low pollen diversity of 8-20 different taxa. The characteristic *Castanea sativa* pollen ranged between 60-93% that is usually overrepresented pollen. The

chestnut honey also contained *Phacelia tanacetifolia*, and *Sambucus nigra* (black elder) pollens more frequently **(Figure 6**).

In linden (TI) honeys the pollen of *Tilia* spp. was identified in 5.3-66.4%. According to the Hungarian legislation the linden honeys should contain at least 30% of *Tilia*, which criteria was not met in the case of three linden honeys. In the linden honeys besides the *Tilia* pollen **(Figure 6**) *Plantago lanceolata* (ribwort plantain), *Papaver rhoeas* (corn poppy), *Helianthemum* (rock rose or sunrose) and *Vicia lathyroides* (spring vetch) were found more frequently. Brassicaceae, *Frangula alnus, Zea mays* (corn), and Asteraceae pollen were also identified in these samples. The pollen diversity of this honey ranged between 13-32 taxa.

Rape honeys (BN) contained a high amount of Brassicaceae pollen, however in this case the *Brassica napus* pollen was not identified separately. The identification of species of the Brassicaceae family is very hard due to their similar morphology. Therefore, in this case, from the amount of the Brassicaceae medium (25-50 micrometer) could we conclude to the amount of the Brassica napus pollen. *Brassica napus* is an overrepresented pollen type, and in these honey types 15-91% of Brassicaceae medium pollen were found. The pollen diversity was between 8-23 taxa, where honeys with higher diversity contained less amount of the characteristic pollen. In these honey types the Brassicaceae pollen, the *Frangula alnus*, and Fabaceae pollen also presented more frequently **(Figure 6**).

In sunflower honeys (HA) the characteristic *Helianthus annuus* pollen can be under-represented. In our study we found that the HA pollen was in the range of 3-83% in the honey types where the pollen diversity ranged between 9-28%. In sunflower honey besides the characteristic pollen, *Phacelia tanacetifolia*, *Zea mays*, *Matricaria* type (chamomiles), *Viola* type, *Chenopodium* (goosefoots), *Caltha* type (marsh-marigold), *Carduus* type (plumeless thistles), *Myosotis* (scorpion grasses), *Rhus typhina* (staghorn sumac), *Plantago lanceolata* and Fabaceae were more abundant. Interestingly, in one of the samples *Nymhoides peltata* (fringed water lily) pollen was found in high amount. This can be due to the geographical origin of this sample that originated from the Lake Tisza region near to Kisköre, where numerous of these waterplants are present **(Figure 6**).

Besides these in most of the samples *Ranunculaceae*, (Buttercup family) *Salix* type (willow), Rosaceae and some of the wind pollinated plants such as *Quercus* (oak), *Artemisia* (mugworts), and *Juniperus communis* (junipers) were found **(Figure 6**).

Figure 6 is printed in a bigger form in the appendices.

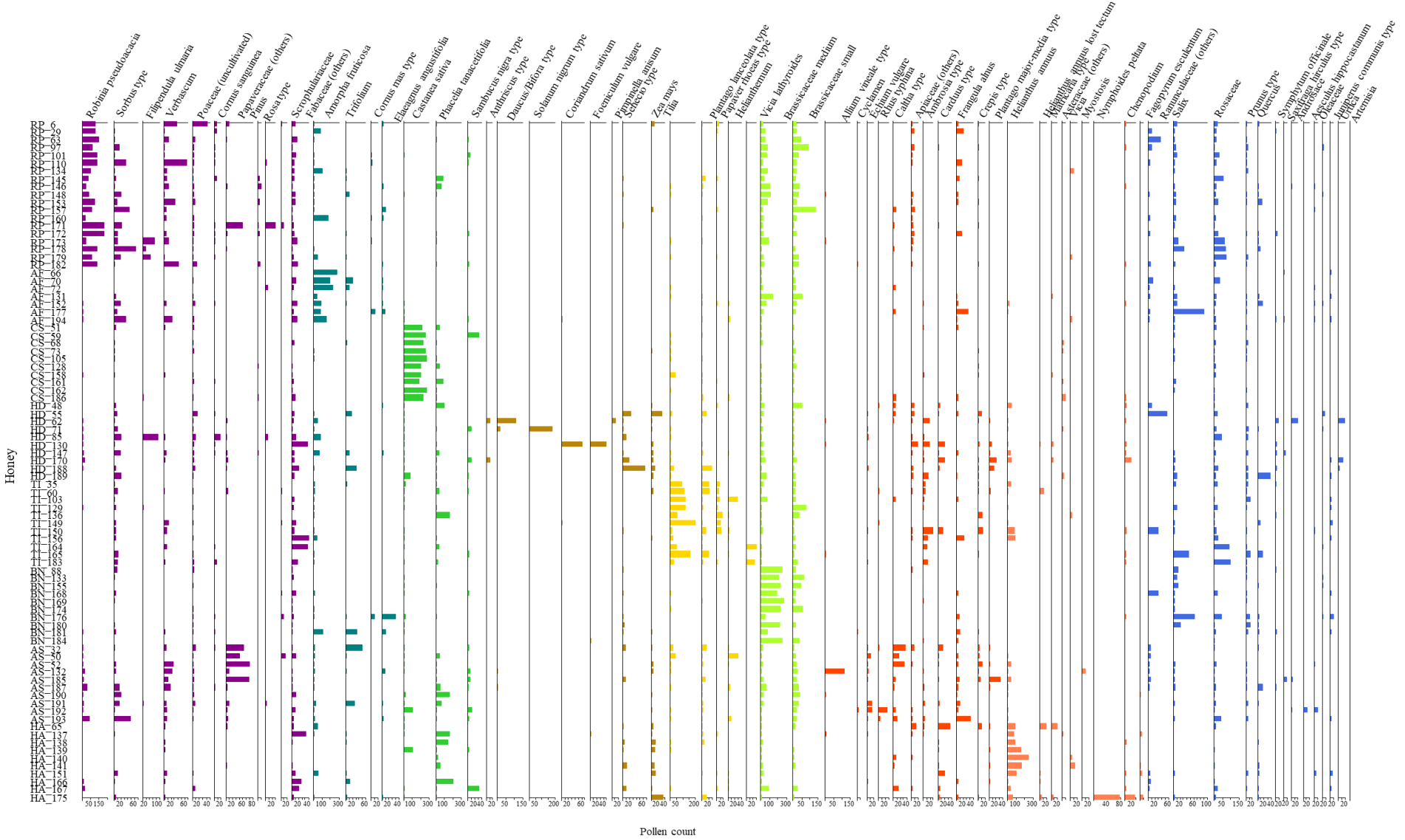

Figure 6. Pollen diagram of the samples of OISWP RP-acacia, CS-chestnut, BN-rape, HA-sunflower, TI-linden AF-bastard indigo

Results of the cluster analysis of the results of the melissopalynological analysis

Results of the cluster analysis of the six honey types showed two main clusters (**Figure 7**). In one of them the chestnut (CS), linden (TI) and sunflower (HA) honeys can be found, while in the other big cluster the rape (BN), acacia (RP), and bastard indigo (AF) honey were found. This is also in line with the physicochemical composition as the chestnut, linden and in some cases the sunflower honeys are richer in nutritive components than the rest of the honey types (Oddo *et al.*, 2004). Within the first big cluster all the chestnut honeys are grouped together, however three of the samples were clustered as a subgroup. These samples had less *Castanea sativa* pollen but contained *Phacelia tanacetifolia* pollen in higher amounts compared to the rest of the chestnut honeys. This can be due to the harvesting time. The end of the flowering period of the chestnut honey is around early July and that time the phacelia plants are also flowering as their flowering period covers July (Farkas and Zajácz, 2007). The second big cluster besides the chestnut is composed of the linden and the sunflower honeys. Under this eight of the linden samples were clustered together, and three of the linden honeys were grouped together with the sunflower honeys. Both sunflower and linden honeys contained the *Phacelia tanacetifolia* pollen in a higher amount, that can be also because of the overlapping with the flowering period of the phacelia plant. Moreover, an important nectar source for the bees between the harvest time of sunflower and acacia. The three linden samples that were clustered with the sunflower honeys contained less of the *Tilia* pollen and higher amount of phacelia or sunflower pollen compared to other linden samples. This can be also because of the harvesting time, probably the linden honeys that contained more sunflower pollen were collected in a later period. The linden plants usually flower in June, and the sunflower plants – depending on the sowing time - can start to bloom in the end of June, which can lead to these pollen amounts (Farkas and Zajácz, 2007). The higher presence of the phacelia can be explained also by the fact that phacelia pollen are over-representing pollen types.

The other big group of the acacia, rape and bastard indigo honeys showed that the first big subgroup is composed of the bastard indigo honey samples, but some of the acacia and rape samples were clustered together with the bastard indigo honey. These acacia and rape honeys contained higher amount of the *Amorpha fruticosa* pollen, which plant typically lives around rivers, and most of these honeys originated from the region of Tisza river and Tisza lake, where the *Amorpha fruticosa* plants are common (Bartha *et al.*, 2021). The second subgroup was of the rape honeys and one of the bastard indigo honeys were clustered together with this rape honey, bastard indigo honey contained a high amount of the Brassicaceae medium pollen, much higher than other samples from this group. The last subgroup composed of all the rest of the acacia

honey samples, however one of the sunflower honeys were classified together with these honey types. The sunflower honey that belonged to this group was low in sunflower pollen (9%).

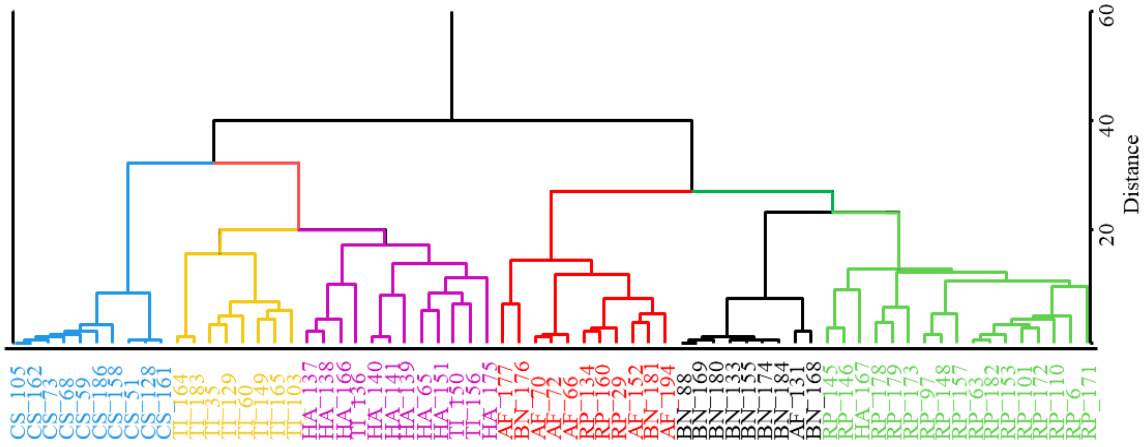

Figure 7. Cluster analysis of the pollen data of the acacia (RP), chestnut (CS), rape (BN), sunflower (HA), linden (TI) and bastard indigo honeys (AF), where colored rectangles denote the main groups

Results of the PCA and PCA-LDA analysis of the melissopalynological analysis

Principal component analysis using the data of the pollen analysis showed separation trend of the different botanical groups where the chestnut honeys completely isolated from the rest of the groups through PC1, that described the 33.2% of the total variance (**Figure 8**).

The *Castanea sativa*, Asteraceae others and *Sambucus nigra* type variables contributed to the chestnut distinction. The group of the rape honey also showed a separation from the rest of the groups along the PC2, that described the 21.2% of the total variance, and the Brassicaceae medium, Brassicaceae small, and *Prunus* type contributed to the separation of this group. The rest of the groups can be seen on the top of the figure. Through PC1 the acacia and bastard indigo honeys showed a discrimination pattern from the linden and sunflower, however, based on the confidence intervals (95%) an overlapping was seen.

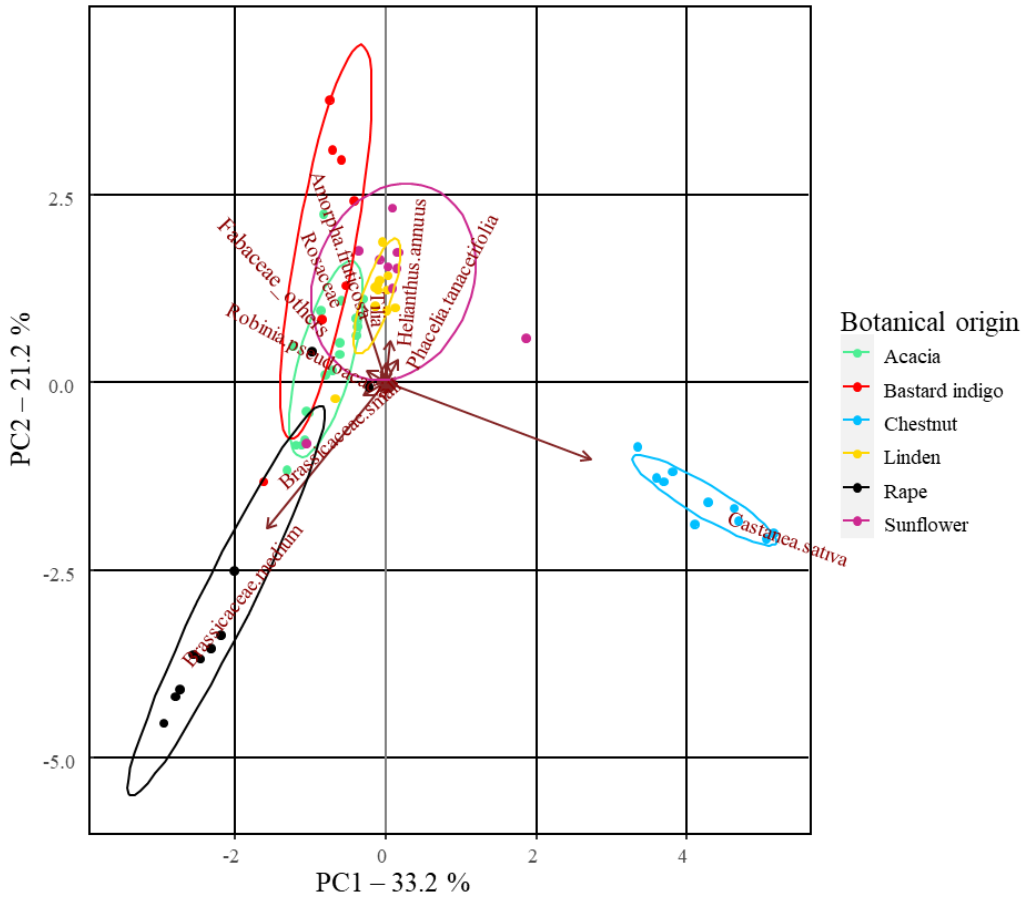

Figure 8. PCA score plot (with the 10 most contributing loadings) of the model of the different honey types using the pollen data

This separation of the groups is more highlighted based on the PCA-linear discriminant analysis model built for the classification of the botanical groups (**Figure 9**). In this case the chestnut is completely separated again from the rest of the groups, and the clear distinction of the linden and sunflower honey group from the rest of the others can be seen. Based on the loadings to the separation of these groups the *Tilia*, *Phacelia tanatecifolia*, *Helianthus annuus*, Ranunculaceae others contributed the mostly. On the top part of the figure, the separation tendency of rape, bastard indigo and acacia honey can be seen. In this case the *Amorpha fruticosa*, Brassicaceae medium and small, *Robinia pseudoacacia*, *Salix* type, contributed the most. Based on the classification tables the average training and validation accuracies were 99.14% and 90.22%, respectively. The training dataset showed the misclassification of acacia sample as belonging to the bastard indigo group in 5.15%, but all the other groups were classified correctly. The classification of the validation dataset showed that the rape and the chestnut groups were classified correctly. The acacia samples showed again misclassification as belonging to the bastard indigo honey group in 5.5%. This was also seen during the cluster analysis where some acacia samples were clustered together with the AF honeys. The bastard indigo samples also showed a misclassification belonging to the rape honeys in 14.16%.

Moreover, 9.02% of the linden group was classified as sunflower honey. These misclassifications were also seen in the cluster analysis, where the honeys were clustered with other types. Sunflower was classified correctly in 69.97%, where the misclassification was found belonging to the linden in 20.12% and to acacia in 9.91%.

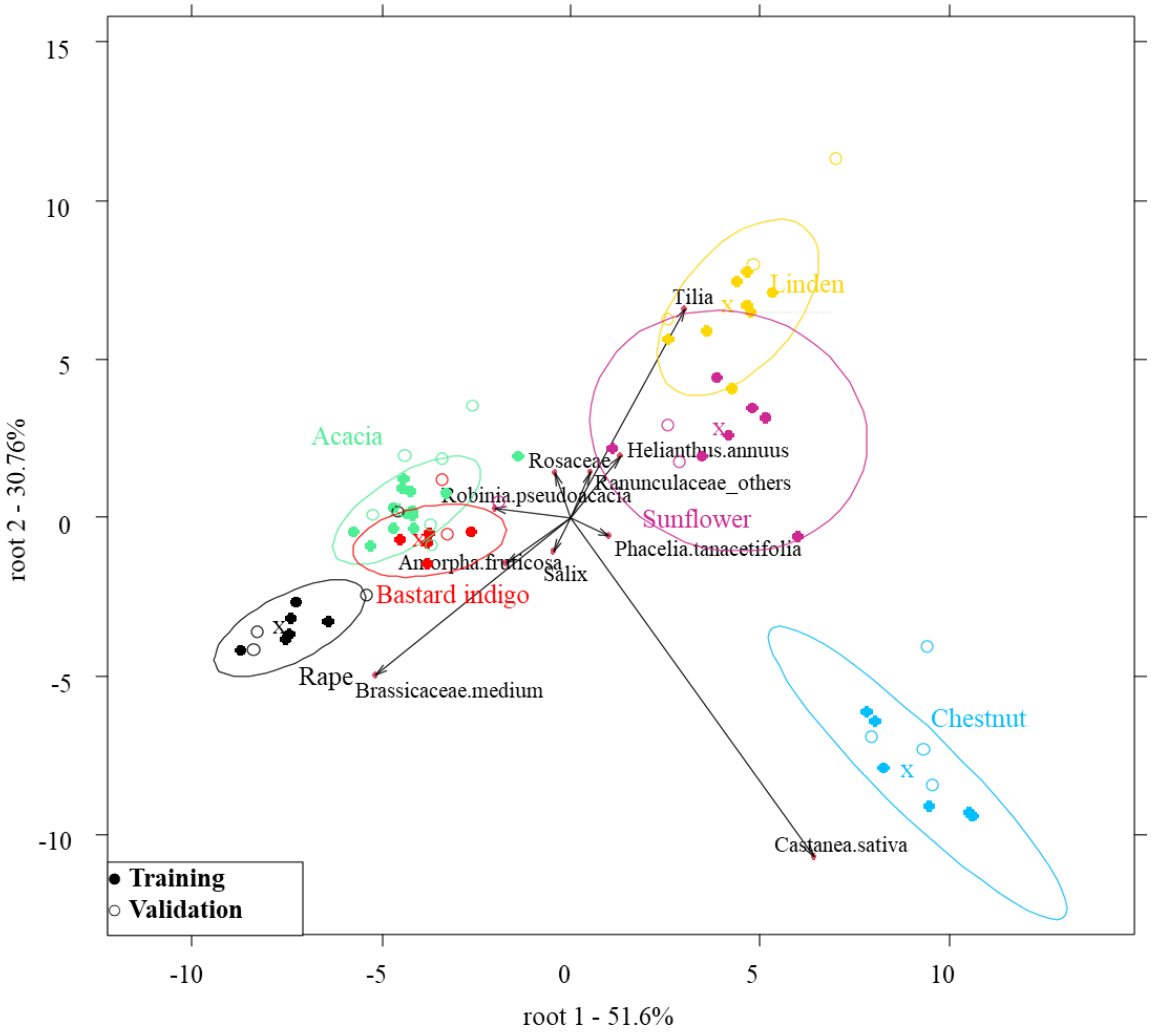

Figure 9. PCA-LDA score plot and with the 10 most contributing loadings of the botanical origin classification model using pollen data

The PCA-LDA model of the geographical origin classification provided weak results of 68.57% and 51.93% of average classification accuracy during the training and the validation. During the validation, the Észak-magyarországi-középhegység was classified correctly, however all the others showed misclassifications. Nyugat-magyarországi-peremvidék was classified correctly in 77.67%, the Dunántúli-középhegység in 67%, Alföld in 66.64%, Dunántúli-dombság in 50.25 and Kisalföld in 50%. This shows that the botanical origin has a bigger effect on the pollen composition than the geographical origin.

5.1.2.3 Results of the NIR spectroscopy

The best classification models for the classifications of botanical origin were achieved using the Savitzky-Golay smoothing (2nd polinom, 21 filter length) + Savitzky-Golay smoothing (2nd polinom, 21 filter length, 1st derivative) pretreatment combination. In this case the PCA and PCA-LDA did not show clear separation tendencies neither for the botanical, nor for the geographical origin **(Figure 10)**. The average classification accuracies of training and validation for the botanical origin classification were 75.52% and 58.14%, respectively. All the groups provided misclassification belonging to each other and the correct classifications were below 70% in each of the botanical types. The detailed classification accuracies can be seen in (**Appendix Table 8**). The model of the geographical origin classification was also not satisfactory, where the average recognition (training) and prediction (validation) accuracies were 68.47% and 58.90%. However, a separation tendency can be seen according to the altitudes, where the samples from lower altitudes can be found on the bottom part and samples from the higher altitudes can be seen in the upper part of the plot (**Figure 10 b**).

The reason for the lack of a better classification accuracy can be the result of the lack of the wavelength range above 1700 nm. The important differences in the sugar composition cannot be reflected in the spectra of our experiment as some of the important bands related to the sugar composition can be found in this range above 1700 nm (Qiu *et al.*, 1999). Chinese researchers also found 85.3% classification accuracy of different botanical groups, which was better than our results, however they also analyzed the higher wavelengths ranges up to 2500 nm (Chen *et al.*, 2012).

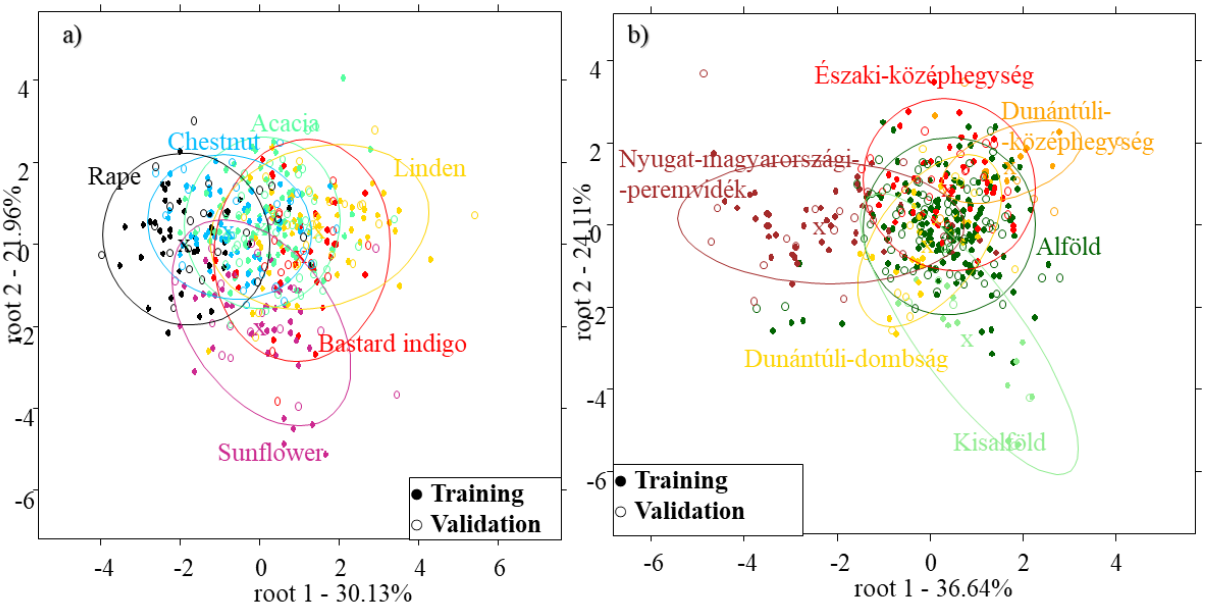

Figure 10. PCA-LDA score plot using for the classification of the a) botanical origin, b) geographical origin using the NIR data of the pollen extended study

5.1.2.4 Results of the fusion of NIR, physicochemical and pollen data

The fusion of the data of the reference methods, pollen and NIR provided improved accuracies both for the classification of the botanical and geographical origin. Based on **Figure 11** we can see that the linden, sunflower, and chestnut samples completely separated from the rest of the groups. Based on the first two factors, the bastard indigo, acacia, and rape samples also show a separation tendency. Electrical conductivity, pH and *Castanea sativa* pollen contributed the mostly to the isolation of chestnut, while in the separation of the linden honeys *Tilia* pollen had the highest role. Moreover, some of the pollen types also showed in the direction of the linden and sunflower samples such as the *Ambrosia* type, *Fagopyrum esculentum*, *Prunus*, *Caltha*, *Carduus* types, Fabaceae, *Papaver rhoeas* and *Helianthus annuus.* The separation of the rape honey can be assigned to the Brassicaceae medium and small pollen. Moreover, the *Robinia pseudoacacia* and *Amorpha fruticosa*, and Quercus pollen also assigned to these directions. From these loading it can be seen that the pollen and the physicochemical data have higher role in the separation of the groups than the NIR dataset, but the fusion of the three dataset provided higher than 99% accuracy, even after the threefold cross validation. In one case misclassification was found, where the acacia sample group showed misclassification as belonging to the bastard indigo group in 4.20% and 4.21% during the training and cross validation. Interestingly, the sample points that were misclassified belonged to the sample that was clustered as bastard indigo with high amount of AF pollen (59.7%) and only 7% of acacia pollen. This can be assigned to the geographical origin of the sample or the later collection of the acacia honey where the bastard indigo started already to bloom.

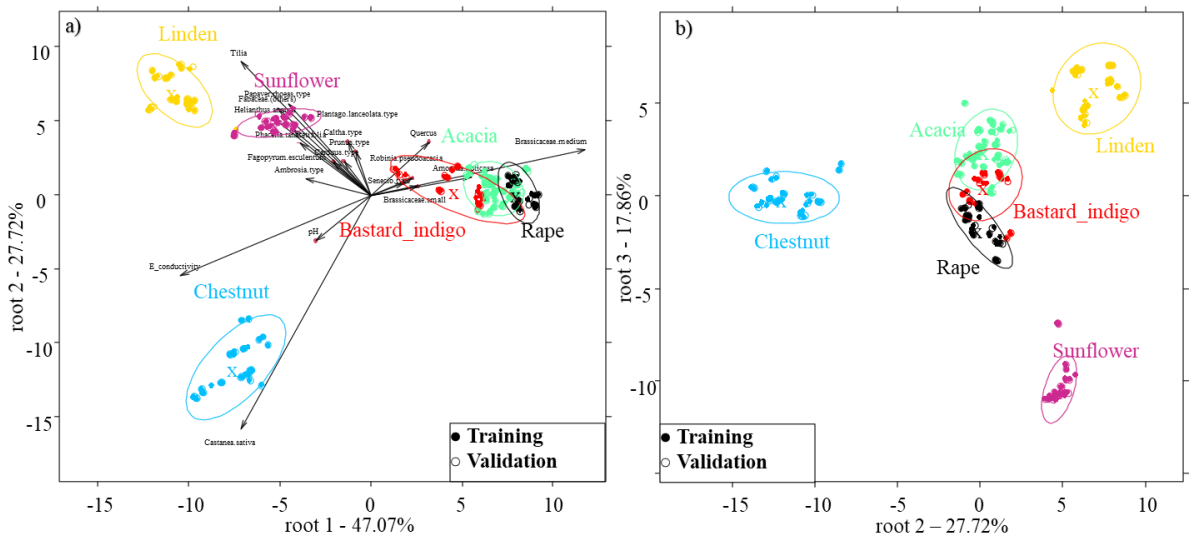

Figure 11. PCA-LDA score and loading plot with the 20 most important loading for the classification of the botanical origin using the fusion dataset of pollen, NIR and reference methods: a) root 1-2, b) root 2-3

The geographical origin classification model provided 100% accuracy during training and validation. Based on the figure we can see (**Figure 12**) that the honeys originating from the Dunántúl region of Hungary (Transdanubian) can be seen on the right side of the figure while the two plains (Alföld and Kisalföld) and the Northern part (Északi-középhegység) can be found on the left side.

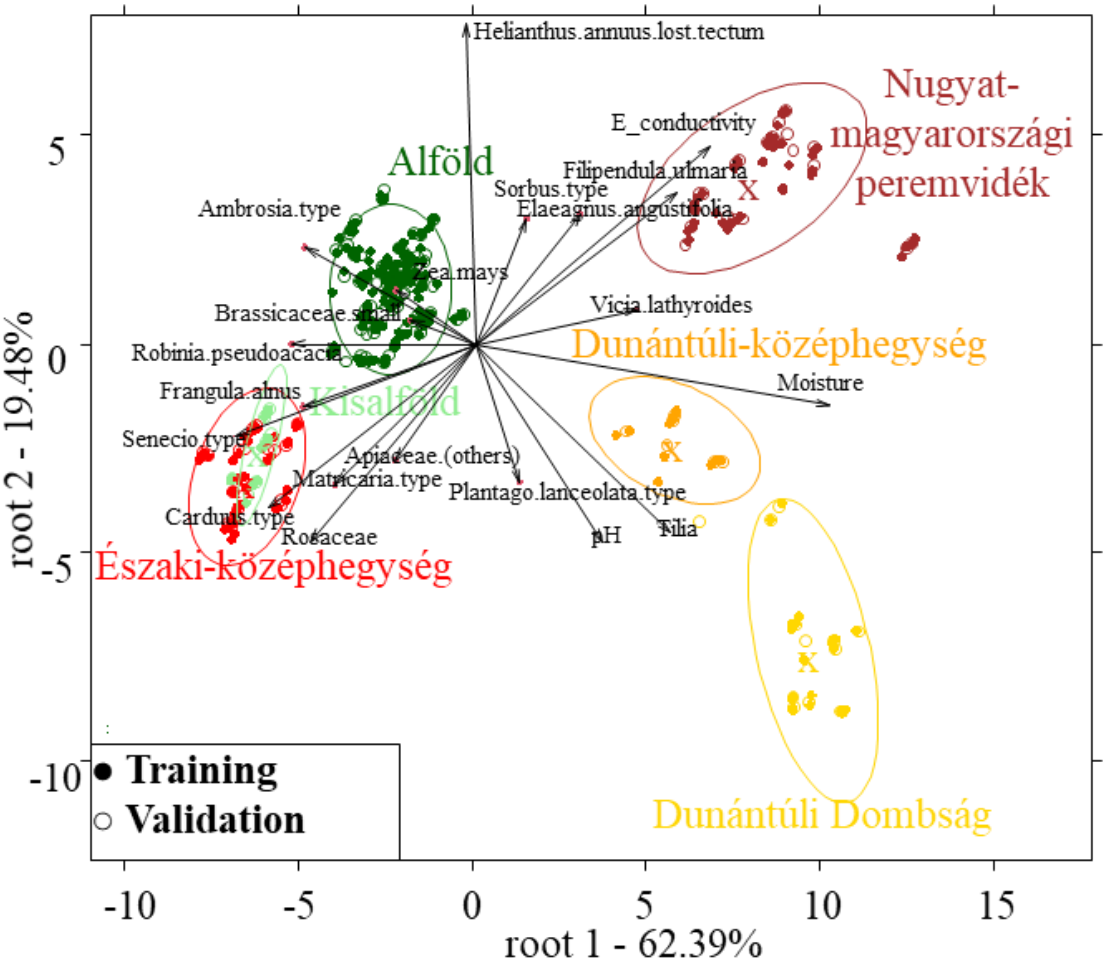

Figure 12. LDA score and loading plot using for the classification of the geographical origin using the fusion dataset of pollen, NIR and reference methods.

5.1.3 Results of the authenticity study

5.1.3.1 Results of the reference methods

The results of the reference methods showed that the pH, electrical conductivity, moisture content, total polyphenol content, CUPRAC and FRAP showed a decreasing tendency with the higher syrup concentration. Moreover, the lightness of the samples increased with the higher syrup content, but the a* parameter showed more greenish tone in case of the linden, but less greenish tone in case of the acacia honey. The yellowness (b*) decreased with the higher syrup concentration. These results are similar to studies of Turkish and Brazilian researchers (Ribeiro *et al.*, 2014; Yilmaz *et al.*, 2014). It can be also seen that in the case of the electrical conductivity

higher ELC was found for the honey adulterated with 10%, that can be due to the fact that the sample that was mixed with the syrup had a higher electrical conductivity (681±1.9 µS/cm) than some of the other linden samples. It was already mentioned that in the case of the physicochemical parameters high variations can be found even within botanical types, which is the explanation why the average ELC of all the acacia samples is lower than the ELC of the honey sample with 10% syrup.

The ANOVA analysis followed by pairwise comparison revealed that the moisture content of all the groups of the acacia honey were different from each other, while for linden honey the honey containing 20% syrup showed significantly higher moisture content compared to the others and, moreover the honey having 50% sugar syrup also had significantly higher moisture content compared to all the other groups. The pH results of the acacia samples showed that the authentic honey samples had significantly higher pH compared to the others. In the case of the linden honey the honey containing 50% syrup had significantly lower pH compared to the rest of the groups, while TI20% had significantly lower pH compared to the authentic, EUnonEU blends and TI10%. In electrical conductivity there was no significant difference between the authentic and the EUnonEU blend samples, however all the other groups showed significantly lower ELC in the case of the acacia honey, while for linden honey all the groups were significantly different from each other. In lightness (L*) none of the sugar syrup blends showed significant difference among each other, but they were significantly lighter than the authentic and EUnonEU samples compared to the acacia honeys. Linden syrup blends were similarly lighter compared to the authentic and EUnonEU linden samples. The A10%, A20% and A50% samples were not significantly greener than the authentic honey, but the honey blends of EUnonEU was significantly greener compared to all the other samples. Linden honeys showed different trend regarding the a*, where the authentic honeys could be characterized with reddish tone, but the EUnonEU blends and sugar syrup containing samples were greenish, therefore the authentic honey was significantly higher in a*, moreover the TI50% was significantly greener compared to the TI10% and TI20% blends. The yellowness results showed that the authentic acacia sample was significantly more yellowish than the blends of syrups. The linden honeys showed that the TI50% was significantly less yellow compared to the authentic samples and the blend with fewer syrup (TI10% and TI20%).

Regarding to the antioxidant parameters RP50% contained significantly less polyphenols than the rest of the samples, except for the RP20%. In the case of linden honey the TI50% had significantly lower total polyphenol content than the other groups, except for the EUnonEU linden. The RP50% also had significantly lower CUPRAC and FRAP values compared to the authentic samples, however the RP10% and RP20% did not differ significantly from the

authentic honey in CUPRAC but had significantly lower FRAP. The linden honey results also showed that the honey containing 50% syrup had lower FRAP and CUPRAC compared to the authentic honey. TI10% and TI20% did not show significantly different CUPRAC compared to the authentic linden but had significantly lower FRAP. Sugar measurements showed that EUnonEU blend acacia and linden honeys showed significantly higher glucose and fructose content compared to the authentic samples.

5.1.3.2 Results of the sensory profile analysis

The sensory profile analysis showed slightly different results for the two types of honey.

In the case of the acacia only four parameters showed that there is a significant difference among the authentic honey and the syrup blends (**Figure 13 a**). The RP10% and RP50% showed significantly less intense sweet and flowery taste, while RP10% had significantly more intense fruity odor. The honeys containing 20% and 50% syrup were characterized by significantly higher caramel taste intensity. Regardless of the lack of significant difference, the honeys containing syrup had less intense odor characteristics (general odor intensity, flowery odor), but had more intense sweet odor (in the case of 10% and 20%), animalic odor, and dry hay odor. Regarding the taste attributes, the adulterated samples were characterized by slightly less intense global taste intensity with the exception of RP20%, that had quite similar value as the reference (reference 50, RP20%: 50.91). The adulterated samples were also considered sourer and had lower taste persistence and dry hay flavor.

In comparison with pure acacia, the linden honeys with syrup differed significantly in eight parameters from the reference sample (**Figure 13 b**). The honey containing 10% syrup was characterized by significantly stronger fresh odor, while TI20% was significantly sourer compared to the reference sample. Moreover, TI50% was scored with significantly less intense bitter and medicinal flavor. All the adulterated samples had significantly less intensely sweet taste, global odor intensity and global taste intensity, and aftertaste persistence. Moreover, the samples with syrup less had intense resinous odor and flavor. Honeys containing syrup were also considered to have more intense refreshing flavor. Regarding astringency, the samples containing 50% syrup had lower astringency score, but the other two had similar values compared to the reference.

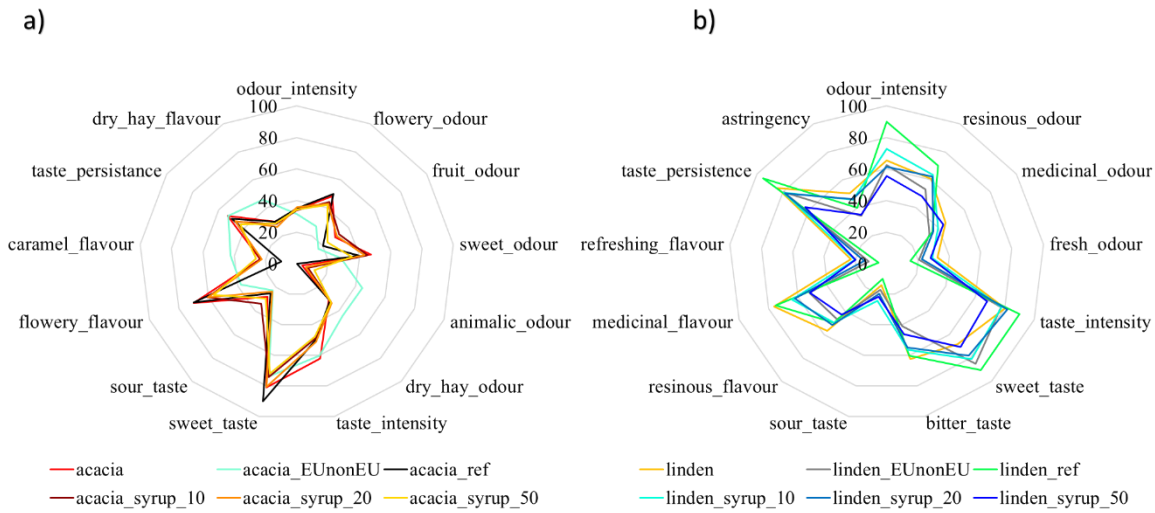

Figure 13. Results of the sensory profile analysis of a) acacia honeys b) linden honeys (Bodor *et al*., 2020)

Our results are in accordance with a Turkish research were the researchers found also significant differences in the odor and taste attributes of authentic and adulterated honeys (Guler *et al.*, 2014).

5.1.3.3 Results of the electronic tongue

The results of the LDA models built for the classification of the authentic, and samples mixed with the syrups showed slight differences between the two types of honey. In the case of the acacia the average training and validation accuracies were 99.22%, where the adulterants were all classified correctly but the control honey showed misclassification in 3.11% belonging to the group of the RP10%. The independent prediction of the EUnonEU acacia honeys showed that only four of the samples were classified correctly as an authentic honey (**Figure 14**). Two of the samples showed misclassification belonging to the 10% adulterant in 7.69% and 4.17%. Three of samples were classified correctly in less than 60%, and the misclassification of these samples was found as belonging to the group of the 20% adulterated samples. One of the samples was completely misclassified in 33.33% as belonging to the 10% and in 66.67% belonging to the 50% syrup-containing honey.

In the case of the linden honey lower average training and validation accuracies were obtained, however in this case the authentic, TI10% and TI50% were classified correctly. The TI20% showed 92.92% correct classification during the training and 71.67% throughout the validation. The misclassification in this case was shown as belonging to the TI50% during the training in 7.08% and belonging to the TI10% and TI50% in the case of the validation in 14.14%. The independent prediction of the linden honeys showed that two of the honeys are classified as authentic, but one of them showed misclassification belonging to the 10% adulteration in 1.28% **(Figure 14**).

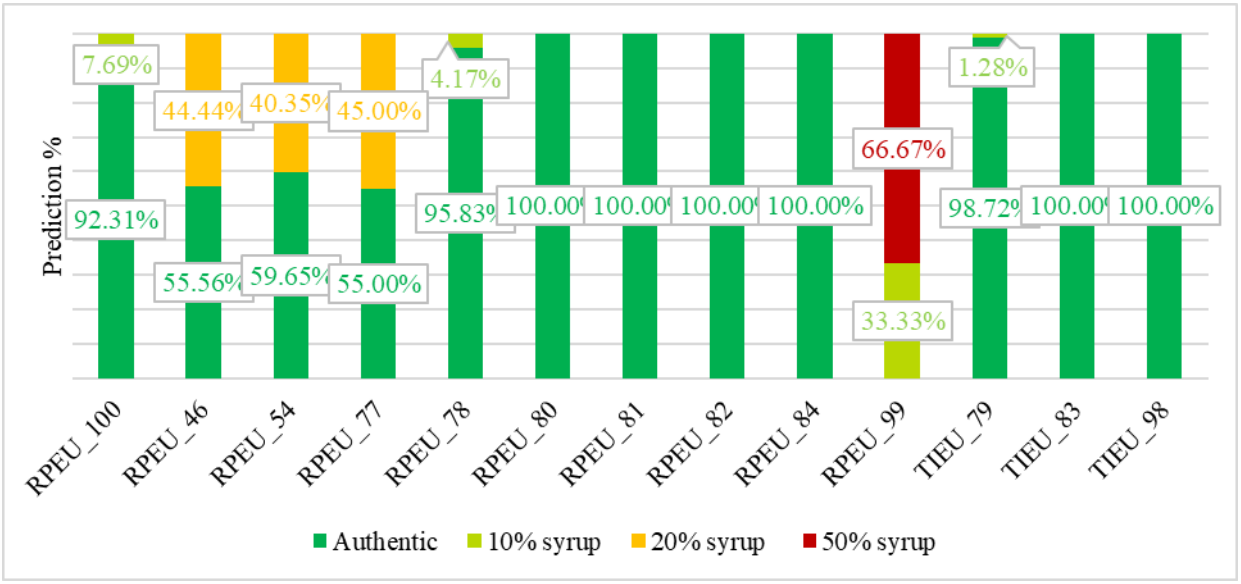

Figure 14. LDA independent prediction results of adulteration level of the EUnonEU acacia (RPEU) and linden (TIEU) honey samples after threefold cross validation using electronic tongue data

Partial least squares models built for the prediction of the sensory properties based on the electronic tongue provided the best results in the case of the flowery taste for the acacia honey (**Figure 15 a**). After the validation, the R^2CV =0.89 was obtained. The sweet taste and caramel taste were weaker, with R^2CV of 0.52 and 0.58. The better result of the flowery taste can be assigned to the fact that acacia honey usually has a strong flowery aroma (Oddo and Piro, 2004), moreover, that electronic tongue is sometimes weak in the prediction of sweet taste. The prediction errors were lower than 6 score. In the case of the linden honey better model parameters were obtained, that can be attributed to the stronger aroma of the linden honey in general (**Figure 15 b**). The best results were achieved in the case of the taste persistence with R^2CV of 0.93. For linden honeys the prediction errors were lower than 4.

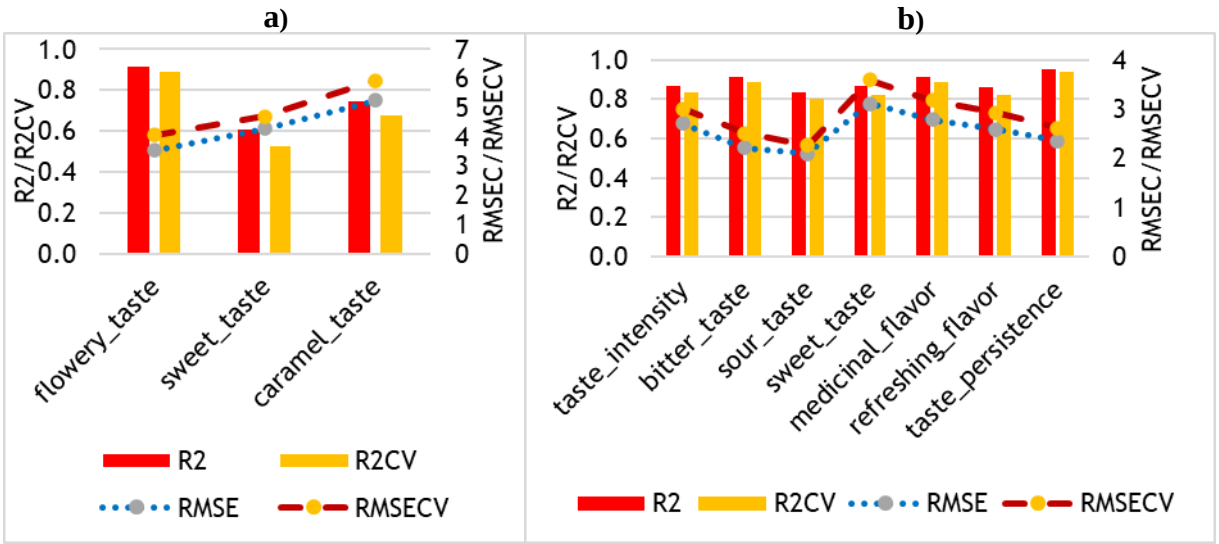

Figure 15. Results of the partial least squares regression for the prediction of sensory parameters using electronic tongue data a) acacia honeys b) linden honeys

5.2 Results of the sugar syrup adulteration study

5.2.1 Results of the preliminary adulteration study

5.2.1.1 Results of the reference methods

Results of the reference methods such as moisture content, did not show unacceptable increase in the case of the blended honey samples. The moisture content of the control sample was 17.20±0.00%, rice syrup showed similar moisture content with 17.28±1.20%, while the beet syrup showed 18.70±0.30%. A slight increase in the moisture content of the samples mixed with beet syrup can be observed on **Figure 16**. However, even at 50% sugar syrup content, the moisture content remained below the limit of the legislation (maximum 20%) (*Codex Alimentarius* Commission, 2001; The European Council, 2001; *Codex Alimentarius Hungaricus*, 2002). In another Hungarian study it was also observed that the type of the syrup produces changes of the reference parameters. Some of the syrups did not induce significant change, while others yes, however none of them was similar to our syrups as those were glucose, fructose/glucose and isosugar syrups (Czipa *et al.*, 2019).

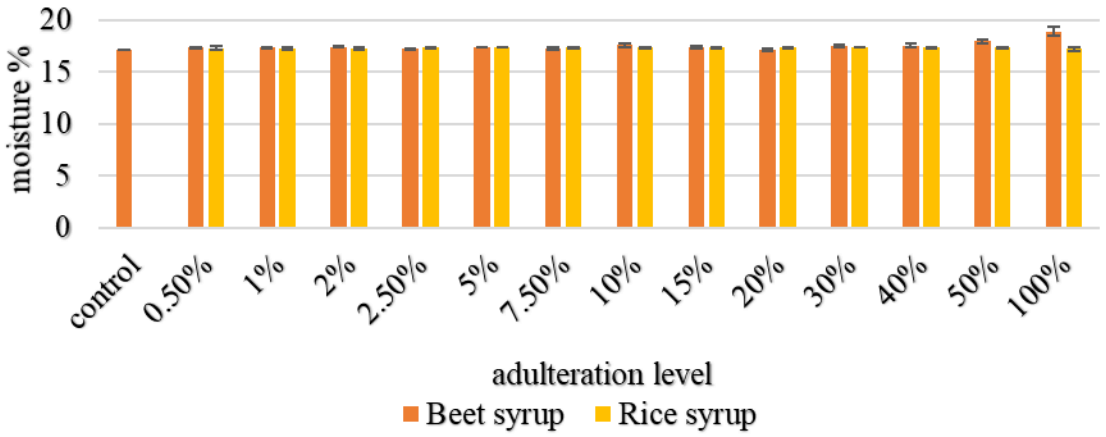

Figure 16. Moisture content of the control and mixed linden honey samples

5.2.1.2 Results of the near infrared spectroscopy

Near infrared raw spectra showed peaks around the 1000 nm, 1200 nm and in the range of the 1400-1500 nm and 1500-1600 nm. Based on the spectra, it also can be seen that the samples mixed with rice syrup (RI) and beet syrup (RS) showed separation especially above 1400 nm (**Figure 17**). This region can be assigned to the first overtone O-H stretching bands (Yang *et al.*, 2020).

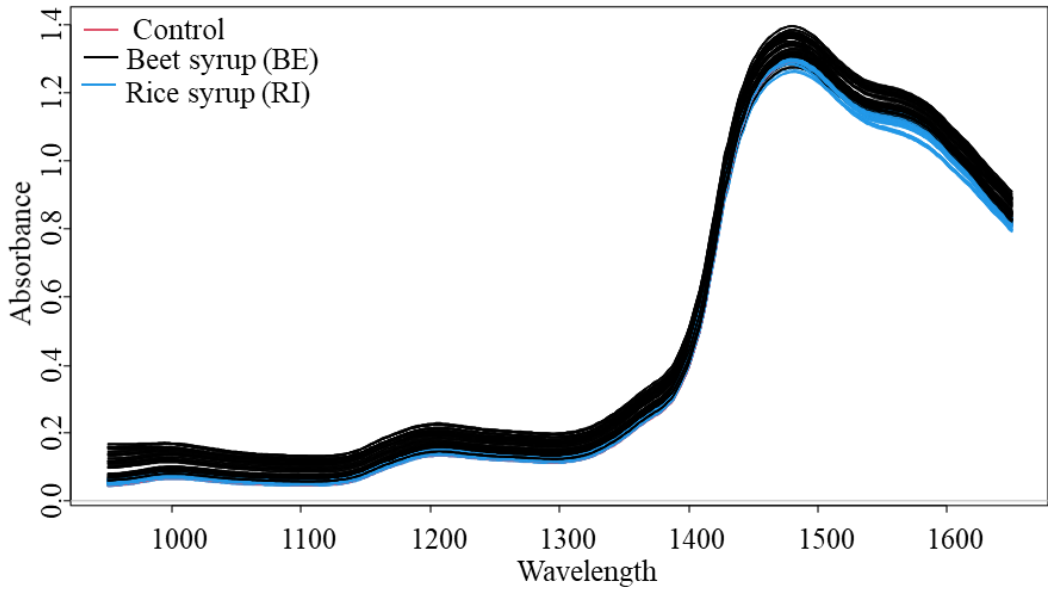

Figure 17. Raw spectra of the linden honey sample and its blends with rice and beet syrup in the range of 950-1650 nm

The results of the PCA built based on the raw spectra showed the first three principal components (PC) described the 99% of the variance. A separation trend of the two types of syrup adulterants was seen based on PC1 that describes the 91.37% of the total variance (**Figure 18**).

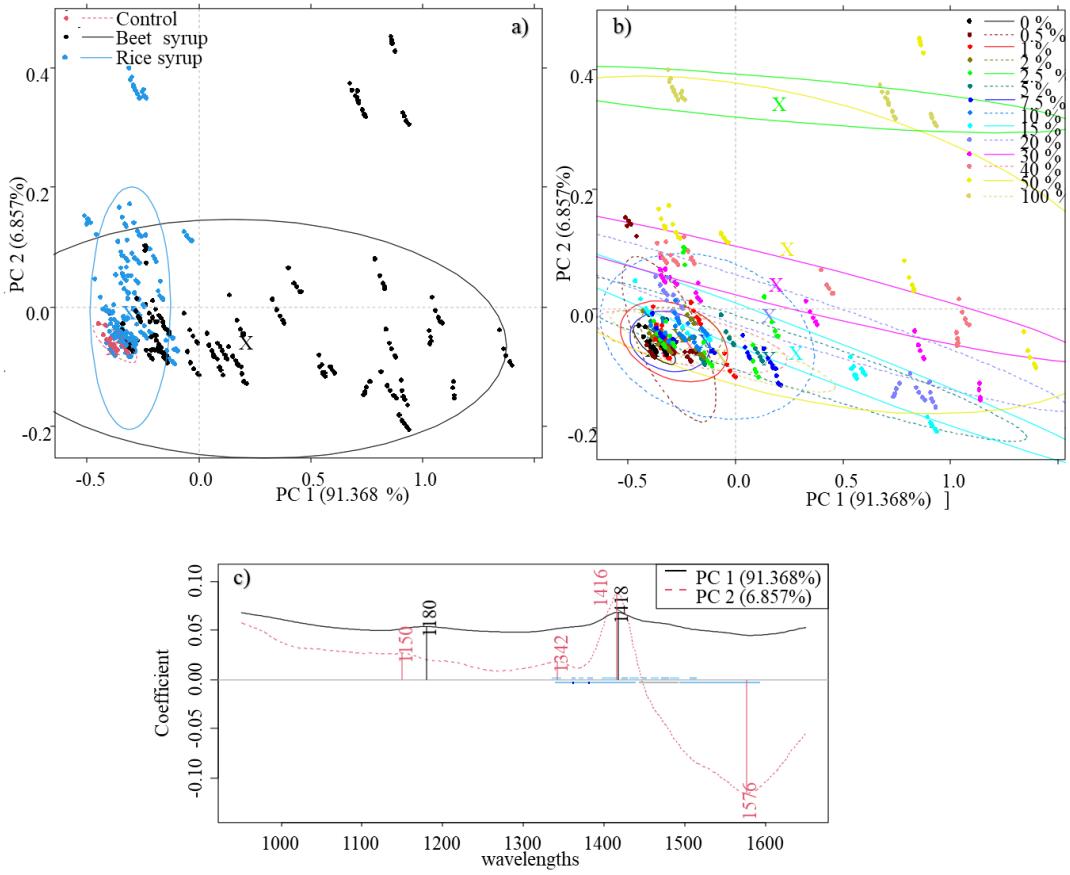

Figure 18. PCA score plot of the linden honey and its blends with rice and beet syrup based on the raw spectra in the range of 950-1650 nm a) colored by the adulterant, b) colored by the adulteration level, c) loadings of PC1-PC2

As the two types of syrups and their blends can be separated, these were analyzed separately as described in the following.

PCA-LDA for the classification of adulteration level (added syrup)

Linear discriminant analysis model for the classification of added syrup level showed a clear separation tendency for both rice and beet syrup (**Figure 19**). Classification model of the rice syrup mixtures showed average classification accuracy of 100% for the training and 98.72% for the validation data set. Control sample was classified correctly, however misclassification was found for the RI0.5 sample in 10% as belonging to the RI001%, and for the RI002 sample as belonging to the RI001% in 6.67%. These results show that low blending levels could not be discriminated completely from each other, but higher levels (>5%) were clearly separated from each other.

Classification model of the beet syrup-blended honey samples also showed 100% average classification accuracy during the training and 98.97% during the validation. In this case the control sample was classified correctly, however misclassification was found for both BE0.5% and BE00%1 sample as belonging to the control sample in 6.67%. Gan *et al.* (2016) also found high accuracy of the discrimination of adulterated honey with corn and rice syrup. They also did not find misclassification as belonging to the ≥5% adulterated honey. Our results are better than a study adulterating rape honeys at 10%, 20% and 40%, where the total classification accuracy was below 96.2% (Li *et al.*, 2017).

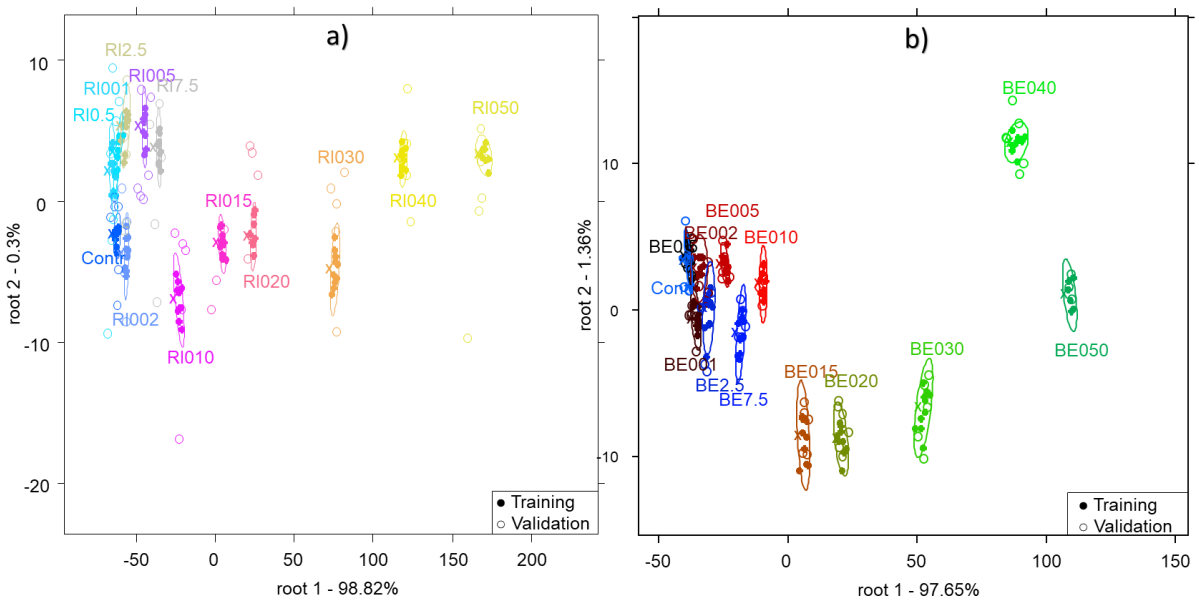

Figure 19. LDA score plot of the classification models of the linden honey a) blended with rice syrup (pretreatment: sgol@2-13-0+sgol@2-13-1) b) blended with beet syrup (pretreatment sgol@2-13-0+sgol@2-17-1)

These results show that in the case of rice syrup all the levels were clearly differentiated from the control, however in the case of beet syrup 0.5% and 1% syrup addition could not be clearly discriminated (nevertheless, it is not a problem in the practice).

Results of the partial least squares regression

Partial least squares regression models built for the prediction of the added syrup % showed strong correlation between the added and predicted sugar syrup concentration. In the case of the rice syrup mixtures R^2C = 0.999 and the R^2CV = 0.998 was obtained with lower than 0.82% error of prediction based on the RMSE values. RDP was also high (>20) showing robustness of the prediction model **(Figure 20 a, c).**

The beet syrup concentration also could be predicted with a high accuracy after the optimization of the model, where R^2C = 0.996, R^2CV = 0.993 were the determination coefficients. The error of prediction was a bit higher than in the case of the rice syrup adulteration, with less than 1.21% after the leave-one-sample-out validation. The RDP values also showed a good robustness of the model, where after the validation RDP >12. These results show that the amount of the added syrup could be predicted with strong model parameters **(Figure 20 b, d).**

The highest scatter in the higher concentration can be attributed to the less frequent concentrations compared to the range under 10%. Therefore, in this case the error could be higher.

From the regression vectors it can be seen that similar wavelengths contributed to the regression models. The detailed spectral assignation is discussed in the next chapter of the extended sugar syrup adulteration study in 5.2.2.2.

Our results are slightly better than a study of Manuka honey adulteration with C3 and C4 sugars in the range of 10-50%. Their results showed the R^2 lower than 0.99 and RMSEC/CV higher than 3% (Yang *et al.*, 2020). Similarly, slightly worse results were obtained for adulteration of rape honeys where the R^2 was below 0.99 and the RMSECV was higher than 1.7% (Li *et al.*, 2017).

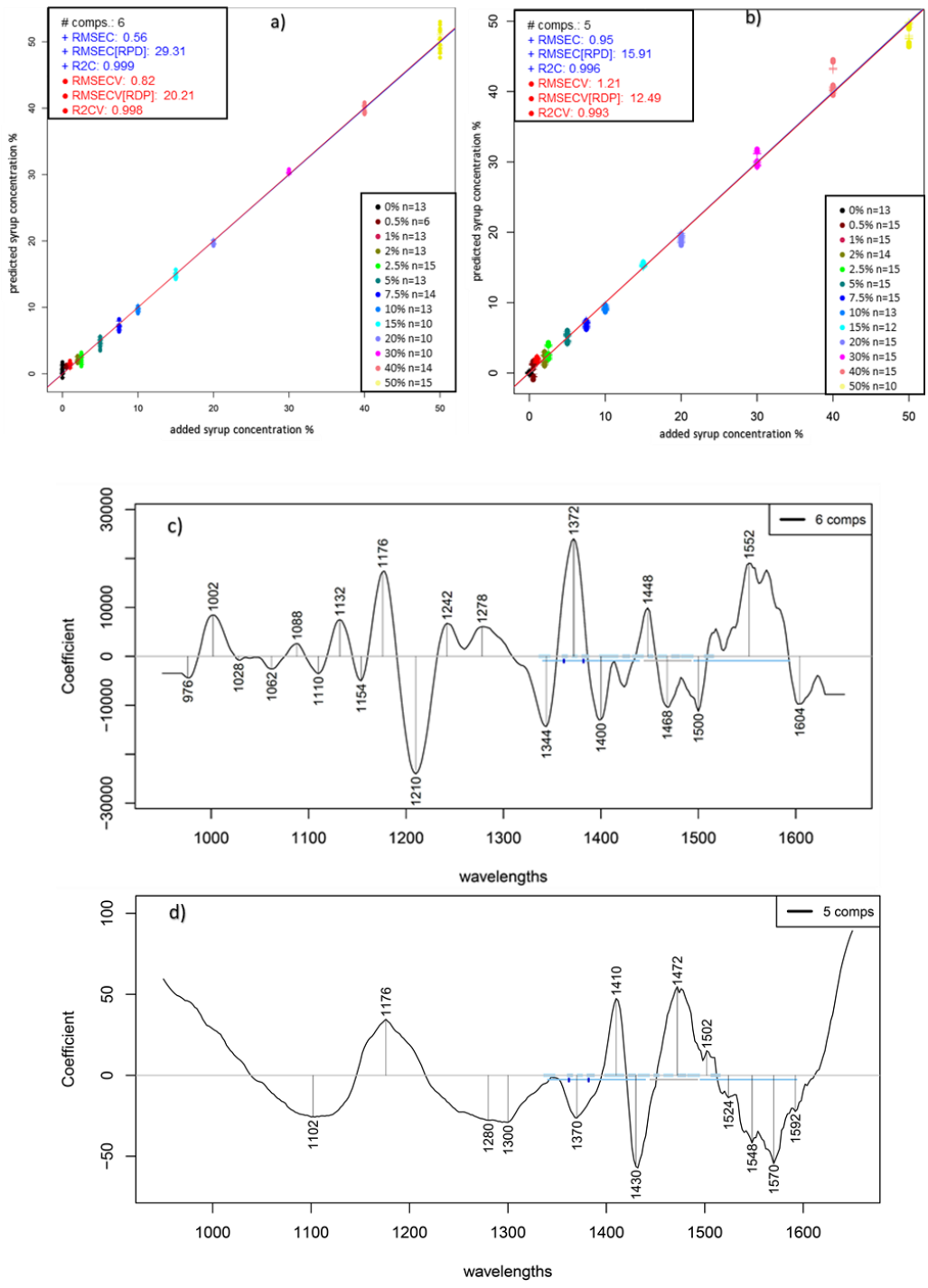

Figure 20. Partial least squares regression model to regress on the added syrup concentration of the linden honey a) blended with rice syrup (pretreatment: sgol@2-21-0+sgol@2-21-2) and b) blended with beet syrup (pretreatment: sgol@2-13-0+MSC) c) regression vectors of the honey blended with rice syrup d) blended with the beet syrup

5.2.2 Results of the extended sugar syrup adulteration study with low concentrations

5.2.2.1 Results of reference methods

Moisture content

Moisture content of the different honeys and their syrup blends were all below the limit of the legislations (20%), moreover two of the syrups also fulfilled this requirement. The F40 syrup has

moisture content of 21.88±0.44 %. The detailed results of moisture content are shown in **Figure 21 a).** The honeys adulterated with the F40 syrup showed an increasing trend with the increasing syrup concentration that is the consequence of the higher moisture content of the F40 syrup. This trend was observable for all the honey types. Similar increasing trend can be seen in the case of the rice syrup mixed samples for the acacia, sunflower, and rape honeys and for the GF syrup in the case of the sunflower and honeydew honeys. These results show that even a low level of syrup addition can affect the moisture content, however this does not cause unacceptable change if the moisture content of the sugar syrup is under or slightly above the limit (20%).

pH

The pH values for all the samples were in the range of 3.18-5.33. The control samples in general had lower pH values compared to the syrups, with the exception of the FS syrup that had pH of 4.02±0.12 %. In contrary with the moisture content, the pH did not show clear increase in the pH of the mixtures, nonetheless the pH of the sugar syrup is higher or lower than the honey's itself (**Figure 21 b**).

Electrical conductivity

The electrical conductivity was quite various in the case of the different control honeys (**Figure 21 c**). It is important to mention that the honeydew honey was below the legislation requirement, as the ELC of the honey was only 508.78±3.07 µS/cm, which is below the 800 µS/cm of the requirement. As mentioned before, this can be due to the fact that sometimes the beekeepers mislabel the honey unintentionally, because in the practice they call honeys "*erdei*" instead of "*mézharmat*". The syrups had very low electrical conductivity: rice syrup 10.73±1.33 µS/cm, F40 syrup 18.72±1.42 µS/cm and the GF syrup 3.42±0.55 µS/cm. In the case of all the honey types and all the case of syrups a decreasing tendency was seen.

Similarly to the results of the other measurements, the moisture content was not highly affected by the addition of the syrup, moreover it did not affect the pH of the samples, but decreasing trend was seen in the electrical conductivity. Similar decrease was found in the Hungarian study however in this other syrup types and much higher adulterant concentrations were applied (Czipa *et al.*, 2019).

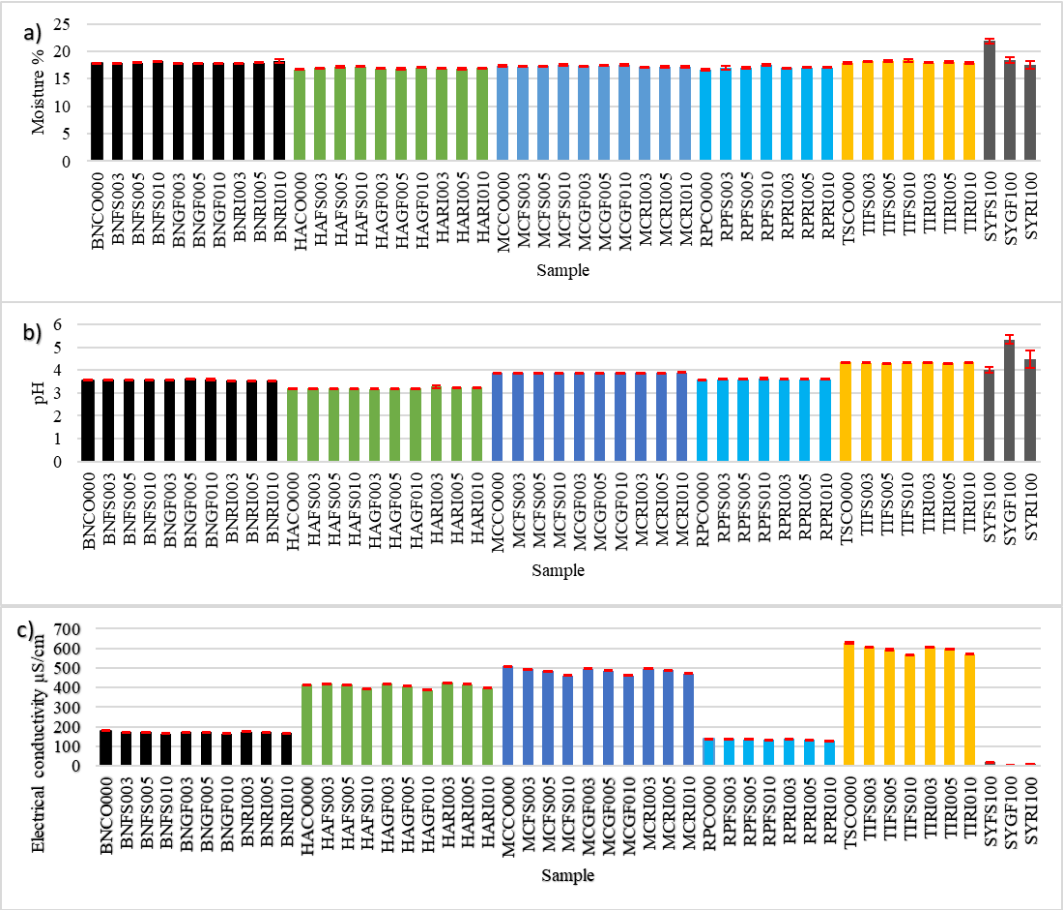

Figure 21. Results of the reference parameters of the extended adulteration experiment a) moisture, b) pH, c) electrical conductivity

5.2.2.2 Results of near infrared spectroscopy

Raw spectra (**Figure 22**) of the extended experiment showed similar peaks to the preliminary experiment (SSAPS) **Figure 17**. The spectra also show higher absorbance values for the sunflower (HA) and for the rape honeys (BN), while the acacia (RP), honeydew (HD), and linden (TI) showed lower absorbance values in general.

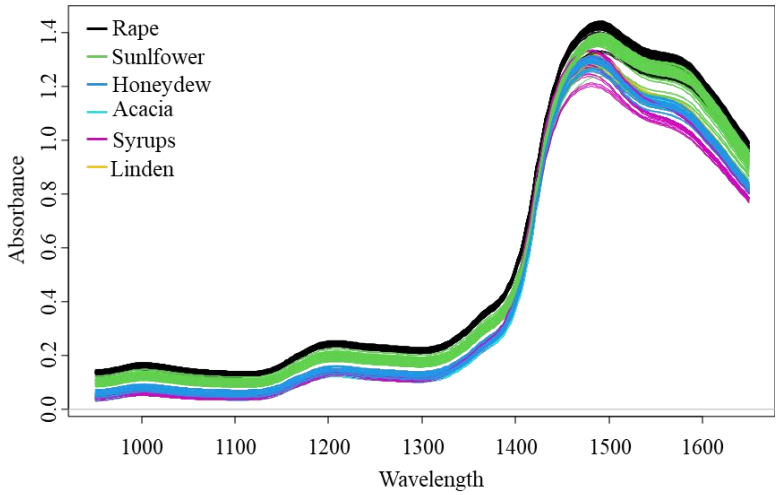

Figure 22. Raw spectra of the honey samples of the extended adulteration experiment in the range of 950-1650 nm

Principal component analysis built based on the raw spectra showed a clear separation tendency of the different botanical groups, where the PC1 described the 96.335% of the total variance and PC2 covered the 2.178% of the total variance (**Figure 23**). Based on the PC1 separation of the sunflower and rape honeys can be seen related to the other honey types and the syrups, while based on PC2 the separation of the syrup from the honeys can be observed.

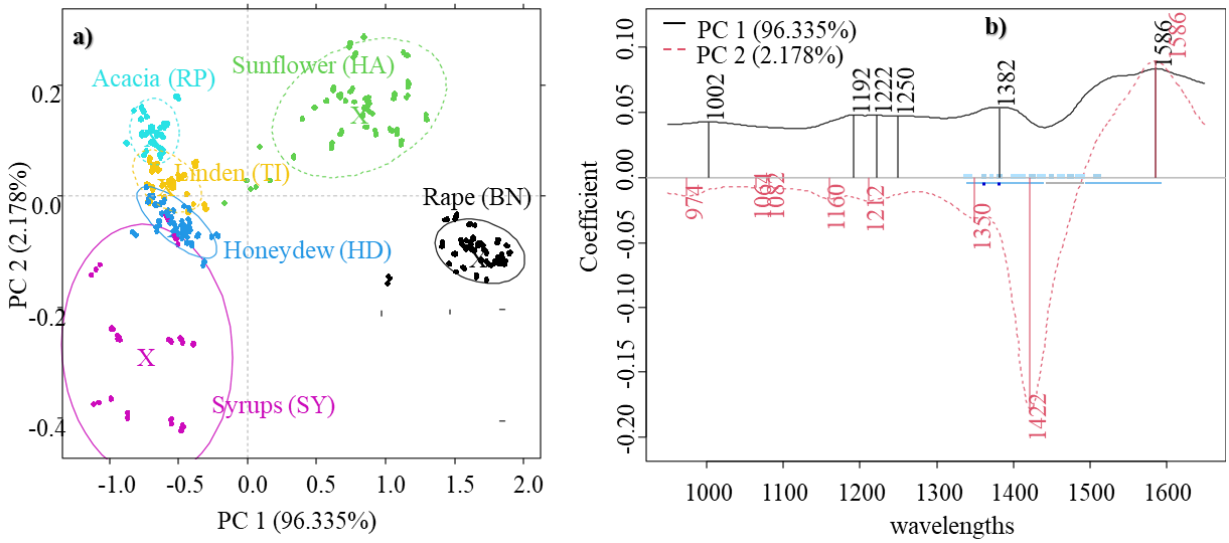

Figure 23. Principal component analysis a) score plot and b) loading plot of the extended adulteration experiment built based on the raw spectra in the range of 950-1650 nm

Owing to this separation trend, the different honey types were evaluated separately

PCA-LDA results of the sugar syrup adulteration study with low concentrations

Linear discriminant analysis models for the different botanical groups showed correct classification of the control sample in all of the models. The average training and validation accuracies were also 100% in most of the cases after the model optimization. On **Table 18** it can be seen that the different models needed different pretreatments.

Results of the acacia honey showed correct classification of all the groups in the case of all the models for the classification of the different levels of syrup concentration.

The models of the linden honey also showed correct classification when data of the honey samples were modelled separately for the two syrup adulterations, however, when the whole acacia dataset was analyzed, the average classification accuracy for the training and validation was 97.62%. In both training and validation data sets the linden honey blended with rice syrup in 5% (TIRI005) was classified correctly in 83.33%, where misclassification was found as belonging to the F40 syrup blended with honey in 5% (TIFS005) in 16.67%.

Similarly to the acacia, the models of the honeydew honey showed 100% correct classification of all the models during training and cross validation.

The classification models of the rape honey provided 100% correct classification of all the groups in the case of the models built for the syrup types separately, where all the syrup levels

could be discriminated from the control and from each other. When all the syrups were analyzed together the average training and validation model accuracy were 100% and 98.89%. During the validation, the rape honey mixed with corn syrup in 3% (BNFS003) showed 88.89% correct classification, where misclassification was found belonging to the 5% F40 syrup mixture (BNFS005).

Compared to the others, the weakest results were obtained in the case of the models of the sunflower honey, where 100% correct classification was obtained for all the groups in the case of the rice (RI) and F40 (FS) syrup models. The model of all sunflower honey containing all the syrups showed 100% average correct classification during the training, but 96.67% average correct classification during the validation. The F40 syrup adulterated honey in 3% (HAFS003) showed 88.89% correct classification, where misclassification was found as belonging to the 5% F40 blended honey (HAFS005). Moreover, sunflower honey mixed with glucose/fructose syrup in 3% (HAGF003) was misclassified as belonging to the 10% blended honey (HAGF010) in 11.11%. The HAGF010 was also misclassified as HAGF003 in 11.11%. The model of the GF syrup blended honeys showed 94.45% average correct classification for the training and validation data set. Both the training and validation data set provided correct classification for the control and 3%, and 5% adulterated honey. Though, the 10% mixture (HAGF010) showed misclassification as belonging to the 5% (HAGF005) honey group in 22.22%. Similar results were obtained in a Chinese study, where also 100% classification was obtained for the classification of adulterated honeys at 5%, 10% and higher levels (Gan *et al.*, 2016).

Table 18. Classification accuracies of the models for the classification of the adulteration levels after choosing the best pretreatment

Honey	Syrup	Pretreatment	Control correct classification Training %	Control correct classification Validation %	Average Training %	Average Validation %
Acacia	all	sgol@2-13-0 + sgol@2-17-1	100	100	100	100
	Rice	sgol@2-13-0 + deTr	100	100	100	100
	F40	sgol@2-17-0 + sgol@2-21-1	100	100	100	100
Linden	all	deTr + snv	100	100	97.62	97.62
	Rice	sgol@2-21-0	100	100	100	100
	F40	deTr	100	100	100	100
Honeydew	all	sgol@2-21-0 + snv	100	100	100	100
	Rice	sgol@2-17-0 + sgol@2-17-2	100	100	100	100
	F40	sgol@2-21-0 + sgol@2-21-2	100	100	100	100

Honey	Syrup	Pretreatment	Control correct classification Training %	Control correct classification Validation %	Average Training %	Average Validation %
	GF	sgol@2-21-0 + msc	100	100	100	100
Rape	**all**	sgol@2-21-0	100	100	100	98.89
	Rice	sgol@2-17-0 + deTr	100	100	100	100
	F40	sgol@2-21-0	100	100	100	100
	GF	sgol@2-13-0 + sgol@2-21-1	100	100	100	100
Sunflower	**all**	sgol@2-21-0 + sgol@2-21-1	100	100	100	96.67
	Rice	sgol@2-17-0 + sgol@2-17-1	100	100	100	100
	F40	sgol@2-13-0	100	100	100	100
	GF	msc	100	100	94.45	94.45

Rice: rice syrup, F40: high-fructose content corn syrup, GF: self-made glucose-fructose syrup

These results are quite satisfactory especially if we take into consideration that in this study low levels of syrup addition *(% w/w* 0-10%) applied and despite these low levels, better or similar classification accuracies were achieved than in other studies (Gan *et al.*, 2016; Li *et al.*, 2017).

PLSR results of the extended adulteration experiment (SSAWLC)

The results of the PLSR built for the prediction of the added syrup concentration of the different honey types according to botanical origin provided various results based on the best prediction models. The models within one botanical type for the syrups altogether and for the different syrups also were different. The summary table of the built models and their contributing wavelengths after choosing the best model can be seen in **(Table 19; Table 20).**

Acacia honey prediction when using the whole dataset (without going down to syrup level) provided R^2CV of 0.98, with lower than 0.5% error. The RPDCV value was above 6. Models built for the prediction of the individual syrups were also good, with RPDCV value of 4.22 and 6.83 for the rice and F40 syrup, respectively. RMSECV was below 1% and the R^2CV values were also higher than 0.94.

In the case of linden honeys slightly weaker model parameters were obtained. In the case of the models using the data of both syrups the R^2CV was 0.92 with error of 1.07, and RPDCV of 3.5. The models of the individual syrups showed better results for the F40, such as in the case of acacia. The R^2CV was 0.80 and 0.86 for the rice and F40 syrup, respectively. For the rice syrup RMSCV=1.64 and RPDCV of 2.27, while for the F40 syrup RMSECV=1.37 and RPDCV=2.73 were obtained.

The models of the honeydew honey showed RPDCV values to be satisfactory in all the cases. Considering all the syrups the R^2CV of 0.88 and RMSECV of 1.07 were achieved. Similarly to the previous two types the F40 syrups had the best model parameters, followed by the rice syrup and glucose/fructose syrup. In the case of the rice and F40 syrup the RMSECV was below 1% and with $R^2CV > 0.94$. The worst results were obtained for the GF syrup, where the R^2CV was only 0.71, RMSECV was 1.85%.

Rape honeys showed similar trend to the honeydew honey, however, in this case the prediction of GF was not satisfactory regarding the RPDCV value that was 1.39. Having all the syrups in the model the model parameters were the following R^2CV was 0.68, RMSECV was 1.80. For the F40 syrup model R^2CV was above 0.95 and the RMSECV was below 1%. For the rice syrup worse results were obtained with R^2CV of 0.88 and RMSECV of 1.13 The model of the GF syrup was the worst where the R^2CV was below 0.5 and the RMSECV was higher than 2%.

Similarly to rape and honeydew the models of sunflower honey showed the best results for the prediction of the F40 followed by the rice syrup, glucose fructose syrup and the model containing all the syrups. Based on the RPDCV values the models were not satisfactory as it was below 1.5. F40 models showed higher determination coefficient than 0.94 and lower RMSECV then 1% and the RPDCV was higher than 4. The rice syrup model was slightly weaker with R^2CV of 0.92 and RMSECV of 1.01.

Summarizing, the F40 syrup could be predicted in with the highest accuracy and models parameters, where the R^2CV was higher than 0.86 with lower RMSECV than 1.4%. Rice syrup prediction provided weaker results as the R^2CV was higher than 0.80 and the prediction error was lower than 1.64. The worst obtained for the GF syrup prediction; the reason could be that the syrup was designed to have the similar sugar ratio to the honeys in general.

Spectral assignations

The wavelengths that were contributing the most in the PLSR models are similar to the study where more levels were analyzed on linden honey (**Figure 20**. **c, d**).

The region of 950-1000 nm can be assigned to the N-H stretch of the second overtone (Zhang *et al.*, 2018). The wavelengths of 1000-1130 nm (10000-8850 cm^{-1}) are assigned to the second overtone O-H stretches. The 1150-1220 (8700-8200 cm^{-1}) nm range might be assigned to the second overtone C-H (CH_2, CH_3) stretches and the 1st overtone of C-H combination (CH_3, CH_2). The region of 1300-1600 nm can be assigned to the first overtone O-H stretches (especially water molecules, while above 1600 nm the characteristic stretches of the carbohydrates can be found (Ozaki *et al.*, 2016; Li *et al.*, 2017; Zhang *et al.*, 2018; Yang *et al.*, 2020). Within this, the

region of 1300-1400 can be assigned to the C-H vibrations and combination bands (Zhang *et al.*, 2018).

Studying deeper the water molecule structures we can also assign the wavelength based on the number of hydrogen bonds of the water: based on this, the region of the 1320-1370 assigned to the less hydrogen bonded water. The bonds around the 1420 nm are related to the less hydrogen bonded free water, while higher wavelengths are assigned to highly bond water (1490-1520). Moreover, the wavelengths above 1580-1590 nm have been assigned to the aqueous solution of sugars such as fructose, sucrose, and glucose (Bázár *et al.*, 2016).

Based on this, we can assume that the addition of the sugar syrup highly affects the water structure of the honey and the sugars, which is very well reflected in the spectra.

Table 19. Summary table of the results of the PLSR models for the prediction of added syrup adulteration level by botanical group using NIR data

Honey	Syrup	Pretreatment	Number of Latent variables	Number of Observations.	R^2C	RMSEC %	RPDC	R^2CV	RMSECV %	RPDCV
Acacia	**all**	sgol@2-13-0+sgol@2-17-1	4	66	0.99	0.29	11.42	0.98	0.49	6.70
	Rice	sgol@2-13-0+deTr	2	41	0.98	0.52	6.79	0.94	0.84	4.22
	F40	sgol@2-17-0+sgol@2-21-1	4	45	0.99	0.36	9.69	0.98	0.50	6.83
Linden	**all**	deTr+snv	4	60	0.97	0.68	5.49	0.92	1.07	3.50
	Rice	sgol@2-21-0	3	37	0.96	0.71	5.25	0.80	1.64	2.27
	F40	deTr	3	50	0.94	0.93	4.03	0.86	1.37	2.73
Honeydew	**all**	sgol@2-17-0+snv	4	57	0.92	0.89	3.53	0.88	1.07	2.95
	Rice	sgol@2-17-0+sgol@2-17-2	4	21	1.00	0.22	14.70	0.95	0.72	4.52
	F40	sgol@2-21-0+sgol@2-21-1	4	27	0.99	0.36	9.32	0.97	0.54	6.20
	GF	sgol@2-21-0+sgol@2-13-2	4	25	0.98	0.54	6.46	0.71	1.85	1.89
Rape	**all**	msc	3	75	0.77	1.53	2.09	0.68	1.80	1.78
	Rice	sgol@2-17-0+sgol@2-21-2	3	29	0.96	0.69	4.80	0.88	1.13	2.93
	F40	deTr	2	27	0.98	0.49	7.14	0.96	0.72	4.91
	GF	sgol@2-13-0+sgol@2-21-2	4	25	0.92	0.99	3.70	0.46	2.62	1.39
Sunflower	**all**	msc	4	76	0.60	2.11	1.60	0.36	2.69	1.26
	Rice	sgol@2-17-0+sgol@2-17-1	4	27	0.99	0.40	9.29	0.92	1.01	3.67
	F40	sgol@2-13-0	4	32	0.99	0.41	8.36	0.94	0.80	4.27
	GF	msc	4	35	0.83	1.50	2.49	0.39	2.89	1.29

Rice: rice syrup, F40: high-fructose content corn syrup, GF: self-made glucose-fructose syrup

Table 20 The contributing NIR wavelengths by botanical and sugar syrup type of the PLSR models built for the prediction of the added sugar syrup concentration

Honey	Syrup	Pretreatment	Wavelengths (nm)
Acacia	**all**	sgol@2-13-0+sgol@2-17-1	992, 1028, 1078, 1152, 1204, 1244, 1290, 1318, 1340, 1362, 1402, 1434, 1500
	Rice	sgol@2-13-0+deTr	1046, 1140, 1284, 1330, 1350, 1422, 1582
	F40	sgol@2-17-0+sgol@2-21-1	988, 1026, 1052, 1078, 1120, 1150, 1212, 1240, 1276, 1324, 1362, 1400, 1440, 1500, 1562
Linden	**all**	deTr+snv	1002, 1114, 1184, 1208, 1264, 1306, 1370, 1420, 1468, 1504, 1564, 1602
	Rice	sgol@2-21-0	974, 1022, 1166, 1198, 1274, 1344, 1366, 1426, 1592
	F40	deTr	1016, 1060, 1164, 1268, 1420, 1492, 1592
Honeydew	**all**	sgol@2-17-0+snv	998, 1120, 1204, 1244, 1320, 1374, 1416, 1456, 1522, 1574
	Rice	sgol@2-17-0+sgol@2-17-2	994, 1036, 1072, 1108, 1130, 1158, 1176, 1196, 1244, 1260, 1278, 1292, 1326, 1346, 1382, 1408, 1448, 1514, 1528, 1546, 1600
	F40	sgol@2-21-0+sgol@2-21-1	978, 1036, 1100, 1164, 1286, 1354, 1396, 1430, 1478, 1540, 1618
	GF	sgol@2-21-0+sgol@2-13-2	992, 1062, 1136, 1190, 1224, 1258, 1270, 1334, 1366, 1388, 1406, 1474, 1494, 1516, 1534, 1552, 1586, 1602, 1618
Rape	**all**	msc	996, 1126, 1218, 1312, 1370, 1422, 1588
	Rice	sgol@2-17-0+sgol@2-21-2	1008, 1054, 1078, 1144, 1186, 1214, 1270, 1324, 1372, 1422, 1470, 1492, 1516, 1536, 1570, 1590, 1606, 1624
	F40	deTr	1030, 1188, 1264, 1342, 1430, 1608
	GF	sgol@2-13-0+sgol@2-21-2	996, 1026, 1078, 1096, 1118, 1158, 1186, 1214, 1258, 1316, 1366, 1396, 1440, 1472, 1490, 1514, 1548, 1564, 1626
Sunflower	**all**	msc	1062, 1102, 1202, 1232, 1272, 1370, 1416, 1466, 1526, 1588
	Rice	sgol@2-17-0+sgol@2-17-1	982, 1032, 1166, 1196, 1226, 1244, 1260, 1304, 1356, 1396, 1432, 1466, 1510, 1550, 1590
	F40	sgol@2-13-0	1122, 1274, 1306, 1414, 1458, 1500, 1516, 1546, 1576
	GF	msc	1160, 1230, 1290, 1406, 1484, 1554, 1614

Rice: rice syrup, F40: high-fructose content corn syrup, GF: self-made glucose-fructose syrup

5.2.2.3 Results of the electronic tongue

PCA results of the adulterated sunflower honey with the GF, rice and F40 syrup showed the separation of the control from the rest of the samples, but the other groups showed overlapping with each other.

Linear discriminant analysis of the electronic tongue data for the classification of adulteration level and syrup type

The linear discriminant analysis model built for the classification of all the groups provided the average training and validation accuracies of 91.13% and 37.77%, respectively after the threefold cross-validation **(Table 21).** During the training, the control sample was classified correctly and none of the samples showed overlapping with the control. However, in the validation the control showed misclassification as belonging to the 3% rice syrup-containing samples in 11%. During the validation also none of the samples were classified as a control, showing that the control could be separated from the adulterated samples. The detailed confusion table shows the misclassification for the other group. During the training the FS10% and RI3% sample was classified correctly, the others showed misclassification. In the validation dataset none of the samples were classified correctly, but none of the samples overlapped with the control. The method could identify the control with a good accuracy, but misclassifications were found among the different concentrations of the same syrup or were classified to another type of syrup.

Table 21 Confusion table of the LDA model built for the classification of the adulterated sunflower honey based on the electronic tongue data

		Control	**FS3%**	**FS5%**	**FS10%**	**GF3%**	**GF5%**	**GF10%**	**RI3%**	**RI5%**	**RI10%**
Average training 91.13%	**Control**	**100**	0	0	0	0	0	0	0	0	0
	FS3%	0	**88.98**	0	0	0	0	0	0	0	0
	FS5%	0	5.51	**77.83**	0	0	0	0	0	0	5.5
	FS10%	0	0	0	**100**	0	0	0	0	0	0
	GF3%	0	0	5.5	0	**88.83**	0	0	0	16.67	0
	GF5%	0	0	0	0	0	**94.5**	0	0	0	0
	GF10%	0	0	0	0	0	0	**83.33**	0	0	0
	RI3%	0	0	0	0	0	0	0	**100**	0	0
	RI5%	0	5.51	16.67	0	11.17	0	0	0	**83.33**	0
	RI10%	0	0	0	0	0	5.5	16.67	0	0	**94.5**
		Control	**FS3%**	**FS5%**	**FS10%**	**GF3%**	**GF5%**	**GF10%**	**RI3%**	**RI5%**	**RI10%**
Average validation 37.77%	**Control**	**89**	0	0	0	0	0	0	0	0	0
	FS3%	0	**0**	0	0	0	0	0	22.33	0	0
	FS5%	0	11.04	**22.33**	0	33.33	0	0	0	66.67	0
	FS10%	0	0	0	**44.33**	0	0	22.33	0	0	0
	GF3%	0	11.04	22.33	0	**0**	11	0	0	0	0
	GF5%	0	0	33.33	0	33.33	**55.67**	0	0	22.33	11
	GF10%	0	0	0	0	0	0	**11**	0	0	22.33
	RI3%	11	44.48	0	0	11	0	0	**77.67**	0	0
	RI5%	0	33.44	11	0	22.33	0	0	0	**11**	0
	RI10%	0	0	11	55.67	0	33.33	66.67	0	0	**66.67**

Columns represent the actual class membership (%) and the rows represent the predicted class membership (%)

FS – F40 HFCS syrup, RI – Rice syrup, GF – Self-made glucose-fructose syrup

The LDA models of the syrup that were built separately for the different syrups showed better results compared to the previous ones (**Figure 24**).

The model of the F40 syrup adulterated honeys provided the average recognition and prediction accuracies of 100% and 75% **(Figure 24 b)**. During the training all the samples were classified correctly, however, throughout the validation the control showed misclassification as belonging to the honey that contained 3% of the syrup in 11%. The FS3% honey showed misclassification as belonging to the FS5% honey in 44.33%. Similarly the FS5% honey could be classified correctly in 77.67% and the misclassification was found into the FS3% group. The FS10% also showed misclassification as belonging to the FS3% honey in 22.33%.

The rice syrup model also provided the average training accuracy of 100%, and better validation accuracy of 88.92% (**Figure 24 c**). During the training all the groups were classified correctly. In the validation dataset the control showed misclassification as belonging to the 3% adulterant in 11%. The 3% mixture was classified correctly but the RI5% showed misclassification as belonging to the RI10% in 22.33%, while the RI10% was misclassified as RI5% in 11%.

Similarly to the results of NIR the GF syrup detection was weaker than the F40 and rice syrup in the case of the sunflower honey. The average training and validation accuracies were 100% and 63.92% (**Figure 24 d**). Similarly to the other two syrups, the training provided correct classification for all the groups, but during the validation misclassification were found except the control, which was classified correctly. The GF3% showed correct classification of only 11% and misclassification were found as belonging to the GF5% in 66.67% and GF10% in 22.33%. The GF5% also showed misclassification as belonging to the GF3% in 11%. The GF10% showed the correct classification of 55.67%, where the misclassification was obtained as belonging to the GF5%.

In a Chinese study similar results were found, the classification accuracy of control sample was 97.74% which is slightly better than our results of 89%, but in our case the misclassification was found as belonging to the 3% honey which is a lower level than their lowest level of 5% (Gan *et al.*, 2016). However, in our case the accuracy of the models of the independent syrups were better.

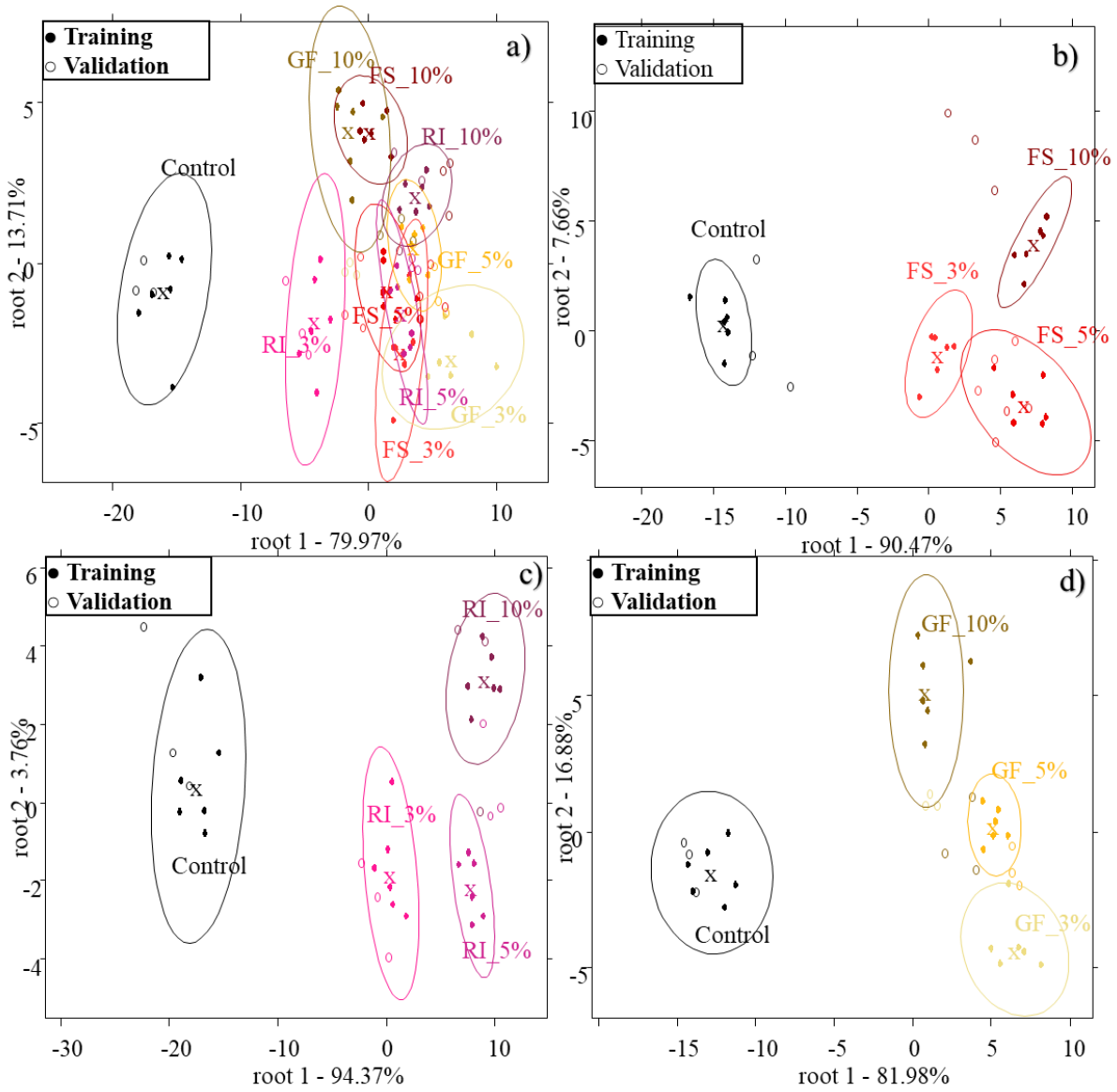

Figure 24. LDA score plots of the models built based on the electronic tongue data of the sugar syrup added sunflower honey a) model of honeys with all the syrups b) model of the honeys with F40 syrup, c) model of the honeys with rice syrup, d) model of the honeys with glucose-fructose syrup

These results are weaker than the classification accuracies during the NIR analysis, only in the case of the 3% adulterant was misclassifications found for the control sample that shows that the control can be distinguished from the adulterants higher than 3%.

Partial least squares regression

PLSR results after using leave-one-sample-out validation showed similar trends to the PLRS models of the NIR data with the exception that in this case the model built for all the syrups provided sufficient results: R^2C was 0.92 while R^2CV was 0.90 with RPD of 3.16. Moreover, in this case the error was somewhat above 1% (**Table 22**). The models of the different syrups showed the best prediction of F40, followed by the GF and RI syrup. GF syrup prediction was also better than in the case of NIR. The error of prediction after the validation was below 2% in

the case of all models and the RPD after the validation was satisfactory as it was in all the cases above 1.5.

The results of a Spanish study showed higher coefficient of determination for the prediction of added syrup brown rice syrup concentration R^2=0.95, but their RMSEP was higher 3.49%. The coefficient of determination of the corn syrup was also higher 0.99 but the RMSEP was also higher 2.662. However, it should be noted that in their study the syrup addition range was higher 2.5-40% (Sobrino-Gregorio *et al.*, 2018).

Table 22. Summary table of the PLSR built to regress on the added syrup concentration using electronic tongue data of sunflower honey

	NrLV	NrObs	R^2C	RMSEC	RPDC	R^2CV	RMSECV	RPDCV
All	4	68	0.92	0.91	3.62	0.90	1.04	3.16
F40	3	29	0.95	0.78	4.50	0.86	1.23	2.76
Rice	3	29	0.86	1.40	2.70	0.72	1.92	1.99
GF	1	36	0.86	1.37	2.69	0.76	1.77	2.06

FS – F40 HFCS syrup, RI – Rice syrup, GF – Self-made glucose-fructose syrup

5.3 Results of heat treatment study

In this section the results of the heat treatment study will be presented. This study was an extended version of a preliminary experiment during my MSc. studies (Bodor *et al.*, 2017; Bodor *et al.*, 2019b).

5.3.1 Results of reference methods of the heat treatment experiment

Moisture content

Similar trends were obtained for all the types of honey in the case of the reference methods (moisture, pH, electrical conductivity) (**Appendix Table 9**). Moisture content of the samples was between 17.6-18.2%, 16.3-17.3% and 16.3-16.8% in the case of the sunflower, bastard indigo, and acacia honey, respectively. Even though moisture content was different at the different heat treatment levels, no clear trend was seen, which can be due to the weak precision of the instrument. The lack of decreasing trend can be explained by the fact that the samples were treated in closed sample holder, not allowing the loss of moisture in the samples. Tosi *et al.* (Tosi *et al.*, 2004) found similar lack of the decreasing trend, while other studies found decrease in moisture content, but in those studies samples were not closed (Chua *et al.*, 2014). In another Hungarian study the moisture content also did not change with the progressing of heat treatment (Czipa *et al.*, 2019).

pH

The pH of the samples (**Appendix Table 9**) was in the acidic range because of the presence of organic acids in honey. The lowest pH range was found in the case of the sunflower honey (pH 3.71-4.11), then acacia (pH 4.21-4.50) and bastard indigo (pH 4.33-4.56). In the case of each

honey type increasing tendency of pH was observed, especially honey heated at 100 °C. The study of Zarei *et al.* (2019) also did not find significant change in the pH value of honey heated at 63°C for 30 minutes that are in accordance with our previous results (Bodor *et al.*, 2017) and the results of our current experiment. In another study of Hungarian researchers showed also slight increase with the rising of the temperature level (Czipa *et al.*, 2019). In contrary to our results, increasing pH was found in a Chinese study when acacia honey was heated at 80°C and 100°C for three and four hours, however, at higher temperature and for longer periods, a decrease of pH was observed that was explained by the formation of organic acids (levulinic, formic, lactic acid) and HMF (Zhang *et al.*, 2012).

Electrical conductivity

Electrical conductivity of the bastard indigo honey was the highest, in the range of 536.6-546.6 µS/cm (**Appendix Table 9**), followed by the sunflower samples (374.8-399.7 µS/cm), then the acacia honey (108-112 µS/cm). The electrical conductivity results also did not show any clear trend, such as in the case of the moisture content. Similar results were found by Hungarian researchers analyzing acacia honey (Czipa *et al.*, 2019).

Hydroxymethylfurfural (HMF)

Similar tendency was found regarding the HMF content of the samples in the case of all the honey types (**Table 23**), where an increasing tendency was obtained with the increasing temperature level. The different honey types had different initial HMF contents: acacia 7.0±0.4 mg/kg, bastard indigo 14.7±1.6 mg/kg, and sunflower 18.5±0.3 mg/kg. These fulfilled the requirement of the legislations demanding that the HMF content of the honey samples (from non-tropic regions) should be below 40 mg/kg (The European Council, 2001). This limit was reached at different heating levels of the different honey types: in case of the acacia and bastard indigo honeys this limit was above honeys heated at 100°C for 120 minutes, with HMF content of 44.7±4.3 mg/kg and 81.4±4.0 mg/kg, respectively. While the honeys heated at 80°C for 240 minute reached this value in the case of the sunflower honey (52.0±2.7 mg/kg).

The ANOVA model built to compare if the different levels are significantly higher compared to the HMF content of the control unheated honeys showed similar results in the case of the sunflower and acacia honey. None of the heat treatment levels of 40°C or 60°C showed significantly higher value compared to the control, the same applies for the honeys heated at 80°C for 60 minutes. All the other levels were significantly different. In the case of the bastard indigo honey only the levels of the 100°C treatment for two or more hours showed significantly higher values compared to the control. Our results are similar to the study of Cozmuta *et al.* (2011), who found also more intense increase at 100°C than in the case of the honey sample heated at 50°C or 80°C. Moreover, Turkish researchers found similar results to ours (Turhan *et*

al., 2008). Moreover, results of Bogdanov (1992) were supported by our results as in our case the 40 mg/kg was not reached at 40 °C and 60°C during the heating period (240 minutes).

Based on the results of the two-way ANOVA built to check if there is a significant effect ($p<0.05$) of heat treatment temperature, time, or their interaction on the HMF content of the heated honeys showed that all of them have a significant effect in the case of all the honey types. Because of the significant interaction, the significant differences among time levels were analyzed by temperature groups and differences among temperature levels were analyzed by time groups:

- the analysis of the honeys heated at 40°C did not show significant difference between the different time intervals.
- honeys heated at 60°C showed different results for the three types of honey. In the case of the acacia no significant difference was found between the time intervals. For the sunflower and bastard indigo honey the trends are not clear.
- In contrary to this, in the case of honeys heated at 80°C, results showed a clear increasing trend with the elevation of the time interval for all the types. In the case of the sunflower and bastard indigo honey significant difference was found between all the treatment times, while in the case of the acacia honey the honeys heated for two and three hours did not show significant difference.
    - The results of the acacia and sunflower honeys heated for one hour showed that honey heated at 100°C showed significantly higher HMF content compared to lower temperature levels. In the case of the bastard indigo honey the HMF content of the honey heated at 80°C was significantly lower compared to the 60°C heated honey. This unexpected result can be due to the limit of sensitivity of the measurement.
    - Honeys heated for 120 minutes showed different results in the case of the three honey types. Significant difference was found between all the temperature levels for the sunflower honey. No significant difference was obtained between the 40°C and 60°C for acacia honey, while the higher temperature levels showed significantly different values compared to all the other temperature levels. Bastard indigo samples had significantly higher HMF content in the case of the honey heated at 100°C compared to lower levels.
    - Honeys heated for 180°C minutes showed that in the case of the sunflower honey the samples heated at 80°C and 100°C had significantly different HMF content compared to the 40°C and 60°C and compared to each other. While acacia and bastard indigo honey had the same trend that they had in the case of the 120 minutes.

- Honeys of the 240 minutes-group showed similar trends for all the honey types: in this case only the honeys of the 80°C and 100°C had significantly different values compared to the others.

Based on these results it can be seen that in some cases there are differences between the honey types that can be the result of the different physicochemical composition with special regard to the sugars and pH and amino acid content that have their role in the Maillard reaction. Studies reported that higher fructose content and higher glucose/fructose ratio accelerate the formation of HMF, especially at pH 4.6 (Shapla *et al.*, 2018). This is in line with our results as it is well known that acacia, has higher glucose/fructose ratio than the sunflower and the relative HMF formation compared to control at the higher temperatures (80°C and 100°C) was stronger in acacia honey. Furthermore, it has been also reported that the free acidity, and total acidity is in high correlation with the HMF formation, however moderate correlation was also fund with the pH and lactones (Shapla *et al.*, 2018).

Table 23. Results of the HMF content of the honey samples of the heat treatment experiment (adapted from Bodor *et al.*, 2022)

Hydroxymethylfurfural content, mg/kg						
		control	**40°C**	**60°C**	**80°C**	**100°C**
Sunflower	**control**	**18.5±0.3**				
	60 min		20.2 ± 1.5^{aA}	16.2 ± 1.0^{aA}	17.6 ± 0.2^{aA}	40.3 ± 0.8^{aB}*
	120 min		17.3 ± 1.3^{aA}	20.5 ± 0.7^{bB}	31.8 ± 1.3^{bC}*	155.1 ± 2.7^{bD}*
	180 min		18.4 ± 1.6^{aA}	19.9 ± 1.8^{bA}	37.2 ± 0.6^{cB}*	241.5 ± 7.4^{cC}*
	240 min		17.5 ± 1.4^{aA}	19.5 ± 2.0^{abA}	52.0 ± 2.7^{dB}*	463.6 ± 28.3^{dC}*
Bastard indigo*	**control**	**14.7±1.6**				
	60 min		14.1 ± 2.8^{aAB}	18 ± 2.3^{abB}	11.9 ± 1.1^{aA}	16.7 ± 0.9^{aAB}
	120 min		15.1 ± 3.5^{aA}	15.8 ± 0.6^{abA}	14.3 ± 1.0^{bA}	81.4 ± 4.0^{bB}*
	180 min		15.7 ± 1.1^{aA}	21.1 ± 3.5^{bA}	19.8 ± 0.6^{cA}	146.4 ± 2.3^{cB}*
	240 min		12.9 ± 1.4^{aA}	13.7 ± 1.3^{aA}	28.2 ± 1.1^{dB}	306 ± 17.8^{dC}*
Acacia	**control**	**7.0±0.4**				
	60 min		9.1 ± 1.3^{aA}	7.7 ± 0.3^{aA}	8.0 ± 0.4^{aA}	16.1 ± 1.7^{aB}*
	120 min		8.0 ± 0.6^{aA}	8.8 ± 1.4^{aA}	13.3 ± 0.9^{bB}*	44.7 ± 4.3^{bC}*
	180 min		8.6 ± 1.0^{aA}	9.6 ± 0.3^{aA}	12.2 ± 0.8^{bB}*	89.1 ± 2.8^{cC}*
	240 min		10.0 ± 1.1^{aA}	9.6 ± 0.9^{aA}	18.8 ± 2.4^{cB}*	211.6 ± 5.0^{dC}*

Letters are representing the significant differences between the samples based on the results of ANOVA test and pair wised comparison $p<0.05$: lowercase stand for the differences between time intervals within a temperature levels, capitals are for the differences between temperature levels within time intervals, * are for the significantly different level compared to the control sample

5.3.2 Results of color analysis of the heat treatment experiment

Similar tendency was obtained for the three types of honeys regarding their color parameters (**Figure 25**). The tendency of changes of L* value was similar in the case of all honey types.

After the heat treatment the honeys of bastard indigo and sunflower type heated at 40°C and the sunflower honey heated at 60°C for one hour showed low values, which can be due to the fact that even after the heat treatment these honeys were in crystallized form. This crystallization can influence the results of L* because of the scattering of the light, therefore the color of honey should be determined in liquid form if we want to keep the good practice. However, b* and a* values seem to be not influenced by the crystals.

The results of the L* parameter showed that honeys heated at 60°C show an increasing tendency of the L* until the three hours of treatment Honeys heated at 60°C showed increasing tendency until 180 minutes-treatment, however samples heated for 240 minutes showed lower L* (darkening) in the case of the sunflower and bastard indigo honey, but the lightening (higher L*) was observed still for at the fourth hour for the acacia honey. The lightening (higher L* value) of the samples during the heating process can be the consequence of the morphological change of the micro-crystal-structure of the honeys. Besides, the darkening is the result of the Maillard reaction and caramelization of sugars (Turkmen *et al.*, 2006; Csóka *et al.*, 2014; Kędzierska-Matysek *et al.*, 2016)

The honeys heated at 80°C resulted in different trends for the three types. Decreasing tendency was found with the elevation of the heat treatment of the sunflower, acacia, and bastard indigo honeys with the exception of the sunflower honeys heated for two hours as they had higher L* compared to the sunflower honey heated for one hour.

All the types of honeys of the 100°C temperature level showed more intense decrease with the increase of the time interval.

Similar tendency of the changes in a* and b* was found in the case of the bastard indigo and sunflower honey. The a* showed an increasing trend with the elevation of the heat treatment level and with the increase of the time. In this case also the 100°C treatment caused the most extreme increase. In the case of the b* similar tendency was found with some exceptions such as honeys heated for four hours at 100°C resulted in lower b* values compared to honeys heated at 120 and 180 minutes in the case of the bastard indigo and sunflower honeys.

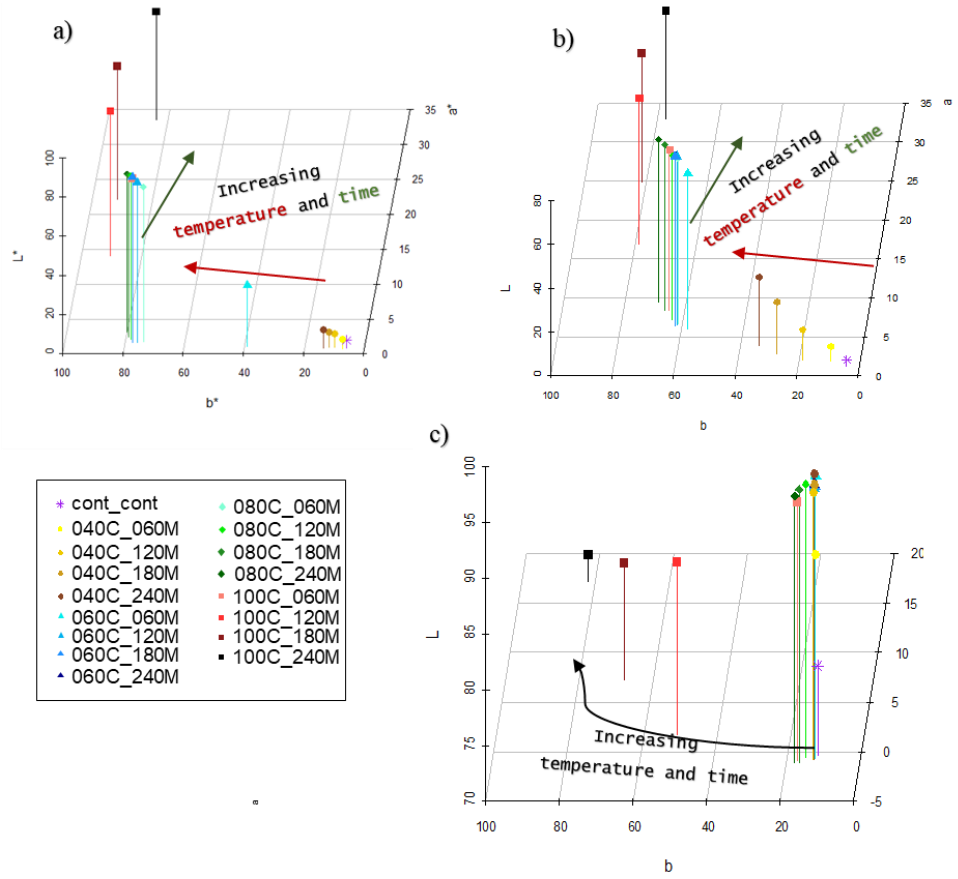

Figure 25 Results of the color measurement of the heat treated samples a) L*a*b* values of the sunflower honey b) L*a*b* values of the bastard indigo honey c) L*a*b* values of the acacia honey n = 5

5.3.3 Results of NIR of the heat treatment experiment

Near infrared spectroscopy results were different in the case of the three honey types. Principal component analysis results showed a tendency of separation according to the heat treatment level mainly through the first PC in the case of the sunflower and bastard indigo honey, but for acacia this trend could not be seen. PC1 described the variance of the data in 99.419%, 99.177%, and 94.666% in the case of the sunflower, bastard indigo, and acacia honeys. The trend according to the temperature was more visible, than according to the time. Figures are not presented here, as the thesis focuses on the discrimination accuracies.

5.3.3.1 Results of the PCA-linear discriminant analysis of NIR of heat treatment experiment

In the case of the honey types similar trends were found regarding the models such as in the case of the electronic tongue, where the time interval classifications were weaker than the temperature and heat treatment level models.

Sunflower

PCA-LDA model built for the classification of the temperature, time and treatment level provided the average classification (validation) accuracy of 84.01%, 62.83% and 80.81%, respectively.

Temperature classification model showed that the control honey was classified correctly even after the validation (**Figure 26 a**), however the 40°C-group showed less than 3% misclassification as belonging to the control. The higher temperature groups (60°C, 80°C, 100°C) could be completely separated from the control honey but showed misclassifications as belonging to each other.

The model of the time intervals showed correct classification of the control (**Figure 26 b**), but the 60 minutes treated sample group showed misclassification as belonging to the control in 2.5% and 5.01%, during the training and validation, respectively. The group of samples treated for longer time intervals did not overlap with the control group.

The classification model of the heat treatment level group (**Figure 26 c**) showed that the control was classified correctly during (**Appendix Table 10**) both training and validation, moreover, no misclassification was found belonging to the control. At higher levels, especially above 60°C and 60 minutes, there were misclassifications.

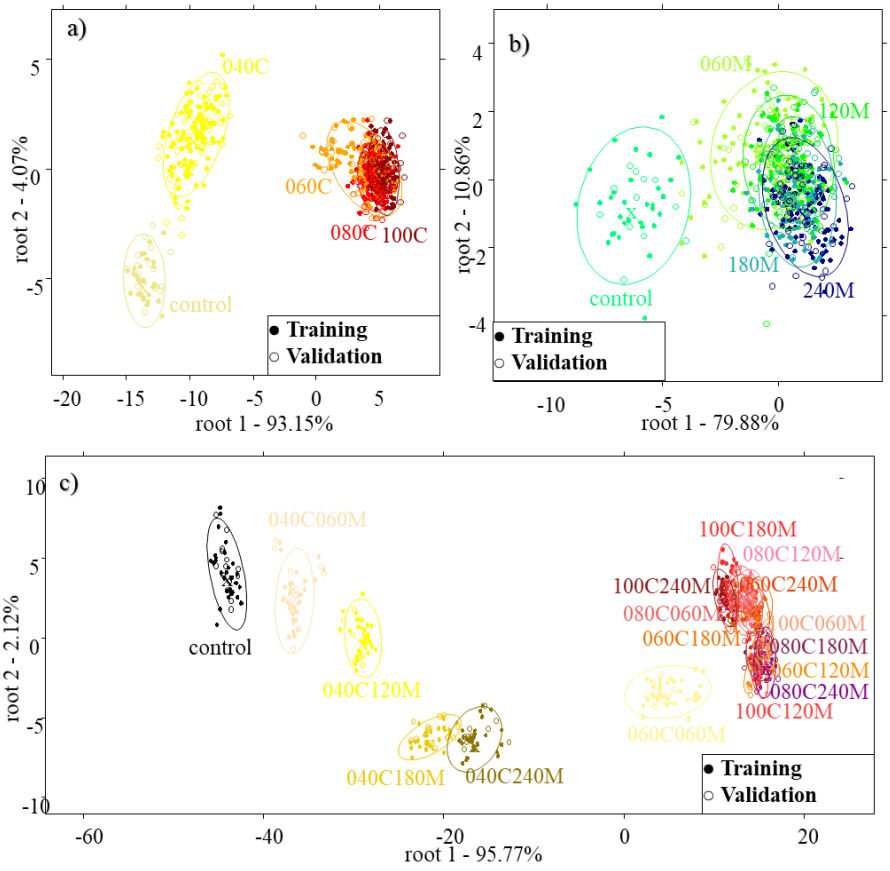

Figure 26. PCA-LDA score plot of the NIR data of sunflower honey built for the classification of a) temperature level, b) time interval and c) heat treatment level

Bastard indigo

The models of the bastard indigo were slightly different from the models of the sunflower honey. The temperature model showed average validation accuracy of 84.93%, the time model provided results of 60.93% and for the level 74.93% average validation accuracy was obtained.

The model built for the classification of the temperature levels showed 100% correct classification of the control honey, and none of the groups showed misclassification as belonging to the control (**Figure 27 a**). However, at higher temperature levels the sample groups showed misclassification as belonging to each other.

The time level classification was weaker, however the control honey showed 100% correct classification (**Figure 27 b**), and all the other honeys showed misclassification as belonging to each other, but none of the groups were misclassified belonging to the control.

The classification model (**Appendix Table 11**) of the treatment levels provided 100% correct classification of the control (**Figure 27 c**) honey and none of the other treatment groups were misclassified as belonging to it. At higher levels, especially in the case of the levels of the 60°C, 80°C and 100°C groups numerous misclassifications were found.

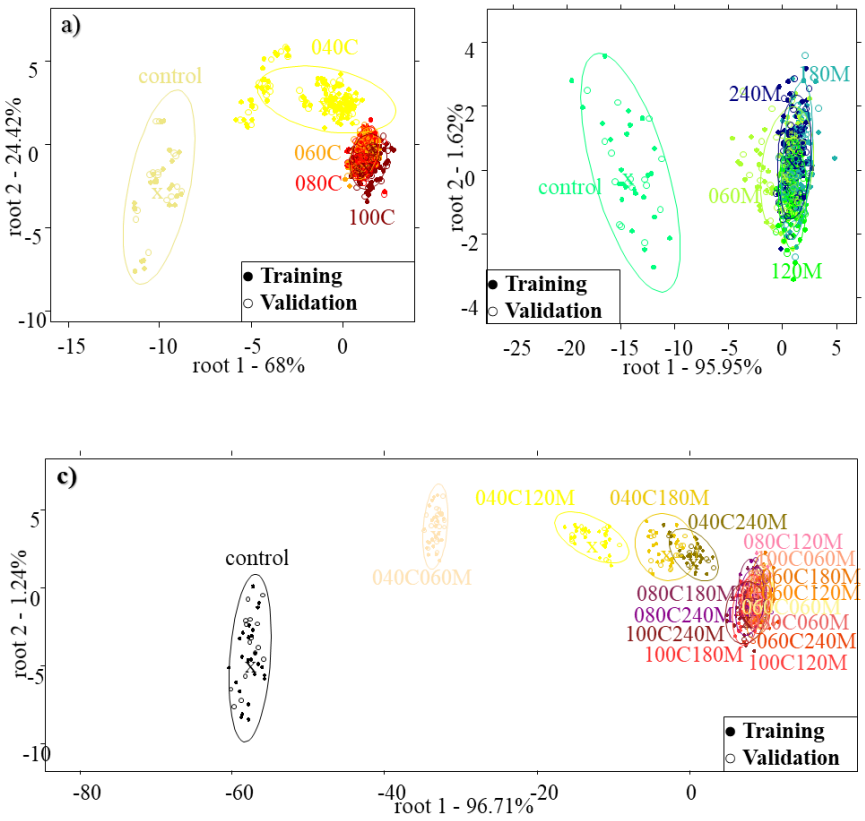

Figure 27. LDA score plot of the NIR data of bastard indigo honey built for the classification of a) temperature level, b) time interval and c) heat treatment level

Acacia

The results of the acacia honey were worse than the other two types. The model of the temperature, time, and the level average correct classification level was 71.71%, 55.59% and 70.56%, respectively

The temperature classification model showed correct classification of the control in 94.79%, but misclassification was found as belonging to the 40°C in 2.61% and to the 60°C in 2.61% (**Figure 28 a**). The 40°C, 60°C, 80°C and 100°C also showed misclassification as belonging to

the control in 4.02%, 0.62%, 2.01% and 0.75%. Furthermore, all the other groups showed misclassifications as belonging to each other.

The model of the time intervals showed the classification accuracy of 84.28% where the misclassification was found as belonging to the 60 minutes, 180 minutes, and 240 minutes in 10.51%, 2.61% and 2.61%, respectively (**Figure 28 b**). Moreover, the group of the 60 minutes and 240 minutes showed 3.82% and 1.32% as belonging to the control, respectively. All the time intervals showed misclassification as belonging to each other. This shows, as in the case of the two other types, that the effect of the temperature is bigger than the effect of the time interval.

The LDA model built for the classification (**Appendix Table 12**) of the heat treatment levels showed that control honey could be classified correctly in 89.49% after the validation, where the misclassifications were belonging to the 040C060M in 2.61%, to the 060180M in 5.2%, and to the 080C060M in 2.61%. The levels of the 040C060M and 060C180M showed misclassification also as belonging to the control in 7.69% and 5.29%, respectively (**Figure 28 c**).

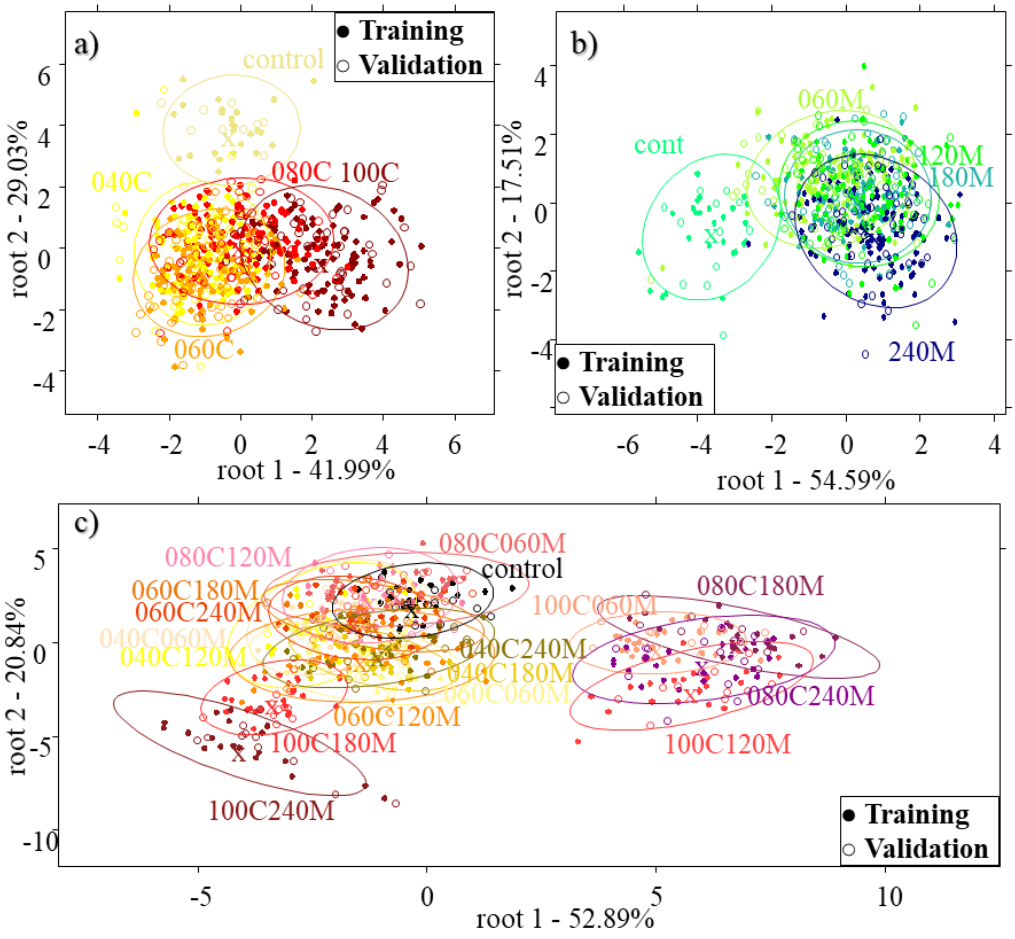

Figure 28. PCA-LDA score plot of the NIR data of acacia honey built for the classification of a) temperature level, b) time interval and c) heat treatment level

All the NIR models showed that the type of the honey has an effect on the results, moreover, the 40°C could be detected in the case of the sunflower and bastard indigo honeys. For acacia worse results were obtained, the 60°C could not be completely discriminated from the control.

Moreover, it can be seen that the honeys from the different crystallization states behaved differently, that can be due to both chemical and physical structure. Similarly, different trends were found in an Italian study who analyzed honeys from three different stages of crystallization at 39°C for 31 minutes and 55°C for 24 hours. Similarly to our results, in an Italian study the higher temperature affected the spectra of the samples much more than the lower level of heat treatment (Segato *et al.*, 2019).

5.3.4 Results of electronic tongue of the heat treatment experiment

Results obtained during the electronic tongue measurement showed different results for the three types of honey. Principal component analysis results showed a tendency of separation according to the heat treatment temperature mainly through the first PC. PC1 described the variance of the data in 63.89%, 73.27%, and 74.59% in the case of the acacia, bastard indigo, and sunflower honeys. Though, the trends according to the time level did not show a clear tendency. This shows that the effect of the temperature was more significant.

5.3.4.1 Results of the linear discriminant analysis

The higher effect of temperature was also proven by the results of the LDA after the threefold-cross validation. where higher classification accuracy was found related to the temperature levels than to for the time levels.

Sunflower

LDA model built for the classification of the temperature, time and treatment level provided the average classification accuracy after the validation of 84.28%, 54.10% and 67.20%, respectively

The model built for the temperature classification showed that the control honey was classified correctly in 96.46% and 85.65% during the training and validation, where misclassification was found as belonging to the 40°C (**Figure 29 a**). The higher temperature groups could be completely separated from the control honey.

The model of the time intervals showed correct classification of the control, however all the other groups showed misclassifications as belonging to each other, therefore the effect of the temperature was higher (**Figure 29 b**).

The classification model (**Appendix Table 13**) of the heat treatment level group showed that the control was classified correctly during the training but the through the validation misclassification was found in 7.08% as belonging to the group heated at 40 °C for 180 minutes sample (**Figure 29 c**).

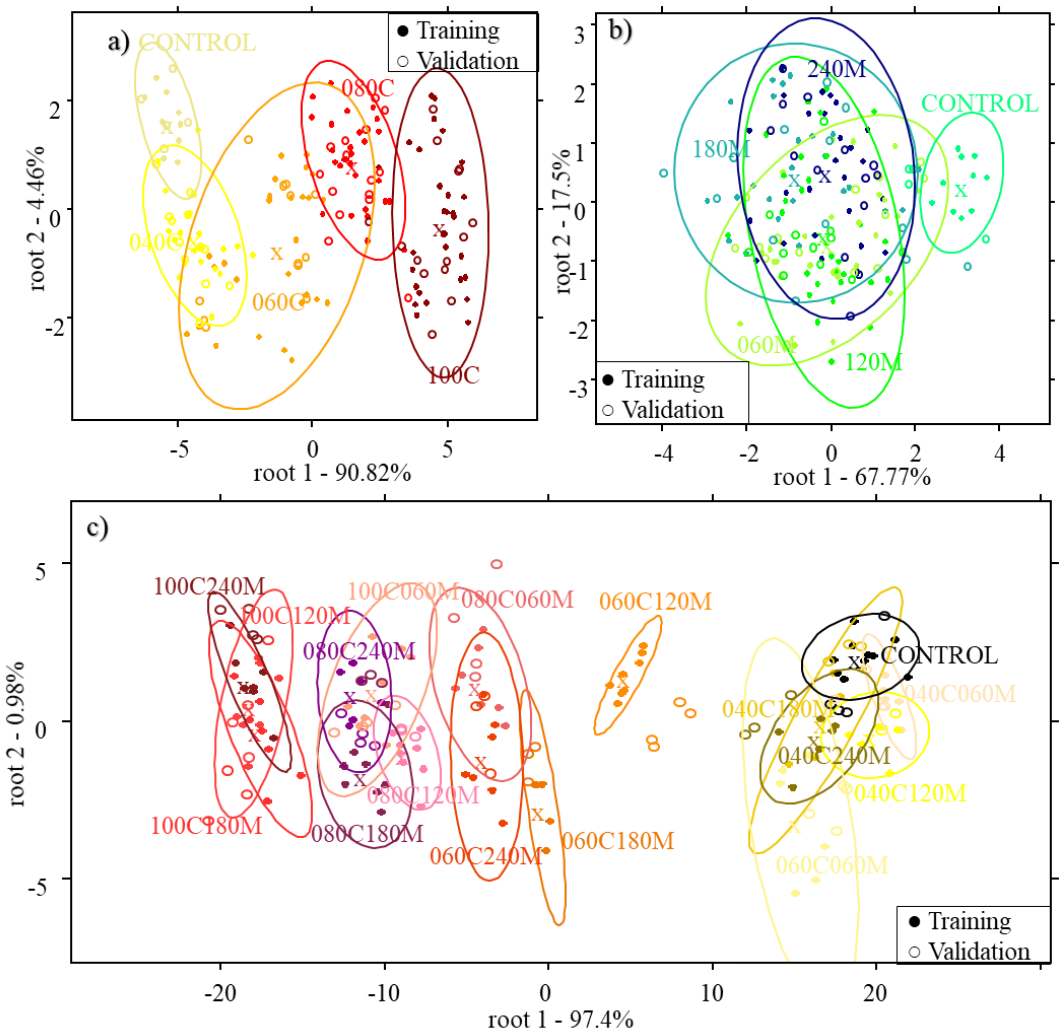

Figure 29. LDA score plot of the electronic tongue data of sunflower honey built for the classification of a) temperature level, b) time interval and c) heat treatment level

Bastard indigo

The models of the bastard indigo were different from the models of the sunflower honey, where the temperature model showed average validation accuracy of 75.57%, the time model provided worse results of 30.29% and for the level 54.43% average validation accuracy was obtained.

Model of the temperature classification showed that after the validation the control was classified correctly in 85.65%, and the misclassification was belonging to the 40°C group. The other honeys did not show misclassification as belonging to the control with the exception of 40°C where the misclassification was 9.28% during the validation (**Figure 30 a**).

The time level classification was very weak, where the control honey showed 56.17% (misclassified to 60 minutes). The other time groups also showed misclassification as belonging to the control during the validation, where the 60 minute samples showed 15.54%, the 120 2.2% and the 180 minutes 2.36% of misclassification (**Figure 30 b**).

The model of the heat treatment levels provided the 85.65% of correct classification (**Appendix Table 14**) of the control, the misclassification was found as belonging to the honey of AF040C060M. Moreover the AF040C060M also showed misclassification as belonging to the control in 8.25% throughout the validation (**Figure 30 c**).

These results show also that only the 40°C (allowed) heat treatment level could not be discriminated from the control, which shows the power of electronic tongue in the detection of the heat treatment.

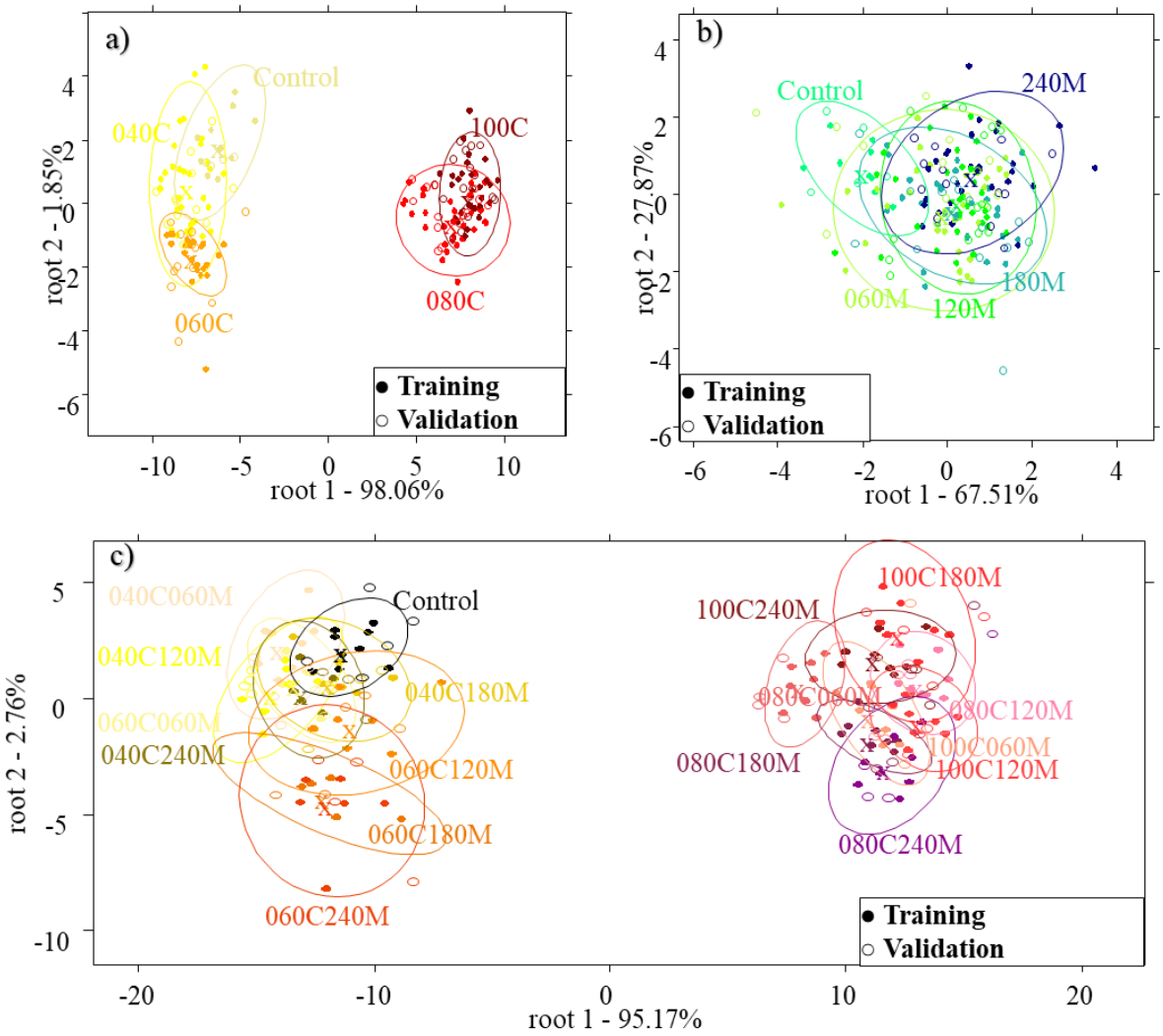

Figure 30. LDA score plot of the electronic tongue data of bastard indigo honey built for the classification of a) temperature level, b) time interval and c) heat treatment level

Acacia

The results of the acacia honey were the worst among the three honey types. In this case the model of the temperature, time, and level classification after the validation was 72.83%, 35.41% and 58.67%, respectively.

The temperature classification model showed correct classification of the control in 31.33% only, where misclassification was found as belonging to the 40°C in 62.48% and to the 60°C in 6.19%. The 40°C also showed misclassification as belonging to the control in 20.56%. All the other groups could be discriminated correctly (**Figure 31 a**).

The model of the time intervals showed the classification accuracy of 56.29%, where the misclassification was found as belonging to the 60 minutes and 240 minutes in 37.52% and 6.19%, respectively. All the other groups showed misclassification as belonging to the control below 13%, with the exception of honeys heated for 120 minutes (**Figure 31** b).

The LDA model built for the classification (**Appendix Table 15**) of the heat treatment levels showed that control honey could be classified correctly only in 43.71%, where the misclassifications were belonging to the 40°C honeys: 040C060M in 6.19%, 040C120M in 12.57%, 040C180M in 31.33% and 040C240M in 6.19%. The levels of the 040C060M and 040C180M showed misclassification also as belonging to the control in 9.91% and 66.67%, respectively (**Figure 31 c**). This worse result of the acacia honey could originate from its weaker aroma content, which reflects in ET results (Oddo and Piro, 2004).

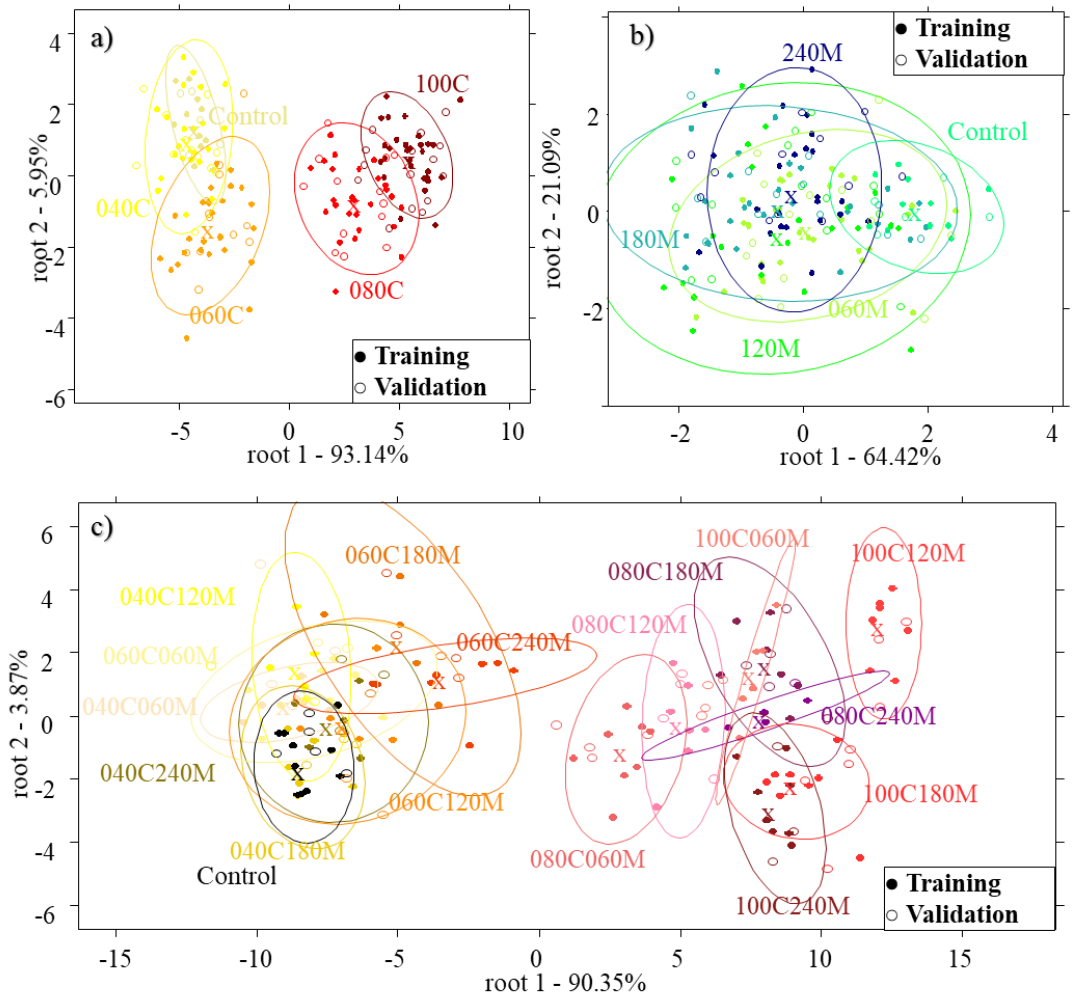

Figure 31. LDA score plot of the electronic tongue data of acacia honey built for the classification of a) temperature level, b) time interval and c) heat treatment level

Summarizing we can see that the results of the electronic tongue was worse than the results obtained for the NIR. However, these results also show that even at 40°C and 60°C there were observable changes in the composition and the taste of honey, which was not observed by the HMF content. These results, moreover, new, as electronic tongue has not been used before for the detection of heat treatment of honey.

6 CONCLUSION AND RECOMMENDATIONS

The thesis focused on the origin identification, adulteration and heat treatment detection of Hungarian honey using reference parameters (physicochemical parameters such as moisture content, electrical conductivity pH, ash content, antioxidant properties – TPC, CUPRAC, FRAP, and color – L*a*b*, sugars, HMF), melissopalynology, and correlative (electronic tongue, and NIR) methods.

Along the basic botanical and geographical origin identification study the descriptive tables of eight unifloral honeys (acacia, rape, bastard indigo, milkweed, sweet chestnut, linden, sunflower, honeydew) and multiflora honeys were prepared including the aforementioned reference methods and the melissopalynology. The results showed that there are significant differences among the unifloral honeys in most of the parameters. Acacia, rape could be characterized by lower ELC, antioxidant power and TPC, and ash content. Acacia honey was also significantly lighter than chestnut, linden, sunflower, and rape. Regarding the color, the chestnut honey was more reddish than acacia, rape, bastard indigo, and linden. Sunflower honeys were more yellowish than the acacia, rape, chestnut, and bastard indigo. In the future the expansion of this database would be useful to have continuous data from the different years.

Alpha Astree potentiometric electronic tongue was used for the botanical and geographical origin identification of the aforementioned honey types. When all the nine honey types were analyzed together the average prediction and recognition abilities of the LDA model was 57.09 % after threefold cross-validation. The high scatter of the honeydew and multiflora honeys could cause this weak classification accuracy. When these honey types left out from the model the classification accuracy was 70.51%. The chestnut honey was classified correctly, while the acacia, rape, and bastard indigo honeys grouped together, providing misclassification belonging to each other. The LDA models using the ET data provided ~59% validation accuracy, where the honeys collected in Alföld obtained the best correct classification of >97%. The reason of the weak model could be the higher effect of the botanical origin. Therefore, the models were analyzed separately for the six honey types and the counties (*megye*), or districts (*járás*) were used as group variables. These models provided better classification accuracies ranging from 70.68%-100% average classification accuracy.

The origin identification study extended with pollen analysis showed that the pollen spectra of the honeys contribute highly to the separation of the botanical groups, where >90% classification accuracy was obtained for the acacia, bastard indigo, rape, chestnut, sunflower, and linden honeys. The geographical origin identification model was not as accurate (~52% classification accuracy), as the botanical model (90.22%). This could be again because of the higher effect of

the botanical origin. Benchtop MetriNIR spectrometer (operating in 740-1700 nm with 2 nm step) did not provide satisfactory results for the geographical and botanical origin classification (~58% classification accuracies). This shows that the NIR all alone cannot be used with good accuracy, therefore in the future it would be worth to check the applicability of an instrument operating in the whole NIR range. Nevertheless, the fusion model of the NIR, melissopalynology and physicochemical properties (pH, EC, moisture) provided higher than 99% classification accuracy. From this we can conclude that the combination of these techniques was more reliable.

In the authenticity study authentic acacia, linden honeys, their blends with syrup in 10%, 20%, and 50%, and EUnonEU blend honeys were analyzed using the reference methods and electronic tongue and sensory profile analysis. The results showed that in the case of the sensory profile analysis the panel could discriminate the adulterated honey from the authentic in four parameters, while in the case of the linden eight parameters showed significant difference. Moreover, the electronic tongue showed that the control sample could be separated completely from the adulterated samples in the case of linden honey. In the case of acacia, the pure honey showed misclassification belonging to the 10% adulterated honey in 3.11%.

As a continuation of the authenticity study linden honey was mixed with rice and beet syrup in the range of 0.5-50%. As extension of this sunflower, linden, acacia, rape, and honeydew honeys were mixed with the high fructose content sugar syrup (F40), rice syrup, and glucose-fructose (self-made) syrup in 3%, 5% and 10%. The samples were analyzed using the benchtop NIR instrument and the sunflower honeys were analyzed with the electronic tongue as well. The electronic tongue results showed that during the validation the LDA model did not provide good accuracy analyzing all the syrups in one model, however if the models were built separately for the syrups, where the control honey was only misclassified with the 3% adulterated group. NIR models were able to differentiate the control from the honeys mixed with 1% syrup in the linden honeys that were mixed with the beet and rice syrup. Moreover, the PLSR models provided >0.99 R^2CV and RPD higher than 17 in these models using leave-one-sample-out cross validation. In the sugar syrup adulteration study extended with lower concentrations acacia, the PCA-LDA models provided in all the cases higher than 94% classification accuracy of the total sample set but, 100% classification accuracy of control. The PLSR models provided higher than 0.94 R^2CV with higher than RPDCV of 4 of acacia, in the case of linden the R^2CV was above 0.80 and RPCV > 2.2. The models parameters of the honeydew honeys were the following: R^2CV ranged between 0.71-0.95 and RPDCV between 1.89 and 6.20. The results of the rape and sunflower honey were weaker: in the case of the honeys blended with GF syrup and the models including all the syrup types were the worst of 0.39-0.68 R^2CV, and the RPDCV was below two. Models of rape and sunflower honeys adulterated with the rice and F40 syrups were better:

R^2CV ranged between 0.88-0.96 and RPCV above 2.9. These results show that the sugar syrup (GF) that was prepared the way to have similar F/G ratio to honey provided the worst models, however the rice and F40 syrups could be predicted and discriminated with high accuracy. In the future it would be useful to expand this database with honeys from different regions from the same type using the syrups.

The heat treatment experiment was performed on acacia, bastard indigo, and sunflower honeys, where the honeys were heated at 40, 60, 80, and 100 °C for 60, 120, 180, and 240 minutes. The samples were analyzed using reference methods such as moisture, pH, electrical conductivity, color and HMF content, and the correlative NIR and electronic tongue. The results showed that in the case of the HMF the honeys heated at 40°C and 60°C could not be discriminated significantly from the control, and the limits of the 40 mg/kg were reached only at 80°C or 100°C, depending on the honey type. In contrary, both electronic tongue and NIR could show differences even at the lowest temperature 40°C in the case of the sunflower and bastard indigo honey, however the models of acacia honey did not provide convincing results (misclassification were found belonging to the 40°C and 60°C groups, moreover, the model parameters were weaker). The reason of this could be that the acacia is less rich in useful components such as minerals, antioxidants, moreover, the sensory characteristics and aroma of this honey is also weaker. NIR was even more accurate comparing with the electronic tongue.

In summary we can conclude that both NIR and electronic tongue could be efficient tools in the origin identification, adulteration, and heat treatment detection (of sunflower and bastard indigo honeys). In the future it would be useful to analyze the sample with both instruments and use these as a fused dataset.

7 NEW SCIENTIFIC RESULTS

For the purpose of these new scientific findings, benchtop spectrophotometer refers to MetriNIR (MetriNIR, Research Development and Service Co., Budapest, Hungary), whereas handheld spectrophotometer refers to NIR-S-G1 (InnoSpectra Co., Hsinchu, Taiwan). E-tongue refers to the Alpha Astree potentiometric electronic tongue (AlphaM.O.S, Toulouse, France) equipped with seven sensors developed for food application (BB, HA, ZZ, GA CA, JE, JB), a reference electrode and a 16-position autosampler. Pollen spectra obtained with melissopalynological using acetolysis method. Sensory profile analysis was performed according to the requirements of International Organization for Standardization (ISO) standards (ISO, 1994, 2003, 2007).

7.1 New scientific findings focusing on botanical and geographical origin identification

1) Reference database was established containing physicochemical parameters (moisture, pH, electrical conductivity, ash content, color (L*a*b*), antioxidant parameters) of 137 Hungarian honey from the most common nine botanical types (acacia, sunflower, linden, chestnut, milkweed, honeydew, rape, bastard indigo, and multiflora) originating from all regions of Hungary (Északi-középhegység, Dunántúli-középhegység, Alföld, Kisalföld, Dunántúli-dombság and Nyugat-magyarországi-peremvidék), and collected mainly between 2015-2020. This database can be used as reference for the authentication of common Hungarian honey types.

2) Pollen spectra based database of 87 Hungarian honey from the most common eight botanical types (acacia, sunflower, linden, chestnut, milkweed, honeydew, rape, bastard indigo) from all regions of Hungary (Északi-középhegység, Dunántúli-középhegység, Alföld, Kisalföld, Dunántúli-dombság and Nyugat-magyarországi-peremvidék), collected between 2015-2020 was established.

3) PCA-LDA models were built for the first time using the low-level data fusion of physicochemical data (pH, moisture, electrical conductivity) pollen spectra, and NIR spectra for the botanical and geographical origin identification of Hungarian authentic honeys from eight botanical origin (acacia, linden, sunflower, chestnut, honeydew, milkweed, sunflower, bastard indigo), and all regions of Hungary (Északi-középhegység, Dunántúli-középhegység, Alföld, Kisalföld, Dunántúli-dombság and Nyugat-magyarországi-peremvidék) collected between 2015 and 2020. The models provided high classification accuracies for botanical and geographical origin identification (99.30% for botanical origin, 100% for geographical origin identification in cross validation).

4) Electronic tongue was used for the first time for botanical and geographical origin identification of 50 Hungarian authentic honeys from nine botanical origins (acacia, linden,

sunflower, chestnut, honeydew, milkweed, sunflower, bastard indigo, multiflora), and from four regions of Hungary (Északi-középhegység, Alföld, Dunántúli-dombság and Nyugat-magyarországi-peremvidék) collected between 2012 and 2016 (mostly from 2015). LDA models provided better classification accuracies for botanical origin identification (70.91% and 70.51% in training and validation, respectively, after excluding the data of honeydew, milkweed, and multiflora honeys), than for the geographical origin identification (59.46% and 59.40% in training and validation accuracy, respectively). Improved accuracies were obtained for the geographical origin identification analyzing the botanical groups separately (training accuracies ranged between 71.85 and 100%, and validation accuracies between 70.68 and 100%).

7.2 New scientific findings focusing on sugar syrup adulteration detection

5) Sensory profile analysis was applied on Hungarian authentic honeys (acacia and linden honeys collected in 2016 from Heves and Pest counties) and their blends with sugar syrup (honeys mixed with sugar syrup at 10%, 20% and 50%). Significant differences were found between the authentic honeys and their blends with 10% sugar syrup in three (fruity odor, sweet and flowery taste), and in five (odor and taste intensity, fresh odor, sweet taste, and aftertaste persistence) sensory parameters for acacia and linden honeys, respectively.

6) Electronic tongue was used for the first time for sugar syrup adulteration detection of Hungarian authentic sunflower honey mixed with different sugar syrups at different levels (rice syrup, F40 – high fructose content sugar syrup, and self-made glucose-fructose syrup (80% of 40/60 glucose/fructose +20% water) each applied at 3%, 5% and 10%, separately). Electronic tongue combined with LDA was able to discriminate the adulterated honeys from the authentic honeys with 100% accuracy and provided misclassification of the authentic honey belonging to samples containing 3% sugar syrup (in 11% belonging to 3% rice syrup and F40 syrup, respectively).

7) Benchtop spectrophotometer was used for the first time for sugar syrup adulteration detection of Hungarian authentic honeys from five botanical origins (acacia, linden, sunflower, rape, honeydew) mixed with different sugar syrups at different concentrations (each honey was mixed with rice and F40 syrups, and rape, sunflower and honeydew honeys were mixed with self-made glucose-fructose syrup (80% of 40/60 glucose/fructose +20% water) in 3%, 5% and 10%, respectively). PCA-LDA models of all the honey types for all the different model variations (models including all the syrups, or syrups separately) provided the complete discrimination of the adulterated and pure honeys (100% classification accuracy was obtained for the control in all the cases). In this regard honeys could be clearly discriminated from the 3% mixtures.

7.3 New scientific findings of the heat treatment study

8) Handheld near infrared spectrophotometer was applied for the first time for the detection of heat treatment (heated at 40°C, 60°C, 80°C, or 100°C, and at each hold for 60, 120, 180, or 240 minutes) of Hungarian authentic honeys (sunflower, bastard indigo honeys). PCA-LDA models of the classification of heat treatment levels, temperatures, and time intervals provided correct classification of the authentic honeys in the case of both honey types.

9) Electronic tongue was applied for the first time for the detection of heat treatment (heating temperatures: 40°C, 60°C, 80°C, or 100°C, time intervals for each temperature: 60, 120, 180, or 240 minutes) of Hungarian authentic honey samples (sunflower, bastard indigo). PCA-LDA models were built for the classification of temperature level, time interval, and heat treatment levels. In the case of sunflower, the control was classified correctly in 85.65%, 100%, and 92.92% in the temperature, time, and heat treatment level model, respectively. Misclassification of the control was found as belonging to 40°C group (temperature model) and to the honey treated at 40°C for 180 minutes (heat treatment level model). In the case of bastard indigo, the PCA-LDA models showed that the control was classified correctly in 85.65% (misclassified to 40°C) in the temperature model, while in time-interval model only in 56.17% (misclassified to 60 min), and the heat treatment level model in 85.65% (misclassified to 40°C 60 minutes honeys). Both honey types showed the complete separation of control from the honeys heated at 60°C or higher temperatures.

8 SUMMARY

Honey is a nutritionally active food product having a high value worldwide. Its composition is rich in sugars, and water, moreover useful components such as minerals, vitamins, amino acids, and antioxidant can be found in it. The amount of these components highly depends on the origin of the samples where the botanical and the geographical origin are influencing. Therefore, according to the botanical origin significant differences can be found among different honey types and even within one unifloral type because of the geographical origin. Owing to this, the origin identification of honey is challenging, which for the most commonly applied practice is the determination of the physicochemical, sensory and pollen characteristics. However, these attributes can be analyzed using different measurements techniques, which can be time consuming, and expensive. Therefore, there is a need for methods which can be used relatively easily and quickly to determine the origin. Another problem is that honey is often a target of adulteration techniques such as direct dilution with sugar syrups or overheating. The detection of the manipulations is also challenging. The aim of this work was to analyze and develop reference and correlative methods (electronic tongue, and NIR) for the botanical and geographical origin identification, adulteration, and heat treatment detection of honeys.

In this study reference methods (physicochemical parameters such as moisture content, electrical conductivity pH, ash content, antioxidant properties – TPC, CUPRAC, FRAP, and color – L*a*b*, sugars, HMF), melissopalynology and correlative (electronic tongue, and NIR) were applied. The study work was separated to three main parts: the origin identification, adulteration detection, and heat treatment detection using the aforementioned methods. In the origin identification study part descriptive tables were established (and a reference database) for the most common honey types of Hungary (acacia, linden, sunflower, chestnut, honeydew, rape, bastard indigo, milkweed, and multiflora). These honey types were also used for the origin identification study using NIR, electronic tongue and melissopalynology, where classification models were built for the identification of the botanical and geographical (regions, counties, districts) origins. Electronic tongue provided >70% classification accuracy in the case of the botanical origin model and ~59% in the case of the geographical origin model (where all the honey types were analyzed together). Better classification accuracy was obtained in the case of the geographical modes built separately for the unifloral honey types. In this case the classification accuracies ranged between 70-100% for the identification of the counties and districts (bastard indigo). Pollen analysis (of the OISWP) showed that the honey types could be separated from each other using PCA-LDA with >90% accuracy, but the geographical origin identification model was worse of 52% average classification. In this study part the NIR

(benchtop) alone did not provide satisfactory results using PCA-LDA for the botanical and geographical origin identification. However, the fusion of the NIR, pH, ELC, moisture and pollen data provided higher than 99% accuracy. In the authenticity study acacia and linden honeys were mixed with sugar syrup, and the results of authentic acacia and linden honeys were compared to EUnonEU honey blends. In this study sensory profile analysis revealed that the sensory panel could discriminate the adulterated honey from the authentic ones in four (acacia) and eight (linden) sensory parameters. In contrary the electronic tongue could achieve higher accuracy, where all the adulterated honeys could be separated with the exception of the acacia where in 3.11% misclassification was found belonging to the 10%.

In the adulteration experiments linden, acacia, sunflower, honeydew, and rape honeys were mixed with different sugar syrups (F40, GF and rice) at 3%, 5%, 10%. Moreover, as a preliminary experiment linden honey was blended with beet and rice syrup in the range of 0.5-50%. The results provided different classification accuracies according to the botanical type and the syrups type based on the NIR models (benchtop), and electronic tongue. In the SSAPS study the honeys containing syrup in more than 1% could be discriminated while in the SSAWLC study the honeys mixed with the three syrups provided higher than 94% classification accuracy during the validation but 100% correct classification of control. The PLRS models of the acacia and linden honeys were the best with RPCV higher than 4 and $R^2CV > 0.8$. In the case of the honeydew the results were similar with R^2CV ranged between 0.71-0.95 and RPDCV between 1.89 and 6.20. The worst models were obtained in the case of the GF syrup. In the case of the rape and sunflower the models of GF syrups and all syrups the R^2CV and RPDCV were below 0.68 and 1.78. Similarly to the honeydew the results of the F40 and rice syrup models of the rape and sunflower honeys the results were better of $R^2CV >0.88$ and $RPDCV > 2.93$.

In the heat treatment experiment acacia, bastard indigo, and sunflower honeys were heated at 40°C, 60°C, 80°C, and 100°C for 60, 120, 180 and 240 minutes HMF was determined at all the heat treatment levels, moreover NIR (Handheld) and electronic tongue analysis were performed besides the color and physicochemical determination. The results showed that the in the case of the HMF only samples heated at 80°C and 100°C showed significant difference compared to control, while in the case of the electronic tongue and NIR the honeys heated at 40°C, 60°C or above could be discriminated with satisfactory accuracy from the control in the case of sunflower and bastard indigo honeys.

The results of the thesis showed that the correlative techniques have a potential in the origin identification, adulteration, and heat treatment detection of honey, especially if they used in combination with the reference methods.

9 LIST OF PUBLICATIONS IN THE FIELD OF STUDIES

9.1 Journal articles

Bodor, Z., Benedek, C., Kaszab, T., Zaukuu, J.-L.Z., Kertész, I., Kovacs, Z., Zinia Zaukuu, J.-L., Kertész, I., Kovacs, Z., **2019.** Classical and correlative analytical methods for origin identification of Hungarian honeys. ***Acta Alimentaria*** 48, 477–487. https://doi.org/10.1556/066.2019.48.4.9 ***Q3 - IF 0.650***

Bodor, Z., Ghdir, C., Zaukuu, J.-L.Z., Benedek, C., Kovacs, Z., **2019**. Detection of heat treatment of honey with near infrared spectroscopy. ***Hungarian Agricultural Engineering Sciences.*** 36, 57–62. https://doi.org/10.17676/HAE.2019.36.57

Bodor, Z., Kovacs, Z., Rashed, M.S., Kókai, Z., Dalmadi, I., Benedek, C., **2020**. Sensory and physicochemical evaluation of acacia and linden honey adulterated with sugar syrup. ***Sensors*** 20, 1–20. https://doi.org/10.3390/s20174845 ***Q2 - IF 3.576***

Bodor, Z., Kovacs, Z., Benedek, C., Hitka, G., Behling, H., **2021.** Origin Identification of Hungarian Honey Using Melissopalynology, Physicochemical Analysis, and Near Infrared Spectroscopy. ***Molecules***. https://doi.org/10.3390/molecules26237274 ***Q1 - IF 4.412***

Bodor, Z., Benedek, C., Aouadi, B., Zsom-Muha, V., & Kovacs, Z. (2022). Revealing the Effect of Heat Treatment on the Spectral Pattern of Unifloral Honeys Using Aquaphotomics. ***Molecules,*** https://doi.org/10.3390/molecules27030780 ***Q1 - IF 4.412***

9.2 Book chapters

Benedek, C., Zaukuu, J.-L.Z., **Bodor, Z**., Zoltan, K., **2021.** Honey - based polyphenols: extraction, quantification, bioavailability and biological activities, in: **Plant-Based Functional Foods and Phytochemicals Traditional Knowledge to Present Innovation.** Apple Academic Press. https://doi.org/10.1201/9781003055419

Bodor, Z., Benedek, C., Kovacs, Z., Zinia Zaukuu, J.-L., **2021.** Identification of Botanical and Geographical Origins of Honey-Based on Polyphenols, in: **Plant-Based Functional Foods and Phytochemicals Traditional Knowledge to Present Innovation**. Apple Academic Press, https://doi.org/10.1201/9781003055419

10 APPENDICES

10.1 References

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10.2 Appendix of the supplementary tables

Appendix Table 1. List of the analyzed samples throughout the thesis

ID*	Botanical origin	Year	District	County	Region	Country	Experiment*
1	Rape	2015	Hevesi	Heves	Alföld	Hungary	B,E
2	Rape	2015	Kunhegyesi	Jász-Nagykun-Szolnok	Alföld	Hungary	B,E
3	Rape	2015	Hevesi	Heves	Alföld	Hungary	B
4	Rape	2015	Hevesi	Heves	Alföld	Hungary	B,E
5	Acacia	2015	Nyírbátori	Szabolcs-Szatmár-Bereg	Alföld	Hungary	B,E
6	Acacia	2015	Debreceni	Hajdú-Bihar	Alföld	Hungary	B,E,P
7	Acacia	2015	Jászapáti	Jász-Nagykun-Szolnok	Alföld	Hungary	B,E
8	Acacia	2015	Hevesi	Heves	Alföld	Hungary	B,E
9	Acacia	2015	NA	Szabolcs-Szatmár-Bereg	Alföld	Hungary	B,E
10	Acacia	2013	Hevesi	Heves	Alföld	Hungary	B,E
11	Sunflower	2015	Hevesi	Heves	Alföld	Hungary	B
12	Sunflower	2015	Hevesi	Heves	Alföld	Hungary	B,E
13	Sunflower	2015	Hevesi	Heves	Alföld	Hungary	B,E
14	Sunflower	2015	Hevesi	Heves	Alföld	Hungary	B,E
15	Linden	2015	Hevesi	Heves	Alföld	Hungary	B,E
16	Linden	2015	Hevesi	Heves	Alföld	Hungary	B,E
18	Shortpod mustard	2015	Hevesi	Heves	Alföld	Hungary	B
19	Multiflora	2012	Pásztói	Nógrád	Északi-középhegység	Hungary	B,E
21	Acacia	2015	Aszódi	Pest	Alföld	Hungary	B,E
24	Multiflora	2015	NA	Zala	Nyugat-magyarországi-peremvidék	Hungary	B,E
26	Honeydew	2015	Bélapátfalvai	Heves	Északi-középhegység	Hungary	B,E
27	Multiflora (Rape-Linden)	2015	Dunakeszi	Pest	Alföld	Hungary	B,E
28	Multiflora	2015	Hevesi	Heves	Alföld	Hungary	B,E
29	Acacia	2015	Salgótarjáni	Nógrád	Észak-magyarországi	Hungary	B,E,P

ID*	Botanical origin	Year	District	County	Region	Country	Experiment*
					középhegység		
30	Chestnut	2015	NA	Vas	Nyugat-magyarországi-peremvidék	Hungary	B,E
31	Sunflower	2015	Szarvasi	Békés	Alföld	Hungary	B,E
32	Milkweed	2015	Kecskeméti	Bács-Kiskun	Alföld	Hungary	B,E,P
33	Rape	2015	Szarvasi	Békés	Alföld	Hungary	B,E
34	Multiflora	2015	Szarvasi	Békés	Alföld	Hungary	B,E
35	Linden	2015	Szigetvári	Baranya	Dunántúli-dombság	Hungary	B,E,P
36	Milk Thistle	2015	Mezőkovács-házi	Békés	Alföld	Hungary	B
37	Honeydew	2015	Monori	Pest	Alföld	Hungary	B,E
38	Acacia	2015	Rétsági	Nógrád	Északi-középhegység	Hungary	B,E
39	Multiflora (Rape-fruit-cream)	2015	NA	NA	Alföld	Hungary	B,E
40	Sunflower	2015	NA	Nógrád	Északi-középhegység	Hungary	B,E
41	Multiflora	2015	NA	Pest	Alföld	Hungary	B,E
42	Multiflora (Linden-Chestnut)	2015	Szobi	Pest	Északi-középhegység	Hungary	B,E
43	Linden	2015	Keszthelyi	Zala	Nyugat-magyarországi-peremvidék	Hungary	B,E
46	EUnonEU Acacia	2015	EUnonEU	EUnonEU	EUnonEU	EUnonEU	A
48	Honeydew	2015	Jászapáti	Jász-Nagykun-Szolnok	Alföld	Hungary	B,E,P
50	Milkweed	2015	Kiskunfélegy-házi	Bács-Kiskun	Alföld	Hungary	B,E,P
51	Chestnut	2015	NA	Vas	Nyugat-magyarországi-peremvidék	Hungary	B,E,P
52	Milkweed	2015	Gyáli	Pest	Alföld	Hungary	B,E,P
54	EUnonEU Acacia	2015	EUnonEU	EUnonEU	EUnonEU	EUnonEU	A
55	Honeydew (Pine)	2015	NA	NA	Északi-középhegység	Hungary	B,E,P
58	Milkweed	2015	Szolnoki	Jász-Nagykun-Szolnok	Alföld	Hungary	B,E,P
59	Chestnut	2015	NA	Zala	Nyugat-magyarországi-peremvidék	Hungary	B,E,P
60	Linden	2015	Szolnoki	Jász-Nagykun-Szolnok	Alföld	Hungary	B,E,P

ID*	Botanical origin	Year	District	County	Region	Country	Experiment*
61	Bastard indigo	2015	Szolnoki	Jász-Nagykun-Szolnok	Alföld	Hungary	B,E
62	Honeydew	2015	Szolnoki	Jász-Nagykun-Szolnok	Alföld	Hungary	B,E,P
63	Acacia	2015	Szolnoki	Jász-Nagykun-Szolnok	Alföld	Hungary	B,E,P
64	Raspberry	2015	Szolnoki	Jász-Nagykun-Szolnok	Alföld	Hungary	B
65	Sunflower	2015	NA	NA	NA	Hungary	B,P
66	Bastard indigo	2016	Szolnoki	Jász-Nagykun-Szolnok	Alföld	Hungary	B,E,P
68	Chestnut	2016	Soproni	Győr-Moson-Sopron	Nyugat-magyarországi-peremvidék	Hungary	B,E,P
69	Buckwheat	2016	NA	NA	NA	Hungary	B
70	Bastard indigo	2016	Kunhegyesi	Jász-Nagykun-Szolnok	Alföld	Hungary	B,E,P
71	Honeydew (Pine)	2016	NA	NA	NA	Hungary	B,E,P
72	Bastard indigo	2016	Karcagi	Jász-Nagykun-Szolnok	Alföld	Hungary	B,E,P
73	Chestnut	2016	NA	Zala	Nyugat-magyarországi-peremvidék	Hungary	B
74	Raspberry	2016	NA	NA	NA	Hungary	B
75	Introduced Sage	2016	NA	NA	NA	Hungary	B
76	Ramsoms	2016	Komlói	Baranya	Dunántúli-dombság	Hungary	B
77	EUnonEU Acacia	2016	EUnonEU	EUnonEU	EUnonEU	EUnonEU	A
78	EUnonEU Acacia	2016	EUnonEU	EUnonEU	EUnonEU	EUnonEU	A
79	EUnonEU Linden	2016	EUnonEU	EUnonEU	EUnonEU	EUnonEU	A
80	EUnonEU Acacia	2016	EUnonEU	EUnonEU	EUnonEU	EUnonEU	A
81	EUnonEU Acacia	2016	EUnonEU	EUnonEU	EUnonEU	EUnonEU	A
82	EUnonEU Acacia	2016	EUnonEU	EUnonEU	EUnonEU	EUnonEU	A
83	EUnonEU Linden	2016	EUnonEU	EUnonEU	EUnonEU	EUnonEU	A
84	EUnonEU Acacia	2016	EUnonEU	EUnonEU	EUnonEU	EUnonEU	A
85	Honeydew	2016	Tokaji	Borsod-Abaúj-Zemplén	Északi-középhegység	Hungary	B,P
86	Multiflora	2016	Hevesi	Heves	Alföld	Hungary	B
87	Oleaster	2016	Jászberényi	Jász-Nagykun-Szolnok	Alföld	Hungary	B

ID*	Botanical origin	Year	District	County	Region	Country	Experiment*
88	Rape	2016	Hevesi	Heves	Alföld	Hungary	B,P
89	Multiflora	2016	Hevesi	Heves	Alföld	Hungary	B
90	Acacia	2016	Hevesi	Heves	Alföld	Hungary	B
91	Honeydew	2016	Bélapátfalvai	Heves	Északi-középhegység	Hungary	B
95	Multiflora	2016	NA	NA	NA	Hungary	B
97	Acacia	2016	Hevesi	Heves	Alföld	Hungary	B,P,A
98	EUnonEU Linden	2016	EUnonEU	EUnonEU	EUnonEU	EUnonEU	A
99	EUnonEU Acacia	2016	EUnonEU	EUnonEU	EUnonEU	EUnonEU	A
100	EUnonEU Acacia	2016	EUnonEU	EUnonEU	EUnonEU	EUnonEU	A
101	Acacia	2016	Egri	Heves	Északi-középhegység	Hungary	B,P,A
102	Linden	2016	Egri	Heves	Északi-középhegység	Hungary	B,A
103	Linden	2016	NA	Pest	Alföld	Hungary	B,P,A
105	Chestnut	2017	Kőszegi	Vas	Nyugat-magyarországi-peremvidék	Hungary	B,P
110	Acacia	2018	Hajdú-böszörményi	Hajdú-Bihar	Alföld	Hungary	B,P
128	Chestnut	2018	Kőszegi	Vas	Nyugat-magyarországi-peremvidék	Hungary	B,P
129	Linden	2018	Szigetvári	Baranya	Dunántúli-dombság	Hungary	B,P
130	Honeydew	2018	Debreceni	Hajdú-Bihar	Alföld	Hungary	B,P
131	Bastard indigo	2018	Hajdúnánási	Hajdú-Bihar	Alföld	Hungary	B,P
132	Milkweed	2018	Kiskunhalasi	Bács-Kiskun	Alföld	Hungary	B,P
133	Rape	2018	Tiszavasvári	Szabolcs-Szatmár-Bereg	Alföld	Hungary	B,P
134	Acacia	2017	Ceglédi	Pest	Alföld	Hungary	B,P
135	Sunflower	2017	Hevesi	Heves	Alföld	Hungary	B
136	Linden	2017	Tabi	Somogy	Dunántúli-dombság	Hungary	B,P
137	Sunflower	2015	Győri	Győr-Moson-Sopron	Kisalföld	Hungary	B,P
138	Sunflower	2016	Győri	Győr-Moson-Sopron	Kisalföld	Hungary	B,P
139	Sunflower	2017	Győri	Győr-Moson-Sopron	Kisalföld	Hungary	B,P,S

ID*	Botanical origin	Year	District	County	Region	Country	Experiment*
140	Sunflower	2017	Békési	Békés	Alföld	Hungary	B,P
141	Sunflower	2017	Paksi	Tolna	Alföld	Hungary	B,P
145	Acacia	2016	Győri	Győr-Moson-Sopron	Kisalföld	Hungary	B,P
146	Acacia	2017	Győri	Győr-Moson-Sopron	Kisalföld	Hungary	B,P
147	Honeydew	2017	Monori	Pest	Alföld	Hungary	B,P,S
148	Acacia	2017	Rétsági	Nógrád	Északi-középhegység	Hungary	B,P,S
149	Linden	2019	Szigetvári	Baranya	Dunántúli-dombság	Hungary	B,P,S
150	Linden	2017	NA	Pest	Északi-középhegység	Hungary	B,P
151	Sunflower	2018	Kunhegyesi	Jász-Nagykun-Szolnok	Alföld	Hungary	B,P,H
152	Bastard indigo	2018	Kunhegyesi	Jász-Nagykun-Szolnok	Alföld	Hungary	B,P,H
153	Acacia	2018	Pétervásárai	Heves	Északi-középhegység	Hungary	B,P,H
155	Rape	2019	NA	NA	NA	Hungary	B,P
156	Linden	2017	NA	NA	Alföld	Hungary	B,P,S
157	Acacia	2017	NA	NA	Alföld	Hungary	B,P
158	Chestnut	2016	Szigetvári	Baranya	Dunántúli-dombság	Hungary	B,P
159	Acacia	2017	NA	Pest	Északi-középhegység	Hungary	B
160	Acacia	2018	Hevesi	Heves	Alföld	Hungary	B,P
161	Chestnut	2019	NA	Zala	Nyugat-magyarországi-peremvidék	Hungary	B,P
162	Chestnut	2019	Veszprémi	Veszprém	Dunántúli-középhegység	Hungary	B,P
164	Linden	2019	Veszprémi	Veszprém	Dunántúli-középhegység	Hungary	B,P
165	Linden	2018	Szigetvári	Baranya	Dunántúli-dombság	Hungary	B,P
166	Sunflower	2019	Balaton-almádi	Veszprém	Alföld	Hungary	B,P
167	Sunflower	2017	Edelényi	Borsod-Abaúj-Zemplén	Északi-középhegység	Hungary	B,P
168	Rape	2017	Edelényi	Borsod-Abaúj-Zemplén	Északi-középhegység	Hungary	B,P
169	Rape	2020	Kőszegi	Vas	Nyugat-magyarországi-peremvidék	Hungary	B,P
170	Honeydew	2018	Váci	Pest	Északi-középhegység	Hungary	B,P

ID*	Botanical origin	Year	District	County	Region	Country	Experiment*
171	Acacia	2019	Encsi	Borsod-Abaúj-Zemplén	Északi-középhegység	Hungary	B,P
172	Acacia	2019	Pápai	Veszprém	Kisalföld	Hungary	B,P
173	Acacia	2020	NA	Zala	Nyugat-magyarországi-peremvidék	Hungary	B,P
174	Rape	2017	Hevesi	Heves	Alföld	Hungary	B,P
175	Sunflower	2017	Füzesabonyi	Heves	Alföld	Hungary	B,P
176	Rape	2019	Füzesabonyi	Heves	Alföld	Hungary	B,P
177	Bastard indigo	2019	Füzesabonyi	Heves	Alföld	Hungary	B,P
178	Acacia	2019	Füzesabonyi	Heves	Alföld	Hungary	B,P
179	Acacia	2017	Ceglédi	Pest	Alföld	Hungary	B,P
180	Rape	2016	Hevesi	Heves	Alföld	Hungary	B,P
181	Rape	2017	Hevesi	Heves	Alföld	Hungary	B,P
182	Acacia	2017	Jászberényi	Jász-Nagykun-Szolnok	Alföld	Hungary	B,P
183	Linden	2019	Veszprémi	Veszprém	Dunántúli-középhegység	Hungary	B,P
184	Rape	2020	Dunaújvárosi	Fejér	Alföld	Hungary	B,P
185	Milkweed	2019	Bajai	Bács-Kiskun	Alföld	Hungary	B,P
186	Chestnut	2019	NA	NA	NA	Hungary	B,P
187	Milkweed	2020	NA	NA	NA	Hungary	B,P
188	Honeydew	2019	NA	NA	NA	Hungary	B,P
189	Honeydew	2019	NA	Pest	Alföld	Hungary	B,P
190	Milkweed	2020	Kunszentmiklósi	Bács-Kiskun	Alföld	Hungary	B,P
191	Milkweed	2019	Kecskeméti	Bács-Kiskun	Alföld	Hungary	B,P
192	Milkweed	2020	Monori	Pest	Alföld	Hungary	B,P
193	Milkweed	2020	Edelényi	Borsod-Abaúj-Zemplén	Északi-középhegység	Hungary	B,P
194	Bastard indigo	2020	Ibrányi	Szabolcs-Szatmár-Bereg	Alföld	Hungary	B,P

A: samples of the authenticity study; B: samples of basic origin identification experiment; H: samples of heat treatment study; E: samples of the electronic tongue experiment (origin); P: samples of the pollen identification study; S: samples of sugar syrup adulteration study; NA: not available
*Missing samples (eg.17) were excluded owing to their origin was not Hungary

Appendix Table 2. The list of applied NIRS pretreatments

Abbreviation	Pretreatment (combination)
sgol@2-13-0	Savitzky-Golay Smoothing 13 sample points, 2nd polynomial no derivation
sgol@2-17-0	Savitzky-Golay Smoothing 17 sample points, 2nd polynomial no derivation
sgol@2-21-0	Savitzky-Golay Smoothing 21 sample points, 2nd polynomial no derivation
SNV	Standard Normal Variate
MSC	Multiplicative scatter correction
detr	Detrending
detr+MSC	Detrending + Multiplicative scatter correction
detr+SNV	Detrending + Standard Normal Variate
sgol@2-13-0+SNV	Savitzky-Golay Smoothing 13 sample points, 2nd polynomial no derivation + Standard Normal Variate
sgol@2-17-0+SNV	Savitzky-Golay Smoothing 17 sample points, 2nd polynomial no derivation + Standard Normal Variate
sgol@2-21-0+SNV	Savitzky-Golay Smoothing 21 sample points, 2nd polynomial no derivation + Standard Normal Variate
sgol@2-13-0 +MSC	Savitzky-Golay Smoothing 13 sample points, 2nd polynomial no derivation + Multiplicative scatter correction
sgol@2-17-0 +MSC	Savitzky-Golay Smoothing 17 sample points, 2nd polynomial no derivation + Multiplicative scatter correction
sgol@2-21-0 +MSC	Savitzky-Golay Smoothing 21 sample points, 2nd polynomial no derivation + Multiplicative scatter correction
sgol@2-13-0 +detr	Savitzky-Golay Smoothing 13 sample points, 2nd polynomial no derivation + Detrending
sgol@2-17-0 +detr	Savitzky-Golay Smoothing 17 sample points, 2nd polynomial no derivation + Detrending
sgol@2-21-0 +detr	Savitzky-Golay Smoothing 21 sample points, 2nd polynomial no derivation + Detrending
sgol@2-13-0 +detr +SNV	Savitzky-Golay Smoothing 13 sample points, 2nd polynomial no derivation + Detrending + Standard Normal Variate
sgol@2-17-0 +detr +SNV	Savitzky-Golay Smoothing 17 sample points, 2nd polynomial no derivation + Detrending + Standard Normal Variate
sgol@2-21-0 +detr +SNV	Savitzky-Golay Smoothing 21 sample points, 2nd polynomial no derivation + Detrending + Standard Normal Variate
sgol@2-13-0 +detr +MSC	Savitzky-Golay Smoothing 13 sample points, 2nd polynomial no derivation + Detrending + Multiplicative scatter correction
sgol@2-17-0 +detr +MSC	Savitzky-Golay Smoothing 17 sample points, 2nd polynomial no derivation + Detrending + Multiplicative scatter correction
sgol@2-21-0 +detr +MSC	Savitzky-Golay Smoothing 21 sample points, 2nd polynomial no derivation + Detrending + Multiplicative scatter correction
sgol@2-21-0 +sgol@2-21-1	Savitzky-Golay Smoothing 21 sample points, 2nd polynomial no derivation + Savitzky-Golay Smoothing 21 sample points, 2nd polynomial 1st derivative
sgol@2-21-0 +sgol@2-21-2	Savitzky-Golay Smoothing 21 sample points, 2nd polynomial no derivation + Savitzky-Golay Smoothing 21 sample points, 2nd polynomial 2nd derivative
sgol@2-21-0 +sgol@2-13-1	Savitzky-Golay Smoothing 21 sample points, 2nd polynomial no derivation + Savitzky-Golay Smoothing 13 sample points, 2nd polynomial 1st derivative

Abbreviation	Pretreatment (combination)
sgol@2-21-0 +sgol@2-13-2	Savitzky-Golay Smoothing 21 sample points, 2nd polynomial no derivation + Savitzky-Golay Smoothing 13 sample points, 2nd polynomial 2nd derivative
sgol@2-21-0 +sgol@2-17-1	Savitzky-Golay Smoothing 21 sample points, 2nd polynomial no derivation + Savitzky-Golay Smoothing 17 sample points, 2nd polynomial 1st derivative
sgol@2-21-0 +sgol@2-17-2	Savitzky-Golay Smoothing 21 sample points, 2nd polynomial no derivation + Savitzky-Golay Smoothing 17 sample points, 2nd polynomial 2nd derivative
sgol@2-13-0 +sgol@2-21-1	Savitzky-Golay Smoothing 13 sample points, 2nd polynomial no derivation + Savitzky-Golay Smoothing 21 sample points, 2nd polynomial 1st derivative
sgol@2-13-0 +sgol@2-21-2	Savitzky-Golay Smoothing 13 sample points, 2nd polynomial no derivation + Savitzky-Golay Smoothing 21 sample points, 2nd polynomial 2nd derivative
sgol@2-13-0 +sgol@2-13-1	Savitzky-Golay Smoothing 13 sample points, 2nd polynomial no derivation + Savitzky-Golay Smoothing 13 sample points, 2nd polynomial 1st derivative
sgol@2-13-0 +sgol@2-13-2	Savitzky-Golay Smoothing 13 sample points, 2nd polynomial no derivation + Savitzky-Golay Smoothing 13 sample points, 2nd polynomial 2nd derivative
sgol@2-13-0 +sgol@2-17-1	Savitzky-Golay Smoothing 13 sample points, 2nd polynomial no derivation + Savitzky-Golay Smoothing 17 sample points, 2nd polynomial 1st derivative
sgol@2-13-0 +sgol@2-17-2	Savitzky-Golay Smoothing 13 sample points, 2nd polynomial no derivation + Savitzky-Golay Smoothing 17 sample points, 2nd polynomial 2nd derivative
sgol@2-17-0 +sgol@2-21-1	Savitzky-Golay Smoothing 17 sample points, 2nd polynomial no derivation + Savitzky-Golay Smoothing 21 sample points, 2nd polynomial 1st derivative
sgol@2-17-0 +sgol@2-21-2	Savitzky-Golay Smoothing 17 sample points, 2nd polynomial no derivation + Savitzky-Golay Smoothing 21 sample points, 2nd polynomial 2nd derivative
sgol@2-17-0 +sgol@2-17-1	Savitzky-Golay Smoothing 17 sample points, 2nd polynomial no derivation + Savitzky-Golay Smoothing 17 sample points, 2nd polynomial 1st derivative
sgol@2-17-0 +sgol@2-17-2	Savitzky-Golay Smoothing 17 sample points, 2nd polynomial no derivation + Savitzky-Golay Smoothing 17 sample points, 2nd polynomial 2nd derivative
sgol@2-17-0 +sgol@2-13-1	Savitzky-Golay Smoothing 17 sample points, 2nd polynomial no derivation + Savitzky-Golay Smoothing 13 sample points, 2nd polynomial 1st derivative
sgol@2-17-0 +sgol@2-13-2	Savitzky-Golay Smoothing 17 sample points, 2nd polynomial no derivation + Savitzky-Golay Smoothing 13 sample points, 2nd polynomial 2nd derivative

Appendix Table 3 Comparative analysis of the physicochemical parameters of the main botanical groups

Botanical origin	Moisture %	pH	Electrical conductivity µS/cm	Ash %
Acacia	17.82±0.94^{a}	3.96±0.21^{ab}	150.3±29.7^{a}	0.0344±0.0208^{a}
Bastard indigo	17.24±1.10^{a}	3.81±0.33^{a}	301.8±158.6^{abc}	0.0614±0.0396^{ab}
Chestnut	16.97±1.25^{a}	4.40±0.22^{c}	695.3±131.6 ^{e}	0.3212±0.0385^{c}
Linden	17.74±1.39^{a}	4.18±0.32^{bc}	544.6±157.2^{de}	0.1114±0.0837 ^{ab}
Rape	18.50±1.20^{a}	4.01±0.14^{ab}	231.2±62.0^{b}	0.0780±0.0123^{b}
Sunflower	18.62±2.16^{a}	3.84±0.35^{a}	440.1±144.0^{cd}	0.1577±0.0520^{b}

Mean ± standard deviation, letters denote the significant difference among the groups based on the ANOVA and pairwise comparison

Appendix Table 4. Comparative analysis of the antioxidant parameters of the main botanical groups

Botanical origin	Total polyphenol content mgGAE/100g	CUPRAC µmol TEQ/g	FRAP mg AAE/100g
Acacia	5.39±2.43^{a}	13.45±5.85^{a}	5.14±2.66^{a}
Bastard indigo	9.63±4.52^{bc}	35.25±13.38^{bc}	19.37±19.23^{abc}
Chestnut	12.43±3.70^{c}	42.87±10.67^{c}	34.72±15.60^{c}
Linden	10.62±1.82^{bc}	39.73±10.20^{c}	25.23±11.43^{c}
Rape	7.64±2.09^{b}	23.95±9.03^{b}	13.73±5.32^{b}
Sunflower	10.40±3.18^{bc}	56.28±29.83^{c}	30.10±18.93^{c}

Mean ± standard deviation, letters denote the significant difference among the groups based on the ANOVA and pairwise comparison

Appendix Table 5. Comparative analysis of the color L*a*b* parameters of the main botanical groups

Botanical origin	L*	a*	b*
Acacia	58.48±2.67^{d}	-1.68±0.73^{a}	13.27±6.61^{a}
Bastard indigo	57.10±1.90^{cd}	-1.18±0.69^{a}	22.33±9.89^{ab}
Chestnut	48.20±5.85^{a}	5.57±6.01^{b}	27.64±7.01^{b}
Linden	52.78±1.83^{ab}	-0.56±1.11^{a}	28.03±5.66^{bc}
Rape	54.60±2.00^{bc}	-1.24±1.25^{a}	23.17±3.10^{ab}
Sunflower	53.16±1.59^{abc}	-0.40±2.49^{ab}	38.45±2.35^{c}

Mean ± standard deviation, letters denote the significant difference among the groups based on the ANOVA and pairwise comparison

Appendix Table 6 LDA confusion table of the honeys for botanical origin classification based on electronic tongue (BBGOIS part)

		Acacia	Bastard indigo	Chestnut	Honeydew	Linden	Multiflora	Rape	Milkweed	Sunflower
Average training 57.07%	**Acacia**	**93.75**	32.08	0	1.33	1.15	16.78	66.67	5.89	0
	Bastard indigo	5.92	**64.18**	0	2.65	0	0	0	8.09	0
	Chestnut	0	0	**88.46**	27.44	2.29	0	0	0	0
	Honeydew	0	0	8.65	**24.33**	21.84	11.19	0	0	0
	Linden	0	0	0.95	9.73	**27.01**	5.59	0	0	14.95
	Multiflora	0	0	1.93	20.35	14.36	**52.80**	4.17	11.03	0
	Rape	0.33	2.24	0	0	2.88	5.24	**26.40**	0	0
	Milkweed	0	1.50	0	6.20	13.79	7.00	2.77	**57.34**	5.74
	Sunflower	0	0	0	7.96	16.67	1.40	0	17.64	**79.31**
		Acacia	**Bastard indigo**	**Chestnut**	**Honeydew**	**Linden**	**Multiflora**	**Rape**	**Milkweed**	**Sunflower**
Average validation 57.09%	**Acacia**	**93.42**	32.84	0	1.78	1.14	16.78	66.67	5.87	0
	Bastard indigo	5.92	**64.20**	0	2.65	0	0	0	8.82	0
	Chestnut	0	0	**86.56**	27.42	2.31	0	0	0	0
	Honeydew	0	0	9.64	**23.89**	23.00	11.89	0	0	0
	Linden	0	0	1.90	9.74	**27.59**	6.29	0	0	13.79
	Multiflora	0	0	1.90	20.36	13.79	**51.04**	4.17	8.82	0
	Rape	0.66	1.48	0	0	2.31	5.60	**26.38**	0	0
	Milkweed	0	1.48	0	7.09	14.93	6.99	2.79	60.30	5.76
	Sunflower	0	0	0	7.09	14.93	1.41	0	16.19	**80.45**

Appendix Table 7. LDA confusion table of the acacia honey for geographical origin classification based on electronic tongue (BBGOIS part)

		Hajdú-Bihar	Heves	Jász-Nagykun-Szolnok	Nógrád	Pest	Szabolcs-Szatmár-Bereg
Average training 71.85%	**Hajdú-Bihar**	**72.25**	9.26	0	2.87	0	2.87
	Heves	0	**90.74**	20.83	45.72	0	11.44
	Jász-Nagykun-Szolnok	0	0	**63.88**	4.28	0	0
	Nógrád	0	0	0	**25.71**	0	7.16
	Pest	0	0	0	0	**100**	0
	Szabolcs-Szatmár-Bereg	27.75	0	15.29	21.42	0	**78.53**
		Hajdú-Bihar	**Heves**	**Jász-Nagykun-Szolnok**	**Nógrád**	**Pest**	**Szabolcs-Szatmár-Bereg**
Average validation 70.68%	**Hajdú-Bihar**	**72.17**	8.65	0	0	0	5.74
	Heves	0	**90.74**	19.43	51.46	0	11.40
	Jász-Nagykun-Szolnok	0	0	**61.13**	2.83	0	0
	Nógrád	0	0	2.75	**25.73**	0	8.57
	Pest	0	0	0	0	**100**	0
	Szabolcs-Szatmár-Bereg	27.83	0.61	16.68	19.98	0	**74.29**

Appendix Table 8. PCA-LDA confusion table of the NIR for the botanical origin classification of honey types (OISWP part)

		Acacia	Bastard indigo	Chestnut	Linden	Rape	Sunflower
Training 75.52%	**Acacia**	**79.41**	13.04	15.00	14.29	7.26	15.52
	Bastard indigo	4.62	**70.65**	2.14	4.29	0.81	4.31
	Chestnut	4.62	2.17	**75.00**	2.14	2.42	2.59
	Linden	4.62	8.70	5.00	**68.57**	1.61	1.72
	Rape	4.20	2.17	1.43	3.57	**87.10**	3.45
	Sunflower	2.52	3.26	1.43	7.14	0.81	**72.41**
		Acacia	**Bastard indigo**	**Chestnut**	**Linden**	**Rape**	**Sunflower**
Validation 58.14%	**Acacia**	**55.46**	19.57	14.29	22.86	8.06	17.24
	Bastard indigo	5.88	**50.00**	2.86	4.29	4.84	6.90
	Chestnut	12.61	6.52	**60.00**	5.71	6.45	1.72
	Linden	10.08	10.87	5.71	**57.14**	6.45	1.72
	Rape	5.88	6.52	8.57	4.29	**69.35**	15.52
	Sunflower	10.08	6.52	8.57	5.71	4.84	**56.9**

Appendix Table 9. Results of the moisture, pH, and electrical conductivity of the heat treatment experiment

	Sunflower			Bastard indigo			Acacia		
Treatment level	**Moisture %**	**pH**	**Electrical conductivity µS/cm**	**Moisture %**	**pH**	**Electrical conductivity µS/cm**	**Moisture %**	**pH**	**Electrical conductivity µS/cm**
Control	18.10±0.19	3.82±0.01	396.9±4.9	16.40±0.17	4.35±0.02	536.6±2.3	16.33±0.20	4.21±0.01	112.2±2.1
040C_060M	18.17±0.07	3.82±0.01	397.3±1.6	16.33±0.26	4.36±0.01	541.2±3.4	16.40±0.20	4.22±0.01	111.8±0.7
040C_120M	18.10±0.09	3.82±0.01	388.8±10.2	16.51±0.36	4.36±0.01	542.2±5.7	16.39±0.16	4.21±0.01	110.8±0.7
040C_180M	17.97±0.24	3.71±0.01	396.3±2.8	16.98±0.04	4.39±0.00	546.6±4.0	16.70±0.15	4.23±0.02	111.6±1.7
040C_240M	18.20±0.22	4.01±0.03	399.7±3.8	16.80±0.21	4.38±0.01	540.7±1.5	16.60±0.00	4.21±0.02	111.2±1.7
060C_060M	17.67±0.15	3.72±0.10	393.2±3.4	17.04±0.10	4.34±0.02	541.7±6.2	16.60±0.00	4.24±0.01	109.1±1.4
060C_120M	17.83±0.12	3.78±0.01	390.4±0.7	17.10±0.10	4.42±0.04	537.9±1.8	16.73±0.20	4.26±0.02	109.6±0.7
060C_180M	17.87±0.15	3.83±0.01	390.6±3.2	17.00±0.27	4.44±0.01	539.4±5.0	16.62±0.04	4.28±0.03	109.9±0.6
060C_240M	17.90±0.09	3.85±0.01	388.0±2.2	17.11±0.15	4.45±0.01	538.0±3.6	16.76±0.19	4.31±0.01	109.3±0.5
080C_060M	17.67±0.15	3.81±0.01	385.3±2.3	16.93±0.19	4.43±0.01	545.2±2.2	16.60±0.00	4.27±0.01	112.7±2.5
080C_120M	17.80±0.11	3.84±0.01	381.4±2.1	16.84±0.26	4.48±0.01	540.2±0.4	16.67±0.10	4.36±0.01	108.3±0.5
080C_180M	17.63±0.17	3.93±0.01	377.4±3.0	17.04±0.24	4.50±0.01	538.1±1.8	16.73±0.20	4.31±0.02	109.6±0.5
080C_240M	17.80±0.24	3.95±0.01	380.4±3.2	17.27±0.20	4.51±0.00	543.3±1.7	16.60±0.00	4.32±0.03	109.9±0.9
100C_060M	17.77±0.07	3.89±0.01	380.8±6.5	17.02±0.12	4.51±0.01	542.9±2.1	16.47±0.20	4.37±0.04	108.0±1.0
100C_120M	17.73±0.13	4.03±0.01	374.8±2.0	17.27±0.13	4.55±0.04	542.4±1.7	16.50±0.16	4.50±0.05	108.0±1.1
100C_180M	18.03±0.19	4.09±0.01	378.1±1.5	16.93±0.22	4.56±0.01	541.9±2.0	16.63±0.05	4.50±0.00	108.2±0.4
100C_240M	17.97±0.19	4.11±0.01	378.8±3.2	16.84±0.30	4.56±0.01	538.4±7.4	16.66±0.20	4.48±0.02	109.0±0.9

Appendix Table 10. PCA-LDA confusion table of the sunflower honey built for the classification of heat treatment level based on NIR data

	Training	040C 060M	040C 120M	040C 180M	040C 240M	060C 060M	060C 120M	060C 180M	060C 240M	080C 060M	080C 120M	080C 180M	080C 240M	100C 060M	100C 120M	100C 180M	100C 240M	control
86.81 %	**040C060M**	**100**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	040C120M	0	**100**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	040C180M	0	0	**100**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	040C240M	0	0	0	**100**	0	0	0	0	0	0	0	0	0	0	0	0	0
	060C060M	0	0	0	0	**100**	0	0	0	0	0	0	0	0	0	0	0	0
	060C120M	0	0	0	0	0	**88.91**	0	0	0	0	12.80	4.29	3.85	0	0	0	0
	060C180M	0	0	0	0	0	0	**88.60**	3.85	0	5.69	0	0	0	0	0	0	0
	060C240M	0	0	0	0	0	0	4.29	**75.65**	9.09	17.04	0	0	0	0	0	0	0
	080C060M	0	0	0	0	0	0	1.41	11.54	**74.23**	9.10	0	0	0	0	0	0	0
	080C120M	0	0	0	0	0	0	5.70	8.96	16.68	**68.17**	0	0	0	0	0	0	0
	080C180M	0	0	0	0	0	5.54	0	0	0	0	**70.91**	4.29	6.42	1.46	0	0	0
	080C240M	0	0	0	0	0	0	0	0	0	0	4.64	**70.00**	12.81	2.96	0	0	0
	100C060M	0	0	0	0	0	5.54	0	0	0	0	9.31	20.02	**71.81**	0	0	0	0
	100C120M	0	0	0	0	0	0	0	0	0	0	2.34	1.41	5.12	**95.59**	0	0	0
	100C180M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**76.74**	4.87	0
	100C240M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23.26	**95.13**	0
	control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**100**
	Validation	**040C 060M**	**040C 120M**	**040C 180M**	**040C 240M**	**060C 060M**	**060C 120M**	**060C 180M**	**060C 240M**	**080C 060M**	**080C 120M**	**080C 180M**	**080C 240M**	**100C 060M**	**100C 120M**	**100C 180M**	**100C 240M**	**control**
80.81 %	**040C060M**	**100**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	040C120M	0	**100**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	040C180M	0	0	**100**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	040C240M	0	0	0	**100**	0	0	0	0	0	0	0	0	0	0	0	0	0
	060C060M	0	0	0	0	**100**	0	0	0	0	0	0	0	0	0	0	0	0
	060C120M	0	0	0	0	0	**74.94**	0	0	0	0	16.26	11.41	2.54	0	0	0	0
	060C180M	0	0	0	0	0	0	**74.29**	5.15	0	9.07	0	0	0	0	0	0	0
	060C240M	0	0	0	0	0	0	11.40	**71.77**	24.27	18.21	0	0	0	0	0	0	0
	080C060M	0	0	0	0	0	0	0	12.85	**57.55**	9.07	0	0	0	0	0	0	0
	080C120M	0	0	0	0	0	0	14.31	10.23	18.18	**63.64**	0	0	0	0	0	0	0
	080C180M	0	0	0	0	0	13.91	0	0	0	0	**60.5**	11.41	7.69	0	0	0	0
	080C240M	0	0	0	0	0	5.58	0	0	0	0	6.98	**51.46**	23.08	8.83	0	0	0
	100C060M	0	0	0	0	0	5.58	0	0	0	0	13.96	22.9	**59.00**	0	0	0	0
	100C120M	0	0	0	0	0	0	0	0	0	0	2.30	2.83	7.69	**91.17**	0	0	0
	100C180M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**76.76**	7.32	0
	100C240M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23.24	**92.68**	0
	control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**100**

Appendix Table 11. PCA-LDA confusion table of the bastard indigo honey built for the classification of heat treatment level based on NIR data

	Training	040C 060M	040C 120M	040C 180M	040C 240M	060C 060M	060C 120M	060C 180M	060C 240M	080C 060M	080C 120M	080C 180M	080C 240M	100C 060M	100C 120M	100C 180M	100C 240M	control
85.12 %	**040C060M**	**100**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	040C120M	0	**100**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	040C180M	0	0	**97.36**	5.12	0	0	0	0	0	0	0	0	0	0	0	0	0
	040C240M	0	0	2.64	**94.88**	0	0	0	0	0	0	0	0	0	0	0	0	0
	060C060M	0	0	0	0	**81.6**	8.96	8.13	4.99	10.28	4.99	0	0	2.79	0	0	0	0
	060C120M	0	0	0	0	9.20	**64.12**	2.34	3.75	0	3.75	0	1.30	2.79	5.87	0	0	0
	060C180M	0	0	0	0	5.25	3.85	**77.91**	0	0	7.50	0	2.65	1.38	1.46	0	0	0
	060C240M	0	0	0	0	1.30	6.42	0	**83.76**	4.41	0	0	1.30	0	2.96	0	0	0
	080C060M	0	0	0	0	2.65	5.12	1.15	1.24	**70.58**	2.51	0	0	1.38	0	0	0	0
	080C120M	0	0	0	0	0	0	4.64	0	2.96	**80.01**	0	0	1.38	0	0	0	0
	080C180M	0	0	0	0	0	10.27	0	0	2.96	1.24	**85.12**	7.90	5.54	0	0	0	0
	080C240M	0	0	0	0	0	1.27	0	0	0	0	2.72	**72.36**	4.17	1.46	0	0	0
	100C060M	0	0	0	0	0	0	0	6.26	8.82	0	6.77	11.84	**80.58**	0	0	0	0
	100C120M	0	0	0	0	0	0	0	0	0	0	5.39	2.65	0	**85.30**	0	3.33	0
	100C180M	0	0	0	0	0	0	5.83	0	0	0	0	0	0	0	**88.75**	11.10	0
	100C240M	0	0	0	0	0	0	0	0	0	0	0	0	0	2.96	11.25	**85.57**	0
	control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**100**
	Validation	**040C 060M**	**040C 120M**	**040C 180M**	**040C 240M**	**060C 060M**	**060C 120M**	**060C 180M**	**060C 240M**	**080C 060M**	**080C 120M**	**080C 180M**	**080C 240M**	**100C 060M**	**100C 120M**	**100C 180M**	**100C 240M**	**control**
74.93 %	**040C060M**	**100**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	040C120M	0	**100**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	040C180M	0	0	**94.71**	10.23	0	0	0	0	0	0	0	0	0	0	0	0	0
	040C240M	0	0	5.29	**89.77**	0	0	0	0	0	0	0	0	0	0	0	0	0
	060C060M	0	0	0	0	**68.43**	17.92	11.65	12.53	11.74	5.03	0	0	2.75	2.91	0	0	0
	060C120M	0	0	0	0	7.89	**48.69**	6.98	9.98	11.74	9.98	0	0	2.75	5.91	0	0	0
	060C180M	0	0	0	0	7.89	5.15	**62.81**	0	0	12.53	2.68	7.90	2.75	0	2.48	0	0
	060C240M	0	0	0	0	5.29	12.85	0	**65.04**	8.83	0	0	2.61	0	2.91	0	0	0
	080C060M	0	0	0	0	5.29	2.54	2.30	2.48	**47.04**	7.50	0	0	2.75	2.91	0	0	0
	080C120M	0	0	0	0	2.60	0	4.68	0	5.91	**60.02**	0	0	0	0	0	0	0
	080C180M	0	0	0	0	0	12.85	0	0	5.91	2.48	**64.94**	7.90	5.59	0	0	0	0
	080C240M	0	0	0	0	0	0	0	0	0	0	10.80	**63.19**	13.93	5.91	0	0	0
	100C060M	0	0	0	0	0	0	2.30	9.98	8.83	2.48	10.80	15.8	**69.47**	2.91	0	0	0
	100C120M	0	0	0	0	2.60	0	0	0	0	0	10.80	0	0	**70.61**	0	2.20	0
	100C180M	0	0	0	0	0	0	6.98	0	0	0	0	2.61	0	0	**82.52**	15.54	0
	100C240M	0	0	0	0	0	0	2.30	0	0	0	0	0	0	5.91	15	**82.25**	0
	control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**100**

Appendix Table 12. PCA-LDA confusion table of the acacia honey built for the classification of heat treatment level based on NIR data

	Training	040C 060M	040C 120M	040C 180M	040C 240M	060C 060M	060C 120M	060C 180M	060C 240M	080C 060M	080C 120M	080C 180M	080C 240M	100C 060M	100C 120M	100C 180M	100C 240M	control
	040C060M	**79.53**	5.18	1.6	1.13	0	0	7.9	5.25	0	1.18	0	0	0	0	0	0	0
	040C120M	0	**74.17**	4.84	0	0	0	2.65	1.30	3.75	0	0	0	0	0	0	0	0
	040C180M	1.27	5.18	**59.68**	15.92	0	3.75	1.30	3.95	0	3.57	0	0	0	0	1.41	0	0
	040C240M	0	1.71	12.92	**63.65**	3.41	13.76	0	0	0	0	0	0	0	0	0	0	0
	060C060M	0	0	3.24	6.82	**79.54**	7.50	5.25	0	0	0	0	0	0	0	0	0	0
	060C120M	0	0	16.12	11.35	17.05	**70.00**	0	0	0	0	0	0	0	0	0	0	0
	060C180M	10.27	1.71	0	1.13	0	0	**68.44**	15.80	2.51	1.18	0	0	0	0	0	0	0
79.34 %	**060C240M**	1.27	1.71	1.60	0	0	0	9.20	**51.34**	4.99	11.9	0	0	0	0	0	0	0
	080C060M	1.27	10.35	0	0	0	0	1.30	17.1	**82.49**	3.57	0	0	0	0	0	0	0
	080C120M	0	0	0	0	0	0	3.95	5.25	6.26	**78.60**	0	0	0	0	0	0	0
	080C180M	0	0	0	0	0	0	0	0	0	0	**90.77**	10.01	8.83	0	0	0	0
	080C240M	0	0	0	0	0	0	0	0	0	0	1.83	**77.47**	5.87	0	0	0	0
	100C060M	0	0	0	0	0	0	0	0	0	0	7.39	6.26	**85.30**	1.50	0	0	0
	100C120M	0	0	0	0	0	0	0	0	0	0	0	6.26	0	**98.50**	0	0	0
	100C180M	1.27	0	0	0	0	3.75	0	0	0	0	0	0	0	0	**94.30**	5.00	0
	100C240M	0	0	0	0	0	1.24	0	0	0	0	0	0	0	0	4.29	**95.00**	0
	control	5.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**100**
	Validation	**040C 060M**	**040C 120M**	**040C 180M**	**040C 240M**	**060C 060M**	**060C 120M**	**060C 180M**	**060C 240M**	**080C 060M**	**080C 120M**	**080C 180M**	**080C 240M**	**100C 060M**	**100C 120M**	**100C 180M**	**100C 240M**	**control**
	040C060M	**84.62**	3.42	0	2.25	0	0	10.51	5.29	0	0	0	0	0	0	0	0	2.61
	040C120M	0	**69.05**	9.68	0	0	0	2.61	5.29	5.03	0	0	0	0	0	0	0	0
	040C180M	0	3.42	**48.4**	15.9	0	0	2.61	2.60	0	7.15	0	0	0	0	0	0	0
	040C240M	0	6.94	16.17	**54.61**	6.82	30.01	0	0	0	0	0	0	0	0	0	0	0
	060C060M	2.54	0	3.19	9.08	**63.64**	9.98	5.29	0	0	0	0	0	0	0	0	0	0
	060C120M	0	0	12.88	15.90	29.54	**52.51**	0	0	0	0	0	0	0	0	0	0	0
	060C180M	5.15	3.42	3.19	2.25	0	0	**52.69**	10.50	2.48	2.36	0	0	0	0	0	0	5.29
	060C240M	0	0	0	0	0	0	7.90	**44.75**	9.98	14.30	0	0	0	0	0	0	0
70.56 %	**080C060M**	0	13.77	0	0	0	0	2.61	18.39	**72.54**	2.36	0	0	0	0	0	0	2.61
	080C120M	0	0	6.49	0	0	0	10.51	13.18	9.98	**73.84**	0	0	0	0	0	0	0
	080C180M	0	0	0	0	0	0	0	0	0	0	**85.22**	9.98	20.56	0	0	0	0
	080C240M	0	0	0	0	0	0	0	0	0	0	3.67	**62.49**	11.74	3	0	0	0
	100C060M	0	0	0	0	0	0	0	0	0	0	11.11	12.53	**67.70**	0	0	0	0
	100C120M	0	0	0	0	0	0	0	0	0	0	0	15	0	**97.00**	0	0	0
	100C180M	0	0	0	0	0	5.03	0	0	0	0	0	0	0	0	**94.26**	13.30	0
	100C240M	0	0	0	0	0	2.48	0	0	0	0	0	0	0	0	5.74	**86.70**	0
	control	7.69	0	0	0	0	0	5.29	0	0	0	0	0	0	0	0	0	**89.49**

Appendix Table 13. LDA confusion table of the sunflower honey built for the classification of heat treatment level based on electronic tongue data

	Training	040C 060M	040C 120M	040C 180M	040C 240M	060C 060M	060C 120M	060C 180M	060C 240M	080C 060M	080C 120M	080C 180M	080C 240M	100C 060M	100C 120M	100C 180M	100C 240M	control
	040C060M	**87.62**	12.55	5.50	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	040C120M	6.19	**87.45**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	040C180M	6.19	0	**61.17**	22.17	0	0	0	0	0	0	0	0	0	0	0	0	0
	040C240M	0	0	11.17	**77.83**	14.99	0	0	0	0	0	0	0	0	0	0	0	0
	060C060M	0	0	5.50	0	**85.01**	0	0	0	0	0	0	0	0	0	0	0	0
	060C120M	0	0	0	0	0	**100**	0	0	0	0	0	0	0	0	0	0	0
	060C180M	0	0	0	0	0	0	**100**	4.95	4.50	0	0	0	0	0	0	0	0
88.41 %	**060C240M**	0	0	0	0	0	0	0	**85.01**	0	0	0	0	0	0	0	0	0
	080C060M	0	0	0	0	0	0	0	10.04	**95.50**	0	0	0	0	0	0	0	0
	080C120M	0	0	0	0	0	0	0	0	0	**100**	9.14	0	8.38	0	0	0	0
	080C180M	0	0	0	0	0	0	0	0	0	0	**72.71**	0	4.12	0	0	0	0
	080C240M	0	0	0	0	0	0	0	0	0	0	4.50	**100**	0	0	0	0	0
	100C060M	0	0	0	0	0	0	0	0	0	0	13.64	0	87.50	0	0	0	0
	100C120M	0	0	0	0	0	0	0	0	0	0	0	0	0	95.05	4.50	9.13	0
	100C180M	0	0	0	0	0	0	0	0	0	0	0	0	0	4.95	**86.36**	9.13	0
	100C240M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9.14	**81.74**	0
	control	0	0	16.67	0	0	0	0	0	0	0	0	0	0	0	0	0	**100**
	Validation	**040C 060M**	**040C 120M**	**040C 180M**	**040C 240M**	**060C 060M**	**060C 120M**	**060C 180M**	**060C 240M**	**080C 060M**	**080C 120M**	**080C 180M**	**080C 240M**	**100C 060M**	**100C 120M**	**100C 180M**	**100C 240M**	**control**
	040C060M	**62.78**	37.45	11.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	040C120M	12.41	**62.55**	0	11.00	0	0	0	0	0	0	0	0	0	0	0	0	0
	040C180M	12.41	0	**33.33**	22.33	20.06	0	0	0	0	0	0	0	0	0	0	0	7.08
	040C240M	0	0	33.33	**33.33**	29.94	0	0	0	0	0	0	0	0	0	0	0	0
	060C060M	0	0	0	33.33	**50.00**	0	0	0	0	0	0	0	0	0	0	0	0
	060C120M	0	0	0	0	0	**91.75**	0	0	0	0	0	0	0	0	0	0	0
	060C180M	0	0	0	0	0	8.25	**85.84**	9.91	0	0	0	0	0	0	0	0	0
	060C240M	0	0	0	0	0	0	14.16	**50.15**	18.26	9.02	0	0	0	0	0	0	0
67.20 %	**080C060M**	0	0	0	0	0	0	0	39.94	**81.74**	0	0	0	0	0	0	0	0
	080C120M	0	0	0	0	0	0	0	0	0	**81.97**	27.25	9.91	16.75	0	0	0	0
	080C180M	0	0	0	0	0	0	0	0	0	9.02	**18.26**	9.91	0	0	0	0	0
	080C240M	0	0	0	0	0	0	0	0	0	0	36.24	**80.18**	0	0	0	0	0
	100C060M	0	0	0	0	0	0	0	0	0	0	18.26	0	**83.25**	0	0	0	0
	100C120M	0	0	0	0	0	0	0	0	0	0	0	0	0	**79.94**	0	8.99	0
	100C180M	0	0	0	0	0	0	0	0	0	0	0	0	0	20.06	**81.74**	18.26	0
	100C240M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18.26	**72.75**	0
	control	12.41	0	22.33	0	0	0	0	0	0	0	0	0	0	0	0	0	**92.92**

Appendix Table 14. LDA confusion table of the bastard indigo honey for the classification of heat treatment level based on electronic tongue data

	Training	040C 060M	040C 120M	040C 180M	040C 240M	060C 060M	060C 120M	060C 180M	060C 240M	080C 060M	080C 120M	080C 180M	080C 240M	100C 060M	100C 120M	100C 180M	100C 240M	control
	040C060M	**79.12**	13.64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.17
	040C120M	20.88	**81.86**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	040C180M	0	0	**54.12**	24.95	0	4.95	0	0	0	0	0	0	0	0	0	0	0
	040C240M	0	4.50	25.00	**62.48**	27.83	10.04	0	0	0	0	0	0	0	0	0	0	0
	060C060M	0	0	8.38	12.57	**72.17**	0	0	0	0	0	0	0	0	0	0	0	0
	060C120M	0	0	4.12	0	0	**85.01**	7.07	0	0	0	0	0	0	0	0	0	0
	060C180M	0	0	0	0	0	0	**78.59**	19.97	0	0	0	0	0	0	0	0	0
75.93 %	**060C240M**	0	0	0	0	0	0	14.35	**80.03**	0	0	0	0	0	0	0	0	0
	080C060M	0	0	0	0	0	0	0	0	**100**	0	0	0	0	0	0	0	0
	080C120M	0	0	0	0	0	0	0	0	0	**70.88**	0	0	0	0	13.64	4.12	0
	080C180M	0	0	0	0	0	0	0	0	0	4.12	**83.27**	16.62	8.36	8.38	4.50	4.12	0
	080C240M	0	0	0	0	0	0	0	0	0	0	0	**66.62**	20.85	4.12	0	0	0
	100C060M	0	0	0	0	0	0	0	0	0	0	8.36	8.38	**70.79**	0	0	8.38	0
	100C120M	0	0	0	0	0	0	0	0	0	4.12	8.36	8.38	0	**87.50**	4.50	8.38	0
	100C180M	0	0	0	0	0	0	0	0	0	8.38	0	0	0	0	**54.57**	4.12	0
	100C240M	0	0	0	0	0	0	0	0	0	12.50	0	0	0	0	22.78	**70.88**	0
	control	0	0	8.38	0	0	0	0	0	0	0	0	0	0	0	0	0	**92.83**
	Validation	**040C 060M**	**040C 120M**	**040C 180M**	**040C 240M**	**060C 060M**	**060C 120M**	**060C 180M**	**060C 240M**	**080C 060M**	**080C 120M**	**080C 180M**	**080C 240M**	**100C 060M**	**100C 120M**	**100C 180M**	**100C 240M**	**control**
	040C060M	**25.00**	36.34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14.35
	040C120M	66.75	**54.64**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	040C180M	0	0	**50.13**	12.36	0	9.91	0	0	0	0	0	0	0	0	0	0	0
	040C240M	0	9.02	33.33	**37.45**	22.33	20.12	0	0	0	0	0	0	0	0	0	0	0
	060C060M	0	0	8.27	25.09	**66.67**	0	0	0	0	0	0	0	0	0	0	0	0
	060C120M	0	0	0	25.09	11	**69.97**	14.16	0	0	0	0	0	0	0	0	0	0
	060C180M	0	0	8.27	0	0	0	**57.08**	39.94	0	0	0	0	0	0	0	0	0
	060C240M	0	0	0	0	0	0	28.76	**60.06**	0	0	0	0	0	0	0	0	0
54.43 %	**080C060M**	0	0	0	0	0	0	0	0	**91.75**	0	0	0	0	0	0	0	0
	080C120M	0	0	0	0	0	0	0	0	0	**25.06**	0	0	0	0	36.24	16.75	0
	080C180M	0	0	0	0	0	0	0	0	0	8.27	**58.4**	25.00	8.27	16.75	0	8.25	0
	080C240M	0	0	0	0	0	0	0	0	0	8.27	8.27	**50.00**	25.06	8.25	0	0	0
	100C060M	0	0	0	0	0	0	0	0	0	0	16.79	0	**58.40**	0	0	16.75	0
	100C120M	0	0	0	0	0	0	0	0	0	8.27	8.27	16.75	0	**66.75**	18.26	8.25	0
	100C180M	0	0	0	0	0	0	0	0	0	25.06	8.27	8.25	0	8.25	**18.26**	0	0
	100C240M	0	0	0	0	0	0	0	0	8.25	25.06	0	0	8.27	0	27.25	**50.00**	0
	control	8.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**85.65**

Appendix Table 15. LDA confusion table of the acacia honey built for the classification of heat treatment level based on electronic tongue data

	Training	040C 060M	040C 120M	040C 180M	040C 240M	060C 060M	060C 120M	060C 180M	060C 240M	080C 060M	080C 120M	080C 180M	080C 240M	100C 060M	100C 120M	100C 180M	100C 240M	control
	040C060M	**74.96**	35.76	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21.86
	040C120M	14.99	**64.24**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	040C180M	0	0	**50.00**	0	0	0	0	0	0	0	0	0	0	0	0	0	12.48
	040C240M	0	0	5.50	**68.73**	4.50	0	0	0	0	0	0	0	0	0	0	0	0
	060C060M	0	0	0	12.55	**95.5**	0	14.35	0	0	0	0	0	0	0	0	0	0
	060C120M	0	0	11.17	12.55	0	**87.62**	0	12.50	0	0	0	0	0	0	0	0	6.29
	060C180M	0	0	0	6.18	0	6.19	**64.24**	8.38	0	0	0	0	0	0	0	0	0
82.46 %	**060C240M**	0	0	0	0	0	6.19	21.41	**79.12**	0	0	0	0	0	0	0	0	0
	080C060M	0	0	0	0	0	0	0	0	**100**	0	0	0	0	0	0	0	0
	080C120M	0	0	0	0	0	0	0	0	0	**100**	0	0	0	0	0	0	0
	080C180M	0	0	0	0	0	0	0	0	0	0	**95.50**	0	0	0	0	0	0
	080C240M	0	0	0	0	0	0	0	0	0	0	4.50	**100**	0	0	0	0	0
	100C060M	0	0	0	0	0	0	0	0	0	0	0	0	**100**	0	0	0	0
	100C120M	0	0	0	0	0	0	0	0	0	0	0	0	0	**100**	0	0	0
	100C180M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**79.12**	16.62	0
	100C240M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20.88	**83.38**	0
	control	10.04	0	33.33	0	0	0	0	0	0	0	0	0	0	0	0	0	**59.38**

	Validation	040C 060M	040C 120M	040C 180M	040C 240M	060C 060M	060C 120M	060C 180M	060C 240M	080C 060M	080C 120M	080C 180M	080C 240M	100C 060M	100C 120M	100C 180M	100C 240M	control
	040C060M	**60.06**	85.84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.19
	040C120M	30.03	**14.16**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12.57
	040C180M	0	0	**11.00**	12.41	0	12.41	0	0	0	0	0	0	0	0	0	0	31.33
	040C240M	0	0	0	**50.00**	36.34	25.19	14.16	0	0	0	0	0	0	0	0	0	6.19
	060C060M	0	0	0	37.59	**63.66**	12.41	0	0	0	0	0	0	0	0	0	0	0
	060C120M	0	0	22.33	0	0	**37.59**	14.16	8.25	0	0	0	0	0	0	0	0	0
	060C180M	0	0	0	0	0	0	**28.76**	16.75	0	0	0	0	0	0	0	0	0
	060C240M	0	0	0	0	0	12.41	42.92	**75.00**	0	0	0	0	0	0	0	0	0
58.67 %	**080C060M**	0	0	0	0	0	0	0	0	**100**	0	0	0	0	0	0	0	0
	080C120M	0	0	0	0	0	0	0	0	0	**90.98**	0	0	0	0	0	0	0
	080C180M	0	0	0	0	0	0	0	0	0	0	**81.97**	19.88	9.91	0	0	0	0
	080C240M	0	0	0	0	0	0	0	0	0	0	9.02	**60.24**	9.91	0	8.27	16.75	0
	100C060M	0	0	0	0	0	0	0	0	0	0	9.02	19.88	**80.18**	0	0	8.25	0
	100C120M	0	0	0	0	0	0	0	0	0	0	0	0	0	**100**	0	0	0
	100C180M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**58.40**	33.25	0
	100C240M	0	0	0	0	0	0	0	0	0	9.02	0	0	0	0	33.33	**41.75**	0
	control	9.91	0	66.67	0	0	0	0	0	0	0	0	0	0	0	0	0	**43.71**

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