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ÚSTAV CHEMIE POTRAVIN A BIOTECHNOLOGIÍ

DETERMINATION OF HONEY AUTHENTICITY AND ADULTERATION BY ANION EXCHANGE CHROMATOGRAPHY

STANOVENÍ PRAVOSTI A FALŠOVÁNÍ MEDU IONTOVĚ VÝMĚNNOU CHROMATOGRAFIÍ

MASTER'S THESIS
DIPLOMOVÁ PRÁCE

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2) Optimization of method based on the determination of carbohydrates profile in honey
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ABSTRACT

The purpose of this thesis is to determine the quality and composition of honey for the investigation of its botanical origin by analysing a broad carbohydrate profile in honey. Honey is a natural product of high quality and due to its high consumption and market value it is also prone to adulteration. In order to facilitate the identification of fraud in honey and identify adulterants originating from sugar syrups, oligosaccharides and polysaccharides based on maltodextrins were analysed.

The theoretical part describes the chemical composition of honey and the main principles of the applied instrumental technique. It provides a literature overview of existing analytical methods for the determination of carbohydrates present in honey as well as for the detection of adulteration. The main characteristics of adulteration and authenticity are defined, including the legislative aspects and description of common type of adulterants.

The experimental part includes the different steps followed in order to develop and optimize the chromatographic conditions and parameters of the electrochemical detector for the determination of carbohydrates and maltodextrins (oligosaccharides and polysaccharides) in honey samples. The analytical technique applied was high-performance anion-exchange chromatography (HPAEC) coupled with pulsed amperometric detection (PAD). The analytical methods were validated in terms of linearity, dynamic range, analytical limits, precision and trueness. The applicability of the analytical methods was successfully evaluated by the analysis of authentic and adulterated honey samples of different botanical origin collected by EU Member-States.

Keywords: honey, carbohydrates, oligosaccharides, polysaccharides, authenticity, adulteration, anion-exchange chromatography

ABSTRAKT

Cieľom tejto diplomovej práce je stanoviť kvalitu a zloženie medu, taktiež stanoviť jeho botanický pôvod na základe profilu sacharidov v mede. Med je prírodný produkt vysokej kvality a vďaka značnej spotrebe a nemalej trhovej hodnote je tiež terčom na falšovanie. S cieľom identifikovať podvody a cudzorodé látky, pochádzajúce z cukrových sirupov, boli analyzované oligosacharidy a polysacharidy na báze maltodextrínov.

Teoretická časť popisuje chemické zloženie medu a hlavné princípy aplikovanej inštrumentálnej techniky. Poskytuje literárny prehľad existujúcich analytických metód na stanovenie sacharidov prítomných v mede a na odhalenie jeho falšovania. Boli definované hlavné body falšovania a autenticity, vrátane legislatívnych aspektov a opisu bežných druhov falšovania.

Experimentálna časť obsahuje postupy, ktoré vedú k vývoju a optimalizácii chromatografických podmienok a parametrov elektrochemického detektora na stanovenie sacharidov a maltodextrínov (oligosacharidov a polysacharidov) vo vzorkách medu. Použitá analytická technika bola vysoko účinná aniónovo-výmenná chromatografia (HPAEC) spojená s pulzným amperometrickým detektorom (PAD). Vyvinutá a optimalizovaná chromatografická metóda bola taktiež validovaná z hľadiska linearity, dynamického rozsahu, analytických limitov, presnosti a správnosti. Na záver bola táto vypracovaná metóda úspešne zhodnotená analýzou autentických a falšovaných vzoriek medu rôzneho botanického pôvodu pochádzajúcich z členských štátov EÚ.

Kľučové slová: med, uhľohydráty, oligosacharidy, polysacharidy, autenticita, falšovanie, aniónovo-výmenná chromatografia

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DECLARATION

I declare that the diploma thesis has been worked out by myself and that all the quotations from the used literary sources are accurate and complete. The content of the diploma thesis is the property of the Faculty of Chemistry of Brno University of Technology and all commercial uses are allowed only if approved by both the supervisor and the dean of the Faculty of Chemistry, BUT.

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student's signature

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CONTENTS

1. INTRODUCTION

Honey is natural product with complex and variable components. According to the definition given by Codex Alimentarius of the Food and Agriculture Organization of the United Nations (FAO), "Honey is the natural sweet substance, produced by honeybees from the nectar of plants or from secretions of living parts of plants, or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature" [1,2].

The European Union definition is similar but specifies the biological species of honey-producing insects: "Honey is the natural sweet substance, produced by *Apis mellifera* bees from the nectar of plants or from secretions of living parts of plants, or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature" [3].

It is the oldest natural sweetening agent and because of its high nutritional value and healing properties its consumption has increased dramatically in the last decades. It is one of the most complex food products by nature and the only sweetening that can be used by the humans without processing. It has been considering being a valuable product since ancient times and having different properties such as a prebiotic, antioxidant, antibacterial and antimutagenic, among others. The potential prebiotic activity is caused by honey oligosaccharides (prebiotic index values between 3,38 and 4,24) by increasing the populations of *Bifidobacteria* and *Lactobacilli* [4,5,6,7,8].

Based on color there are two types of honey: light and dark. The dark honey is considered more nutritious such as richer in minerals. Honey can be also classified as honeybee (*Apis mellifera*) and stingless bee (*Meliponini)* honey. The different between bees is in sting and size. Honeybees are using sting for defend and they are bigger in size. Honey produces by these bees is sweet. On the other hand, stingless honey bees do not sting, they exhibit other defensive behaviors and mechanisms and they are smaller in size. Honey produced by these bees is a mixture of sweet and sour taste [9,10,11].

2. THEORETICAL PART

2.1 Honey composition

Honey contains lots of nutritious compounds that are essential for human body, including sugars and water as the main chemical constituents of honey (more than 95 %) and the minor components represented by amino acids, organic acids, carotenoids, vitamins, minerals and numerous volatile compounds [3,12].

The honey compositions, color and flavor are rather variable and depend primarily on its floral and geographical source as well as on external factors, such as thermal processing, packing and storage conditions (fermentation, oxidation and thermal processing). For example, 5-hydroxymethylfurfal (5-HMF), which is product of Maillard reaction, can be formed when honey is submitted to a long storage time or heat treatment. 5-HMF can be also produced by dehydration of sugars in an acidic environment, such as honey. This compound can become volatile and toxic, depending on its concentration [6,13,14,15].

The other products of sugar degradation produced during heat treatment in presence of amino acids are 2-acetylfuran, isomaltol, 3,5-dihydroxy-2-methyl-5,6-diidropiran-4-one and maltol. These compounds are contributing to the change in color, taste and odor of honey [16].

One of the natural honey products is propolis that is waxy and resinous. Propolis is rich in carbohydrates (49 %) and crude fiber (44 %). The rest of components are moisture (23 %), crude fat (21 %), ash (4 %) and crude protein (3 %) [9,17].

2.1.1 Sugars

The sugar is formed from the nectar and honeydew by using bee enzyme diastase (amylases) and invertase (α-glucosidase) during storage and maturation in the beehive. During the process, diastase and invertase catalyze the conversion of the sugars of nectar and honeydew into fructose and glucose which represent the main constituents of the sugars. The final mixture contains about 70 % monosaccharides and 10-15 % disaccharides composed of glucose and fructose with the glycosidic bond in different positions and configurations. The minor components consisting of oligosaccharides (three to ten monosaccharides linked together) and dextrins (polymers of D-glucose units) [6,18].

The percentage sugar content ranges from between 30,91 and 44,26 for fructose and between 22,89 and 40,75 for glucose. So generally, fructose is the dominant component and only in very few honey types, such as rape (*Brassica napus*) and dandelion (*Taraxacum officinale*), the glucose fraction become greater than fructose. The fructose/glucose ratio (F/G) is typically between 0,76 and 1,86. This ratio is important parameter to predict the crystallization tendency of honey. Honey samples, which do not crystalize for a long time have F/G ratio greater than 1,33, and if the ratio is less than 1,11, the honey crystallizes quickly. The F/G ratio is recommended to determinate this phenomenon because glucose is less water soluble than fructose. Glucose crystallizes as α-D-glucose monohydrate below 50 °C, while anhydrous forms of α and β-D-glucose are stable at 50-80 °C. Glucose changes from the monohydrate to

the anhydrous form at temperatures below 30 °C when saturated with fructose. The other important factor is ratio of glucose and water (G/W) and storage temperature. Slow crystallization is indicated when G/W in honey is less than 1,7 and when the ratio is greater than 2,0 the phenomenon is fast and complete [12,19,20,21,22].

2.1.2 Proteins

The difference in protein and amino acids composition of honey is related to the species of the honeybees as well as to animal and vegetal sources, including fluids and the nectar secretions of the salivary glands and pharynx of honeybees. But the main source of protein is the pollen [23].

Amino acid content in honey is about 1 % among which proline is the main one (50-80 %). It originates mainly from the salivary secretions of honeybees during conversion of nectar into honey. Beside proline the most common are glutamic acid, alanine, phenylalanine, tyrosine, leucine and isoleucine [24,25].

A small fraction of the proteins in honey is presented by enzymes such as invertase, α- and β-amylases. A-amylase is responsible for hydrolyzing starch chains in the α-D-(1→4) linkages and produces dextrin. B-amylase hydrolyzes starch chain at the end and leads to the production of maltose. Whereas invertase is responsible for hydrolysis of sucrose into fructose and glucose. Another present enzyme is glucose oxidase that converts glucose into δ-gluconolactone, which is hydrolyzed to gluconic acid. This enzyme also produces hydrogen peroxide, which has bactericidal action [14,26].

2.1.3 Organic acids

Honey has a slight acidity as a result of approximately 0,57 % organic acids. They are produced by honeybees using enzymes during transforming the nectar into honey or can be obtained directly from nectar [27,28].

Organic acids can be also used for the determination of the botanical and geographical origin. These acids are related to the color and flavor of honey and they are responsible also for chemical properties such as acidity, pH and electrical conductivity.

The main acid in honey is represented by gluconic acid (from activity of glucose oxidase as explained in previous chapter). Other organic acids present in honey (such as levunilic, formic, acetic, citric acid, isocitric, 2-hydroxybutyric, tartaric, lactic, malonic, methylmalonic and others) are also important, because their concentration together with gluconic acid is used as parameter to differentiate floral honey from honeydew [27,29].

2.1.4 Vitamins

Honey contains small amounts of vitamins, which are from the pollen grains in suspension. Mainly B complex include thiamine (B1), riboflavin (B2), nicotinic acid (B3), pantothenic acid

(B5), pyridoxine (B6), biotin (B8 or H) and folic acid (B9). Vitamin C is also found almost in all type of honey and it is adding value due to its antioxidant effect. But it is not good indicator because of its instability. It is very vulnerable to chemical and enzymatic oxidation and has an accelerated degradation rate due to various factors such as light, oxygen or heat [30,31].

2.1.5 Minerals

The mineral content in honey is from 0,04 % in light honeys to 0,2 % in dark honeys. This composition reflects the chemical components of the plants from which the honeybees collect their food. It depends also on the type of soil in which they were found [23,24].

The most abundant element is potassium. Usually is corresponding to one third of the total mineral content in honey. Potassium with calcium and sodium represent macro elements of minerals in honey. In smaller quantities also other minerals at trace levels are present such as sodium, iron, copper, silicon, and manganese. Both groups perform a fundamental function in biological systems including the following: maintaining normal physiological responses, inducing the overall metabolism, influencing the circulatory system and reproduction, and catalyzing in various biochemical reactions [24].

The mineral elements in comparison with vitamins and amino acids are not subjected to degradation by exposure to heat, light, oxidizing agents, extreme pH or other factors that affect organic nutrients. That means they are indestructible [32].

2.1.6 Phenolic compounds

Phenolic groups are a chemically heterogeneous group, with approximately 10 000 compounds, which are grouped into different classes according to their basic chemical structures. They contain an aromatic ring with one or more hydroxyl groups and they can vary from a simple to a complex molecule phenolic polymer of high molecular weight. They can be divided into two groups: non-flavonoids (phenolic acid) and flavonoids (flavones, flavonols, flavanones, flavanols, anthocyanidin, isoflavones and chalcones) [33,34].

The main functional components of honey are flavonoids. They significantly increase total antioxidant activity of honey and bring beneficial effects for human health. Their antioxidant activity in most cases depends on the number and position of hydroxyl groups, other substituents and the glycosylation of flavonoid molecules [35].

2.1.7 Volatile compounds

Volatile compounds in honey are responsible for aroma of honey. They may come from the nectar or honeydew collected by bees and from the plant of honey origin. They can be transferred from the plant or honeybees can also produce or convert plant constituents in other compounds with volatile properties. Moreover, these compounds can be affected by postharvest

processing, from the presence of microorganisms. The may differ depending on the nectar, processing conditions, origin and storage [16,25,36].

Volatile compounds are present in honey in very low concentrations, which generally belong to monoterpenes, C13-norisoprenoid, sesquiterpenes, benzene derivates and to a lower content of superior alcohols, esters, fatty acids, ketones, terpenes and aldehydes. Alcohols are a large and important class of volatile compounds present in honey. They provide freshness to honey by methyl groups. Examples of such compounds are 3-methyl-3-butene-1-ol and 2-methyl-2-buten-1-ol [36,37].

The different flavor that can range from spicy to rancid is depending on length of the carboxylic acids carbon chains. The short chain such as acetic acid has a spicy aroma and flavor, while the butanoic and hexanoic acid cause rancid aroma [15].

2.2 Authenticity

The major concern of honey quality is to ensure that honey is authentic in respect to the legislative requirements. According to the definition of the Codex Alimentarius and other international honey standards honey shall not have added any food ingredient than honey to it nor shall any particular constituent be removed from it. Honey shall not have any objectionable matter, flavor, aroma or taint from foreign matter during its processing and storage. The honey shall not have begun to ferment or effervesce. No pollen or constituent particular to honey may be removed except where this is unavoidable in the removal of foreign inorganic or organic matter. Honey shall not be heated or processed to such an extent that its essential composition is changed and/or its quality impaired [2,3].

Honey made primarily from the nectar of one type of flower is called monofloral honey, whereas honey made from several types of flowers is called polyfloral honey. Monofloral honey has a high commercial value in the marketplace due to characteristic flavor. However, most commercially available honey is a blend of honeys differing in floral source and geographic origin because honeybees usually feed on various plants [6,38].

Honey authenticity has two main aspects such as origin of honey and the mode of production of the honey. The origin of the honey includes geographical origin and botanical origin, while production is related to the harvesting of honey hives and processing.

The carbohydrates' composition in honey depends on the nectar collected from the flowers by the bees and the regional climatic conditions. Beside fructose and glucose, about 25 different oligosaccharides have been detected in the composition of honey [38,39].

2.3 Adulteration

As a result of the high nutritional value and the unique flavor characteristics of natural bee honey, its cost is much greater than of any other sweetener. That makes honey perfect target of adulteration especially for economics gains. Adulteration includes addition with low cost and nutritional value substances or mislabeling regarding the botanical origin. Adulteration also influences the safety of honey by adding chemicals with lower medicinal values as well as may

contain compounds which harm the consumers. The main type of adulteration is shown in **Figure 1**. [1,40].

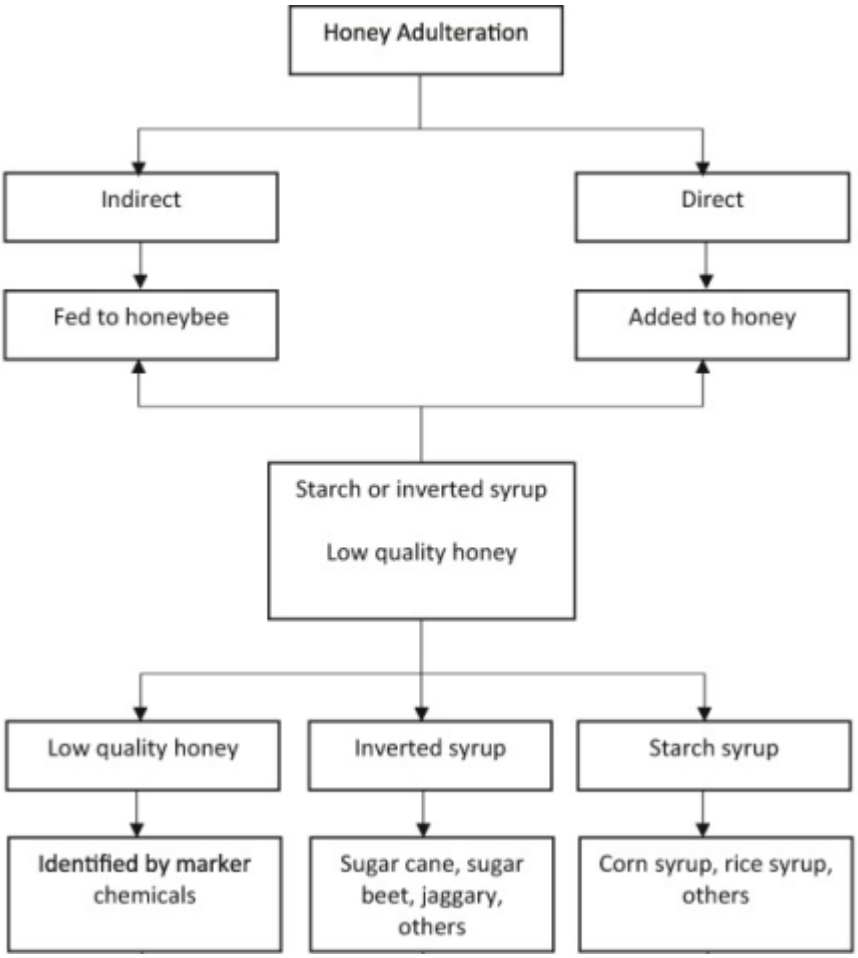

Figure 1 *Type of adulteration*

2.3.1 Photosynthetic pathway of the plants

Honey adulterated by plant sources are categorized as C3, C4 and CAM as per their carbon metabolism during photosynthesis, which are shown in **Figure 2**. Plants that are categorized as C3 fix carbon dioxide via Calvin (C3) cycle and use enzyme called ribulose bisphosphate carboxylase/oxygenase (RUBISCO), which has similar isotopic composition to natural honey but is creating number of oligosaccharides and polysaccharides and represent about 90 % of plants. Whereas C4 plants fixing carbon dioxide using the Hatch-Slack (C4) cycle and it has influence on isotopic composition but not produce oligosaccharides neither polysaccharides. They are typically originated from hot climates. The third groups of plants are the CAM plants, which have a unique metabolism called the "Crassulacean Acid Metabolism". They generally use C4 pathway, but they can also use the C3 pathway. CAM plants are mostly from very arid environments. Examples of plants following different photosynthetic pathways: C3 plants:

sugar beet, rice; C4 plants: corn, sugar cane, maize; CAM plants: pineapple, cacti, agave [41,42,43].

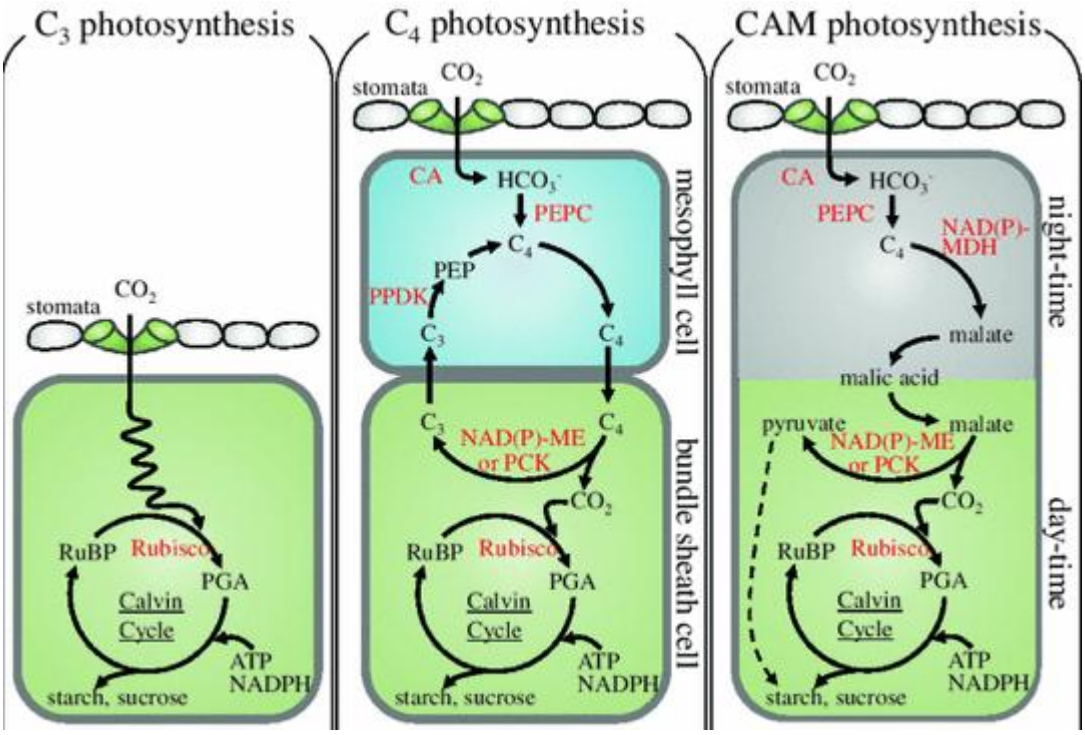

Figure 2 *Photosynthetic pathways*

2.3.2 Direct Adulteration

The most common adulteration is direct one, when low cost substances are added into honey. Mostly inexpensive sweetening products such as corn syrup, saccharose syrups from sugar beet and cane, invert syrup, glucose syrup or high fructose corn syrup (HFCS), are evolved from the basic addition of sugar and water to these specially produced syrups. According to hydrolysis, they contain variable mixture of sugars such as glucose, maltose, maltotriose, dextrins, and so on. This carbohydrates profile can be manipulated to resemble the carbohydrate profile of honey [44,45,46,47,48].

The resulting mixture of fructose and glucose in inverted sugar syrups is caused by hydrolysis of sucrose catalyzes by β-fructofuranosidase. Related to invertases are sucrases, which give the same mixture of glucose and fructose. Invertases cleave the O-C (fructose) bond, whereas the sucrases cleave the O-C (glucose) bond. The small addition of invert syrups does not change the normal ranges of concentration for fructose and glucose. This fact is making detection of adulteration difficult by common methods [49].

Inverstase is usually derived from yeast or it is also synthesized by bees, which use it to make honey from nectar. The optimum reaction temperature is 60 °C and optimum pH is 4,5. Typically, sugar is inverted with sulphuric acid.

HFCS is cheaper product than unadulterated honey while its composition is similar to natural honey. Three different enzymes (α-amylase, glucoamylase, and gluco-isomerase) are needed to transform corn starch into simple sugars glucose and fructose. After a complex fractionation and combination process, mixtures with various amounts of fructose can be obtained for HFCS.

High fructose inulin syrups (HFIS) contain mainly oligofructose, which is a mixture of oligosaccharides of various chain lengths having fructose units linked by β-(2→1)- bonds to a final single glucose unit. Inulin is also used for the production of other syrups for adulteration with different degrees of polymerization. They are produced by partial enzymatic hydrolysis of inulin [50,51,52].

The jaggery is another possible adulterant. The normal composition of jaggery is up to 50 % sucrose, up to 20 % invert sugars, and up to 20 % water together with insoluble matters such as proteins and fibers. The jaggery is considered like perfect adulterant due to its low cost and natural brown color which makes distinguishing from nature honey very hard [53].

Oligosaccharides and polysaccharides can be present in sugar syrup as a result of incomplete enzymatic hydrolysis of starch. They are composed only of glucose monomers with alpha (1-4) and/or alpha (1-6) linkage. The degree of polymerization (DP) for oligosaccharides is from DP 3-11 and for polysaccharides from DP 12 and higher. In pure honey (linden, manuka, polyfloral) is possible to detect oligosaccharides between DP 3-10. In adulterated samples they can appear between DP 12-19 and higher. The example of such a syrup is rice syrup, which is coming from C3 plants following a similar Calvin cycle of photosynthesis as the plants from which natural honey is made [18,54,55].

Another possibility of honey adulteration is mixing high-cost honey with less expensive honey. For example, Acacia honey is frequently adulterated by rape honey. Acacia honey is coming from *Robinia pseudoacacia* blossoms and it has quite transparent pale-yellow color and does not crystallize. While rape honey from rape flower is sweet, alight amber color and it is easy to crystallize. Because of their similar colors, rape honey is often used as adulterant [56,57].

Plant syrups, obtained by heat concentration of vegetable juices or plant sap, can also be used like adulterants. There are three types of plant syrups such as “miel de palma” (palm syrup or honey), “arrope” (must syrup) and “miel de cana” (sugar cane syrup), which are reported from Spain. While palm syrup is made by making cuts in the central bud at the top of the trunk of the endemic palms (*Phoenix canariensis*) and the sap which drips is concentrated by heating to 12,5–20 % of the initial weight. The result of this process is brown syrup with high viscosity and nice aroma. Must syrup is made from the fresh grape juice that is heat-concentrated to 20-25 % of the initial volume. Moreover, sugar cane syrup is produced from crushed sugar cane which is heated rapidly and then decanted and filtered. By further heating it becomes similar to dark honey. These products are potential direct or indirect adulterants [58].

2.3.3 Indirect adulteration

Bees need additional feeding at certain moments during year, particularly in winter. This feeding is needed to maintain breeding activities and to meet food requirements. The traditional substitute is a sucrose solution in ratio 3:2 (sugar: water), sometimes 1:1. Different sucrose-based bee feeding products exist and they differ in their composition depending on their uses (winter feeding, spring stimulation feed or early winter feeding). Beside sucrose there are other commercially available sugar syrups for feeding bees such as syrups from starch, sugar cane, sugar beet, agave or syrups of natural origin such as maple. Commercial starch-based products are made up of differing amounts of glucose and maltose as well as maltooligosaccharides and isomalto-oligosascharides (they are of higher molecular weight). Of these constituents, only glucose occurs in large quantities in natural honeys, whereas maltose can account for up 8 % and oligosaccharides are present in smaller concentrations [6].

Indirect adulteration is considered feeding bees during the main nectar flow period. Detection of adulteration in this case is also required, but it brings lots of challenges, since it is still unknown how honeybees use commercial sugar syrups after being fed and how they convert these sugars [59,60].

Proline has been used as criterion for the evaluation of the maturation of honey, and in some cases, adulteration with sugars. A minimum value of 180 mg kg^{-1} of proline is accepted as the limit value for pure honey [61].

2.4 Analytical techniques

Various analytical techniques to identify fraud in honey samples have been applied and more are being developed. Recent review papers present a wide range of analytical methods developed for the detection of adulteration in honey. To be able to detect this adulteration different type of methodologies were developed based on the detection of markers such as, oligosaccharides, polysaccharides and linked to specific syrups including 2-acetylfuran-3-glucopyranoside (AFGP), difructose anhydrides (DFAs) and inulin [47,62].

2.4.1 High-Performance Anion-Exchange Chromatography coupled with Pulsed Amperometric Detection

Analytical methods using liquid chromatography for analysis of carbohydrates have often utilized silica-based amino-bonded or polymer-based, metal-loaded, cation-exchange columns, with refractive index (RI) or low-wavelength ultraviolet (UV) detection. Disadvantages of those methods are that they require attention to sample solubility, sample concentration and, in the case of the metalloaded cation-exchange columns, also require column heating. Moreover, RI and UV detection methods are sensitive to eluent and sample matrix components. That's why using gradient is not possible and proper sample cleanup prior to injection is required. As a solution of these problem High-Perfomance Anion-Exchange Chromatography coupled with

Pulsed Amperometric Detection was developed to effectively separate carbohydrate fraction [63,64,65].

2.4.1.1 Principle

The technique enables separation of all classes of alditols, amino sugars, mono-, oligo- and polysaccharides, based on structural features such as size, composition, anomericity and linkage isomerism. The main parameters affecting the separation are the number of hydroxyl group, anomerism, positional isomerism and the degree of polymerization. Monosaccharides possess several potentially ionisable hydroxyl groups with, the following hierarchy of acidity:
1-OH > 2-OH ≥ 6-OH > 3-OH > 4-O [63,64,65,66].

This chromatography takes advantage of weakly acidic nature of carbohydrates to give highly selective separations at high pH using a strong anion-exchange stationary phase. The range of pKa values is from 12 to 14 (the examples are shown in **Table 1**). At high pH values their hydroxyl groups are partially or totally transformed into oxyanions, enabling this class of compounds to be selectively eluted as anions [63,64,65,66,67].

Table 1 *Dissociation constants of some common carbohydrates (in water at 25 °C)*

Sugar	**p*K*a**
Fructose	12,03
Mannose	12,08
Xylose	12,15
Glucose	12,28
Galactose	12,39
Dulcitol	13,43
Sorbitol	13,60
α-Methyl Gluoside	13,71

2.4.1.2 Columns

This technique, on high pH-resistant polymeric-based strong anion-exchange columns specifically designed for carbohydrate analysis, enables selective elution of carbohydrates. Under alkaline conditions, carbohydrates are readily separated by quaternary-ammonium-bonded pellicular anion-exchange columns (**Figure 3**), where the order of increasing retention is correlated with decreasing pKa value. The most common columns employed in HPAE-PAD, specifically designed for carbohydrate anion-exchange chromatography, are manufactured by Thermo Fisher Scientific. The type of columns and characteristics are shown in the **Table 2** [66,67,68].

Table 2 *Most employed columns for HPAEC-PAD analysis*

Column	Specifications	Samples	Notes
CarboPac MA1	7.5 μm diameter vinylbenzylchloride-divinylbenzene macroporous substrate fully functionalized with alkyl quaternary ammonium groups (15% crosslinked)	Reduced mono- and disaccharide alditol analyses	Other carbohydrates: fucose, N-acetyl-(D)-glucosamine, N-acetyl-galactosamine, mannose, galactose
CarboPac PA1	10 μm diameter substrate (polystyrene 2% crosslinked with divinylbenzene) agglomerated with MicroBead quaternary ammonium functionalized latex (5% crosslinked)	Mono- and disaccharides, oligosaccharides	Column well suited for the analysis of food for nutrition labelling
CarboPac PA10	10 μm diameter substrate (ethylvinylbenzene 55% cross linked with divinylbenzene) agglomerated with a 460 nm MicroBead difunctional quaternary ammonium ion (5% crosslinked)	Mono- and disaccharides, glucosamine, galactosamine; sialic acids	Ideal for the analysis of mono- and disaccharides in foods, drugs, and plants
CarboPac PA20	6.5 μm diameter substrate (ethylvinylbenzene 55% crosslinked with divinylbenzene) agglomerated with a 130 nm MicroBead difunctional quaternary ammonium ion (5% crosslinked)	Mono- and disaccharides	High resolution regarding the six monosaccharides commonly found in mammalian glycoproteins
CarboPac PA100	8.5 μm diameter substrate (ethylvinylbenzene 55% crosslinked with divinylbenzene) agglomerated with a 275 nm MicroBead quaternary amine functionalized latex (6% crosslinked)	Separation of closely related oligosaccharides, oligosaccharides released from glycoproteins which can differ in size, charge, branching, and linkage	Separation of neutral and charged oligosaccharides in the same run Separation of closely related oligosaccharides
CarboPac PA200	5.5 μm diameter substrate (ethylvinylbenzene 55% crosslinked with divinylbenzene) agglomerated with a 43 nm MicroBead quaternary amine functionalized latex (6% crosslinked)	Oligosaccharides	Separations based on size, charge, degree of branching, and linkage isomerism
CarboPac SA10	6.0 μm diameter substrate (ethylvinylbenzene 55% crosslinked with divinylbenzene) agglomerated with a 55 nm MicroBead quaternary ammonium ion (4.5% crosslinked)	Mono- and disaccharides	Fast analysis of mono- and disaccharides of food interest.

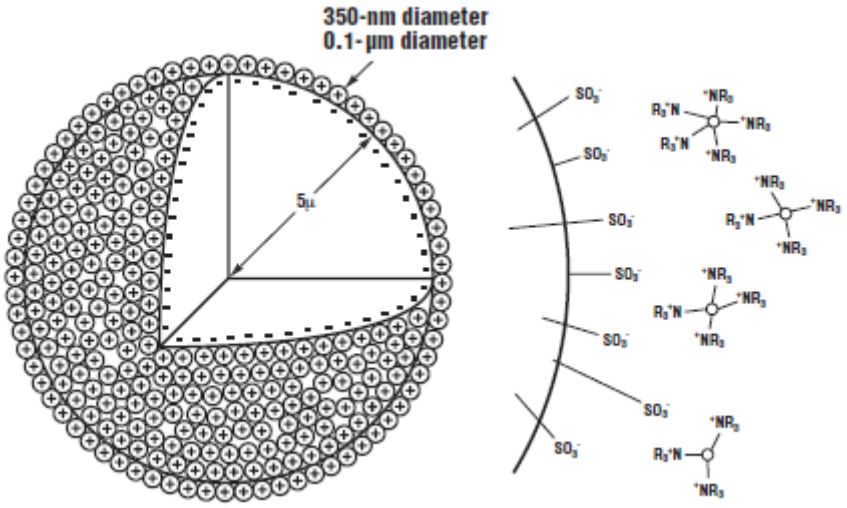

Figure 3 *Pellicular anion-exchange resin bead*

2.4.1.3 Detector

The choice of detector permits direct quantification of nonderivatized carbohydrates at low-picomole levels with minimal sample preparation and cleanup. The compatibility of electrochemical detection with gradient elution coupled with the high selectivity of the anion-exchange stationary phases allows mixtures of simple sugars, oligo- and polysaccharides to be separated with suitable resolution in a single run [63,64,65,66].

The advantage of the applied pulsed amperometry detector is its detection of carbohydrates with excellent signal-to-noise ratio without requiring derivatization. The detector is measuring the electrical current generated by their oxidation at the surface of a gold electrode. The cleaning process is required as well because the products of this oxidation reaction poison the surface of the electrode. This is accomplished by first desorption of the carbohydrate oxidation products by raising the potential to a level sufficient to oxidize the gold surface.

The detection thus includes a repeating sequence of three potentials (**Figure 4**). E_1 is the first potential at which carbohydrate oxidation is measured.

The second potential is E_2, and it is a more positive potential that oxidizes the gold electrode and cleans it of products from the carbohydrate oxidation. It has to be high and long enough to oxidize the electrode surface fully, but not too excessive because otherwise gold oxidation will occur, and the electrode will wear too rapidly.

The last potential E_3 reduces the gold oxide on the electrode surface back to gold. This step allows detection during the next cycle at E_1. It must be low enough for reducing the oxidized surface completely without being so low that chemical reductions will occur. This case can lead to baseline disturbances during subsequent measurement.

These three potentials are applied for fixed durations referred to as t_1, t_2 and t_3. The step from one potential to the next produces a charging current that is not part of the analyte oxidation current, so the analyte oxidation current is measured after a delay that allows the charging current to decay. The detector response is measured in coulombs by integrating the cell current after the delay. Alternatively, the amperes can be used as well in the case when the average current during the integration period is reported.

Optimal potentials can be determined by electrochemical experiments such as cyclic voltammetry, in which the applied potentials are slowly scanned back and forth between positive and negative potential limits [67].

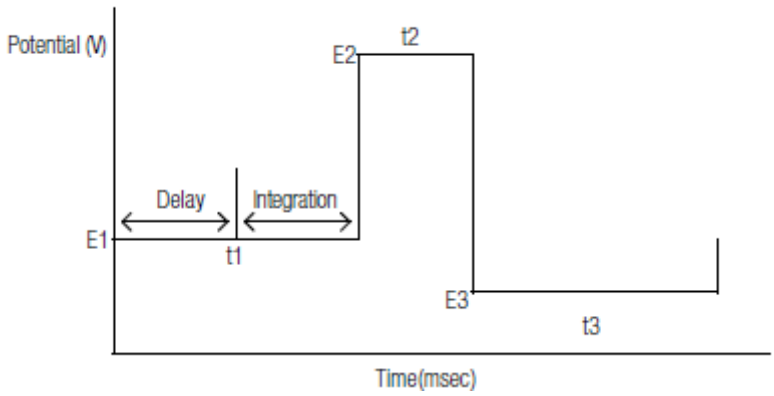

Figure 4 *Diagram of the pulse sequence for carbohydrate detection*

2.4.2 Existing methods for determination of carbohydrates and adulteration in honey

Different chromatographic methods such as high-performance liquid chromatography, gas chromatography as well as HPAE-PAD have been developed for evaluating the sugar content of honey and for detecting adulteration. For detecting adulteration analytical platform based on isotopic measurements have been developed. Rapid analytical methods for determining honey quality have been applied, such as, nuclear magnetic resonance, infrared spectroscopy and Raman spectroscopy [69].

2.4.2.1 Thin-layer chromatography

Thin-layer chromatography (TLC) is the first official method for detection honey adulteration and perfect method for detecting HFCS in honey. The carbohydrates have to be separated first to achieve the required degree of sensitivity. On the other hand, charcoal-Celite column is used for isolation of fraction containing oligo- and polysaccharides. The fraction is then examined by TLC on silica gel. The pure honey samples bring only 1 or 2 blue-grey or blue-brown spots at Rf values above 0,35, while the adulterated samples yield an additional series of spots or blue streaks [70].

High performance thin-layer chromatography (HPTLC) is a modern and simple analytical method based on the similar principles as TLC. Chromatographic separation is made on HPTLC silica gel plates, followed by developing twice with ethyl acetate:pyridine:water:acetic acid, 6:3:1:0,5. The last step is done by dipping in an immersion solution [71].

Although the identification of adulterant by the TLC-based methods is simple and fast its application is limited, because it produces false positives results due to hydrolysis of oligosaccharides. These results are present mainly because of higher amount of smaller oligosaccharides in pure honey. The increase in variety of adulterants apart from HFCS led to development of new methods [71].

2.4.2.2 Gas chromatography

Gas chromatography (GC) is used for analysing mono-, di-, and trisaccharides in honey with a relatively high resolution and sensitivity. This technique is used mainly for detection of adulteration by HFCS, maltose and isomaltose. It was successfully used to detect fraudulent addition of industrial syrups (5-10 %) [72,73].

Difructose anhydrides (DFAs) are not detected in pure honey samples. These compounds are formed during caramelization and they contain two fructose residues. They are considering like pseudo-disaccharides. The caramelization is reaction occurs during heating of sugars and it leads to a smaller fraction of volatile compounds and higher level of non-volatile components (90-95 %), including DFAs. The DFAs content depends on food composition and processing. GC was the first method which detected HFCS, DFAs and invert sugars in honey samples [73].

Studies have shown that to detect HFCS and invert sugar syrups in honey samples, require the step to remove major sugars such as monosaccharides. To detect DFAs new methodology has been developed. This methodology is based on yeast (*Saccharomyces cerevisiae*) treatment and detection of DFAs is achievable at a concentration higher than 5 % (w/w). Incubation of such honey samples, with yeast and adulterated by one of the mentioned syrups, concentrates the level of DFAs, which are then easily detected by GC [74].

Inulotriose is good marker in adulteration with HFIS since it is not detected in pure honey samples. HFIS is detected by gas chromatography coupled with mass spectrometry (GC/MS).

GC/MS analysis of HFCS first showed the presence of fructosyl-fructoses and some unknown carbohydrates, related to fructosyl-glucoses. Honey from caged bees fed with HFCS contain fructosyl-fructoses, while honey from free-flying bees or fed by sucrose don't. This leads to easy detections of honey adulteration by using GC/MS, based on the present of fructosyl-fructoses [52,60].

GC is a first-choice technique to detect HFCS adulteration, because of its high sensitivity. This technique is also considered suitable for detection HFIS adulteration, based on present of marker inulotriose. The detection of HFCS is mainly based on DFAs, but under yeast treatment condition, which is not essential in other advanced techniques. The risk of using DFAs as marker is linked to the formation of DFAs under storage conditions with temperature higher than room temperature [74].

2.4.2.3 High-performance liquid chromatography

Starch syrup as adulterant can be easily detected by using high-performance liquid chromatography (HPLC) coupled with a refractive index detector (RID) by detecting the oligosaccharides peak as a syrup indicator. This is done by overlapping peak of oligosaccharides with degree of polymerization higher than DP5. This characteristic peak is identified by comparing the chromatographs of starch syrup and a series of standard mono-, di- and non-separated oligosaccharides of DP3-7 [75].

Another technique for detection of adulterant is HPLC coupled with diode array detection (HPLC-DAD). It is based on using 2-acetylfuran-3-glucopyranoside (AFGP) as marker of adulteration. This compound is not present in pure honey, while is observed in rice syrups. HPLC-DAD can rapidly detect honey samples with added rice syrup from 10 % [76].

HPLC has proven to be a widely accepted simple method to detect both C3 and C4 starch syrup as well as rice syrup [75,76].

2.4.2.4 Stable carbon isotope ratio analysis and detection of honey adulteration

Based on different chemical pathways during photosynthesis we can divide plants into C3, C4 and CAM plants. Considering these facts different methods based on stable carbon isotope ratio analysis were developed. Isotope ratio mass spectrometry (IRMS) is nowadays considered one of the best techniques for detection of honey adulteration. This technique enables the precise and accurate measurement of variations in the natural isotopic abundance of stable

isotopes of carbon ($^{13}C/^{12}C$). Isotopic ratios are measured relative to a working reference gas calibrated using internationally accepted standards and the results are reported using delta notation (δ) and expressed in units per mill (‰). The delta notation is defined as:

$$\delta^{13}C\ (‰) = \left[R\ (Sample) / R\ (Standard) - 1\right] \times 1000 \quad (2.1)$$

where R is the ratio $^{13}CO_2/^{12}CO_2$. C3 plants yield values ranging from -23 to -28 ‰, whereas C4 plants exhibit the values ranging from -9 to -15 ‰ [43].

Adulterants like sugar syrups produced from C4 plants as corn and sugar cane are detected by IRMS based on elemental analysis (EA-IRMS). Sugar syrups from C3 plants can not be detected by this method.

IRMS coupled with liquid chromatography (LC-IRMS) determinate $\delta^{13}C$ isotopic ratios of individuals sugars present in honey (glucose, fructose and sucrose) and it is able to detect adulteration with C3 plants (sugar beet) but also with C4 sugars (sugar cane, isoglucose syrup and HFCS). The adulteration with rice syrup (C3 plants) can be detected only by using the percent area of the oligosaccharides peak parameter. When the adulterants are carefully selected to have similar isotopic patterns, alternative analytical methods, which are more specific and more sensitive for detection oligo- and polysaccharides are required [43].

2.4.2.5 Quadrupole time-of-flight mass spectrometry

Quadrupole time of-flight mass spectrometry (Q-TOF-MS) provides accurate mass measurements of full-product ions, thereby ensuring sensitive and accurate analyte identification. TOF MS and Q-TOF MS operate in full scan or product ion scan modes with high sensitivity, enabling identification and quantification of targeted analytes and investigation of metabolites, degradation products and unknown contaminants in samples, based on accurate mass, elemental composition and/or isotopic ratio. Q-TOF-MS is used to provide structural characterization of the major compounds in raw and processed honey, to identify changes in chemical compositions that could cause differences in their tonic effect. Ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF MS) is used for untargeted metabolomics approach to discriminate honey of various floral and geographical origins. Mono- and polyfloral honeys within one region could be differentiated, as well as some monofloral honeys from various geographical origins. This method is able to detect the presence of different sugar syrups, using different markers such as polysaccharides, DFAs and AFGP. Q-TOF-MS coupled with ultra-high-performance liquid chromatography (UHPLC) can effectively detect residual syrups in honeys produced by honeybees after being fed with syrups for three days [77,78,79,80].

2.4.2.6 Nuclear magnetic resonance

Nuclear magnetic resonance (NMR) provides structural information with excellent repeatability and reproducibility and can help in understanding the structural components in

food system. It is fast technique which requires no calibration with internal standards or components extraction prior to analysis. Another advantage is that it detects all organic materials simultaneously and quantitatively in their native state without any tampering or previous separation. NMR is widely used to identify the floral origin of unifloral honeys by specific fingerprints or to identify specific markers. The dilution effect can be the only way to detect adulteration with C3 plant sugars, which are devoid of marker peaks. NMR has great potential for fast detection of adulterants in honey, generated in a short time. This technique is also highly advantageous for screening larger numbers of samples faster [81,82,83,84,85].

2.4.2.7 Infrared-based spectroscopy

Infrared-based spectroscopy (IR) is an optical sensing technique, which has the advantages in being rapid, non-destructive and user-friendly method. Characteristic absorbance in the mid-infrared (MIR) region is based on the fundamental vibrational modes of the corresponding chemical groups in food compounds. The following result is MIR spectra from the stretching, bending, and rotational vibrations of the sample molecules, whereas the near-infrared region (NIR) spectra is result from complex overtones and high frequency combinations of fundamental vibrations at shorter wavelengths [86,87].

IR spectroscopy is able to detect wide range of adulterants, such as corn syrups, HFCS, inverted beet syrup, cane sugar syrup and others. It is an easy solution in detection of adulteration. IR-based methods require just small sample volume and minimal or no sample preparation. The equipment can be made portable, which made this technique perfect solution in the field for fast authentication of honey. The major challenge is in developing advanced chemometric methods, such as discriminant analysis, for the data analysis [88].

2.4.2.8 Raman spectroscopy

The principle of Raman spectroscopy is in detecting Raman signals, which arise from the inelastic scattering of incident light by sample molecules, and the frequency of the scattered light shifts depending on characteristic molecular vibrations. The big advantage of Raman spectroscopy is no interference from water present in the sample and no interference of the sample matrix when using NIR lasers as the light source. Similar as in IR spectroscopy the sampling and measurement is easy, and the method is simple and rapid. As well can be used for on-site testing in field applications [89,90,91,92].

For distinguishing between pure honey samples and adulterated ones, different absorption of energy depending on different isotopes is used. In honey adulteration, sugars of different origin will differ in their stable carbon isotope ratio as well as in the deuterium to hydrogen ratio. Raman spectroscopy can detect adulterants including HFCS, maltose syrup, beet and inverted cane sugar [92,93].

3. EXPERIMENTAL PART

3.1 Instrumentation

For this master thesis a HPAE-PAD system (BioLC system ICS-2500) was used including:

- Autosampler AS50 (Dionex, Sunvyle, USA)
- 6-port injection valve ((Dionex, Sunvyle, USA)
- Gradient pump GS50 (Dionex, Sunvyle, USA)
- Thermal stabilizer (Dionex, Sunvyle, USA)
- Analytical column CarboPac PA200, 3 × 250 mm (Thermo Fisher Scientific, Merelbeke, Belgium)
- Electrochemical Detector ED50A (Dionex, Sunvyle, USA)
- Software for data acquisition, instrument control and integration Chromeleon 6.8 (Dionex, Sunvyle, USA)

The overall instrumentation is shown in the following **Figure 5**:

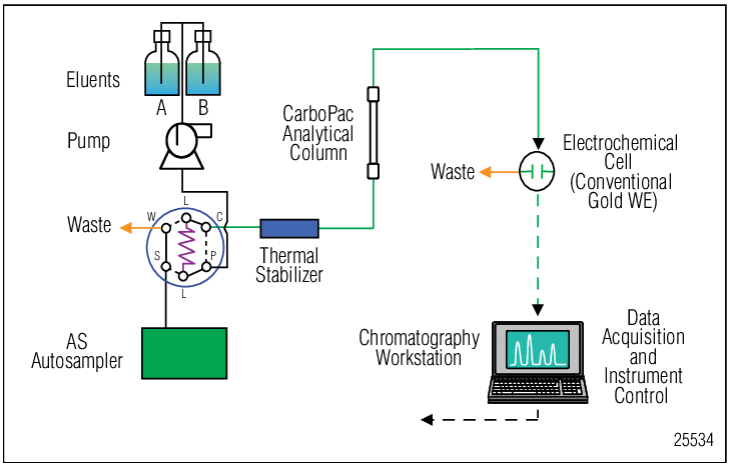

Figure 5 *Instrumentation scheme for HPAE-PAD*

3.2 Equipment

For the preparation of the chemical solutions and pre-treatment of samples the following equipment was used:

- Automatic pipettes 0.5-10 µl, 10-100 µl, 100-1000 µl and 1-10 ml, Eppendorf (Aarschote, Belgium)
- Milli-Q (> 18 MΩ) water purification system (Millipore, Molsheim, France)
- Vortex (VWR, Leuven, Belgium)
- Roto-Shake Genie (Scientific Industries, New York, USA)
- Analytical Balance AX504, (Mettler Toledo, Zaventem, Belgium)
- Branson 5510R-MT Ultrasonic Cleaner (Marshall Scientific, New Hampshire, USA)

3.3 Materials

- Acetonitrile (LC-MS grade), Merck (Darmstadt, Germany)
- Sodium acetate (NaOAc) anhydrous > 99 % (Sigma-Aldrich, St. Louis, USA)
- Sodium hydroxide (NaOH) solution 50-52 % in water (Sigma-Aldrich St. Louis, USA)
- High purity water produced by Milli-Q
- Individual maltodextrins of analytical grade with a degree of polymerization (DP) 2 to 10 Elicityl-OligoTech (Crolles, France)
- Maltodextrin mixtures, dextrose equivalent to 4,0-7,0, 13,0-17,0 and 16,5-19,5 Sigma-Aldrich (St. Louis, MO, USA)
- Analytical grade standards: Trehalose, Arabinose, Glucose, Fructose, Melibiose, Isomaltose, Maltulose, Sucrose, Gentobiose, Turanose, Melezitose/Palatinose, Raffinose, Stachyose, Kestose, Maltose, Erlose, Panose, Nystose and Maltotriose (Sigma-Aldrich, St. Louis, USA)
- Filters-syringe, acrodisc nylon, 25 mm, 0,2 µm (VWR)
- Disposable Omnifix 3 ml syringes (VWR, Leuven, Belgium)

- 1,5 ml amber injection vials, septum: silicone white/PTFE red (VWR, Leuven, Belgium)

- BD Falcon 50 ml centrifuge tubes, polypropylene (VWR, Leuven, Belgium)

3.4 Method Development and Optimization

3.4.1 Stock solutions

Primary stock solutions of individual carbohydrates were prepared by dissolving 10 mg of each reference standard in 10 ml of a solution of acetonitrile/water (50/50, v/v). The solutions were sonicated for 10 min until crystals of the standards were dissolved. Intermediate stock standard solutions (100 μg/ml) and individual working standard solutions (10 μg/ml) were prepared by appropriate dilutions with acetonitrile/water (50/50, v/v). Dilutions were prepared each time at a volume ratio of 1:10 in order to achieve more precision and less uncertainty in the final concentration values. The working standard solutions were always freshly prepared. All stock standard solutions were stored -20 °C.

Individual working standard mixtures for carbohydrates and oligo-/polysaccharides were prepared at a concentration of 10 μg/ml. The mixture for carbohydrates included the following: trehalose, arabinose, glucose, fructose, melibiose, iso-maltose, maltulose, sucrose, gentobiose, turanose, melezitose, palatinose, raffinose, stachyose, kestose, maltose, erlose, panose, nystose and maltotriose. The mixture for maltodextrins included DP 1 to 11. DPs higher than DP11 were not available as individual analytical standards. A qualitative mixture of DP 1 to 24 was also prepared based on maltodextrin mixtures with dextrose equivalent to 4,0-7,0, 13,0-17,0 and 16,5-19,5. All standard mixture solutions were stored in the dark at -20 ^{o}C. These solutions were used in the development and validation phases of the method and are also required when applying the method under routine conditions.

3.4.2 Stability of compounds

Carbohydrates, oligosaccharides and polysaccharides are sensitive to light, natural or artificial, and under extreme conditions of temperature, humidity and pH they can degrade. As a result, their concentration and consequently the intensity of the detector response is reduced. In order to avoid the above, these solutions must be handled carefully, not to remain exposed to light and ambient temperature but to be maintained at the required temperature.

The stability of these compounds has been studied over time at two different concentration levels 0,5 ng/ml and 5 ng/ml. Two different temperatures were evaluated 2-8 ^{o}C and -20 ^{o}C for standard solutions prepared in water or acetonitrile/water (50/50, v/v). To determine the stability of the standard solutions, the peak area of both the fresh and the older solution (3 replicates) was evaluated. The peak area of the old solution was subtracted from the peak area

of the fresh solution and divided by the peak area of the older solution, as shown in equation 3.1. The criterion set is that the result should be less than 5 %.

$$\frac{[\text{Area peak response (new)} - \text{Area peak response (old)}]}{\text{Area peak response (old)}} \text{X}100\% \leq 5\% \qquad (3.1)$$

After a period of 4 weeks Glucose and DP10 degraded more significantly in water than in acetonitrile/water (50/50, v/v) at 2-8 ºC. At -20 ºC no changes were observed for both solvents. The optimum conditions for storing standard solutions were at -20 °C and using acetonitrile/water (50/50, v/v) as a solvent.

3.4.3 Selection of column and mobile phase

The choice of the appropriate mobile phase and analytical column are of great importance for achieving the best possible separation of the compounds with the optimal peak shape and intensity. In ion chromatography, except of the chromatographic separation, the adjusted detector parameters can influence the overall performance of the method.

The most common columns designed for carbohydrate anion-exchange chromatography, are based on CarboPac family manufactured by Thermo Fisher Scientific. The CarboPac PA200 column is the latest addition to the CarboPac family of columns for carbohydrate separations. This column has been specially developed to provide high resolution separations of charged and neutral oligosaccharides and is the recommended column for such applications. The CarboPac PA200 columns are packed with a hydrophobic, polymeric, pellicular anion exchange resin stable over the range of pH 0–14. This unique pH-stability of the packing material allows the use of eluent compositions that are conducive to anodic oxidation of carbohydrates at gold electrodes. This pellicular resin structure permits excellent mass transfer, resulting in high-resolution chromatography and rapid re-equilibration compared to CarboPac PA100 column. Sodium hydroxide eluents can provide a different chromatographic selectivity than the previously developed CarboPac columns, which used potassium hydroxide eluent.

Hence, CarboPac PA200 column was selected as the most suitable analytical column for separating carbohydrates and oligo-/polysaccharides. Additionally, a guard column was used before the analytical column to prevent sample contaminants from eluting onto the analytical column.

For the separation of carbohydrates an isocratic elution was selected based on sodium hydroxide which is less corrosive than potassium hydroxide. Different composition of sodium hydroxide was used in combination of different flow rates of the mobile phase. The optimum separation, peak response and shape for 20 carbohydrates was achieved by applying isocratic elution of 100 mM sodium hydroxide with a flow rate of 0,45 ml/min, with the exception of melezitose and palatinose which were eluting as one peak. The total run was 30 min. In order to enforce stability of the chromatographic system a temperature of 30 ºC was applied for the column throughout the analysis. Moreover, the temperature of the autosampler tray was

maintained at 10 ºC to achieve better stability for the honey samples. Such chromatography in combination to the detection method is sensitive enough to allow the determination of lower concentrations of carbohydrates, while also being robust enough to handle higher concentrations of the major components, glucose and fructose.

In order to achieve high resolution separation of a broad range of oligo-/polysaccharides (DP1-24) a gradient elution was applied. To maintain baseline stability, it was important to keep the sodium hydroxide (NaOH) concentration constant during the sodium acetate (NaOAc) gradient, because acetate has no buffering capacity at high pH. This was achieved by making the eluents as follows:

Eluent A: x mM NaOH
Eluent B: x mM NaOH, y mM NaOAc

The optimum compositions for the eluents selected were (A) 100 mM NaOH and (B) 100 mM NaOH / 1000 mM NaOAc. Eluent A was prepared by adding 10,4 ml of 50 % NaOH in 2000 ml of high purity water. Sodium hydroxide was added rapidly for avoiding any possible carbonation. Eluent was mixed in special closed plastic bottle. Eluent B was produced by dissolving 164,08 g in 2000 ml of high purity water. The procedure was performed by sonication for 10 min in order to obtain rapid and effective dissolution. Additionally, 10,4 ml of 50 % NaOH was added by following the same steps as eluent A.

Efficient separation of the oligo-/polysaccharides from the matrix components was achieved with the following gradient program: 95 % eluent A initially (t = 0 min), increased linear to 68 % (t = 30 min, held for 2 min), increased to 95 % (t = 1 min, held for 1 min) and equilibrated for 7 min at the initial conditions (**Table 3**).The total run was 40 min and the flow rate was kept at 0,45 ml/min. The temperature for the column was set to 30 ºC and the temperature for the autosampler tray was maintained at 10 ºC.

Table 3 *Program of gradient elution*

t (min)	(A): 100mM NaOH	(B): 100mM NaOH with 1000mM NaOAc
0,0	95 %	5 %
30,0	68 %	32 %
32,0	68 %	32 %
33,0	95 %	5 %
40,0	95 %	5 %

3.4.4 Detector parameters

Carbohydrates separated by high pH anion exchange chromatography are detected by pulsed amperometric detection. Amperometric detection is used to measure the current or charge resulting from oxidation or reduction of analyte molecules at the surface of a working electrode. During oxidation reactions electrons are transferred from molecules of electroactive analytes, such as carbohydrates, to the working electrode in the amperometry cell. Detection is sensitive and highly selective for electroactive species, since many potentially interfering species cannot be oxidized or reduced, and are not detected.

Different electrochemical cells are used for the detection based on the type of the electro-active species. Hence, for carbohydrates and amino acids a gold working electrode is suitable, for cyanide, bromide, iodide, thiocyanate, sulfide, thiosulfate a silver electrode and for alcohols, aldehydes, ketones, carboxylic acids a platinum electrode. Therefore, pulsed amperometric detection at a gold working electrode was selected as it provides a reproducible and sensitive method for carbohydrates and oligo-/polysaccharides.

The carbohydrate oxidation at gold electrodes is made by a rapid sequence of potentials (waveform) adjusted between the working electrode (gold) and the reference electrode (silver/silver chloride). Resulting currents are measured by integration during a short time interval of the detection waveform. The standard, recommended carbohydrate waveform by Thermo Fisher Scientific was applied, as shown in **Table 4**.

Exposure of working electrode to alkaline solutions, such as flowing sodium hydroxide, caused the 3 M potassium chloride electrolyte inside the reference electrode to gradually become alkaline and the silver chloride layer on the Ag wire in the electrode to dissolve or be converted to a mixture of silver oxide and silver hydroxide. As a result, the reference potential shifted, become increasingly unstable and reduced sensitivity. Hence, it is recommended to change the working electrode every four months when analysing complex samples such as honey samples.

Table 4 *Carbohydrate Quadrupole Waveform*

Time (sec)	Potential (V)	Integration
0,00	+ 0,1	
0,20	+ 0,1	Begin
0,40	+ 0,1	End
0,41	- 2,0	Electrode Cleaning
0,42	- 2,0	
0,43	+ 0,6	Au Oxide Formation
0,44	- 0,1	Au Oxide Reduction
0,50	- 0 1	Electrode Activation

The final Retention Times (RTs) for all compounds are shown in the following **Table 5** and **Table 6**. At **Figure 6** the HPAE-PAD chromatogram of a standard solution of carbohydrates at a concentration of 0,5 ng/ml is shown. At **Figure 7** the HPAE-PAD chromatogram of a standard solution of carbohydrates at a concentration of 0,5 ng/ml is enlarged. The HPAE-PAD chromatogram of a standard solution of maltodextrins at a concentration of 0,5 ng/ml is also shown in **Figure 8** and a HPAE-PAD chromatogram of DP 1 to 24 mixture based on maltodextrins with dextrose equivalent to 4,0-7,0, 13,0-17,0, and 16,5-19,5 is shown in **Figure 9**.

Table 5 *Retention times for carbohydrates*

Compound	**RT (min)**
Trehalose	2,29
Arabinose	2,70
Glucose	2,93
Fructose	3,18
Melibiose	3,38
Iso-maltose	3,84
Maltulose	3,96
Sucrose	4,07
Gentobiose	4,99
Turanose	5,14
Melezitose/Palatinose	5,32
Raffinose	5,74
Stachyose	6,15
Kestose	6,66
Maltose	7,48
Erlose	9,69
Panose	15,18
Nystose	19,93
Maltotriose	22,33

Table 6 *Retention times for maltodextrins*

Compound	**RT (min)**
DP1	4,10
DP2	2,43
DP3	4,10
DP4	6,54
DP5	8,27
DP6	9,95
DP7	11,8
DP8	12,83
DP9	14,05
DP10	15,18
DP11	16,24
DP12	17,22
DP13	18,15
DP14	19,04
DP15	19,89
DP16	20,71
DP17	21,51
DP18	22,28
DP19	23,01
DP20	23,0
DP21	24,33
DP22	24,92
DP23	25,47
DP24	26,012

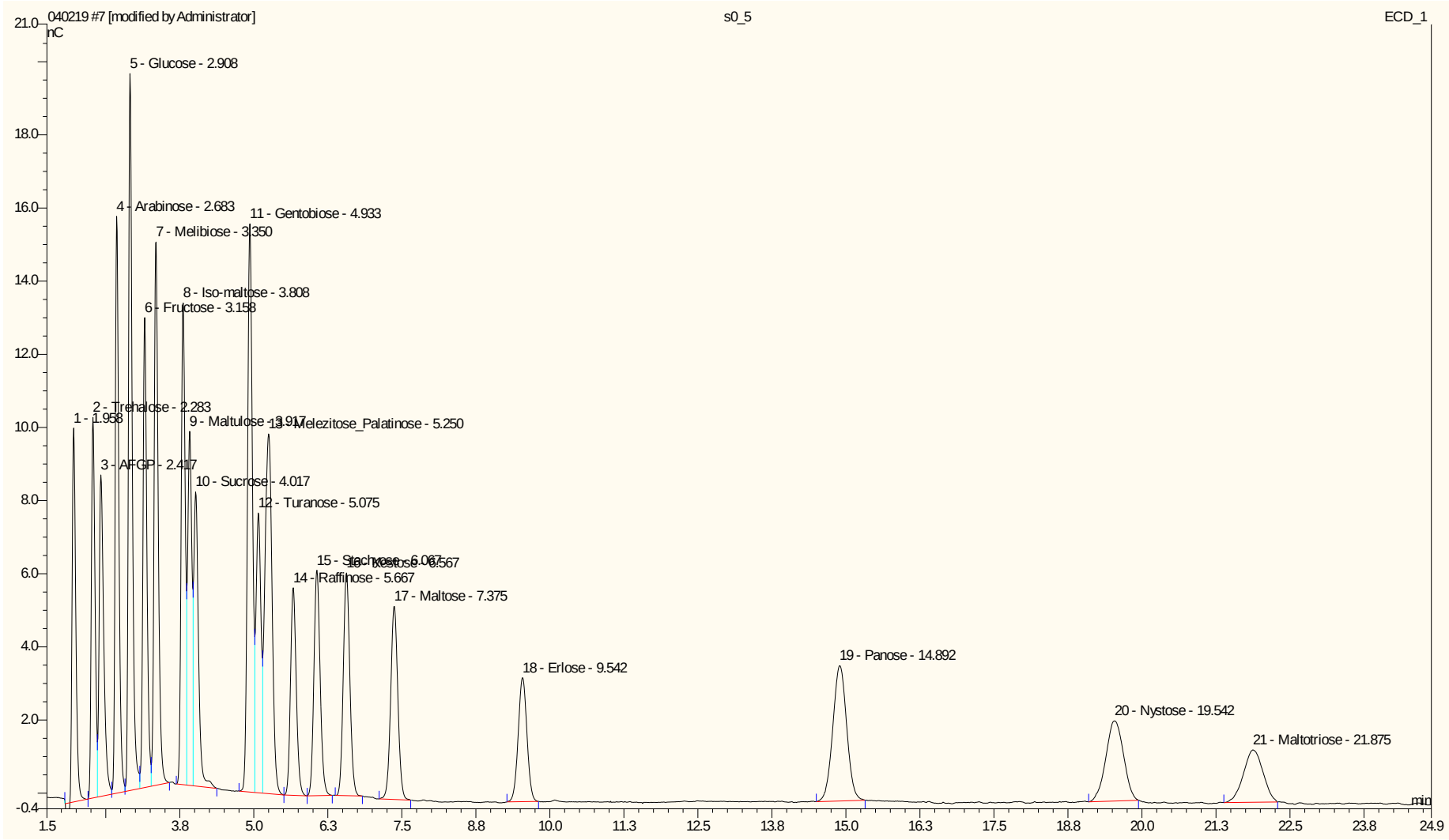

Figure 6 *HPAE-PAD chromatogram of a standard solution of carbohydrates at a concentration of 0,5 ng/ml*

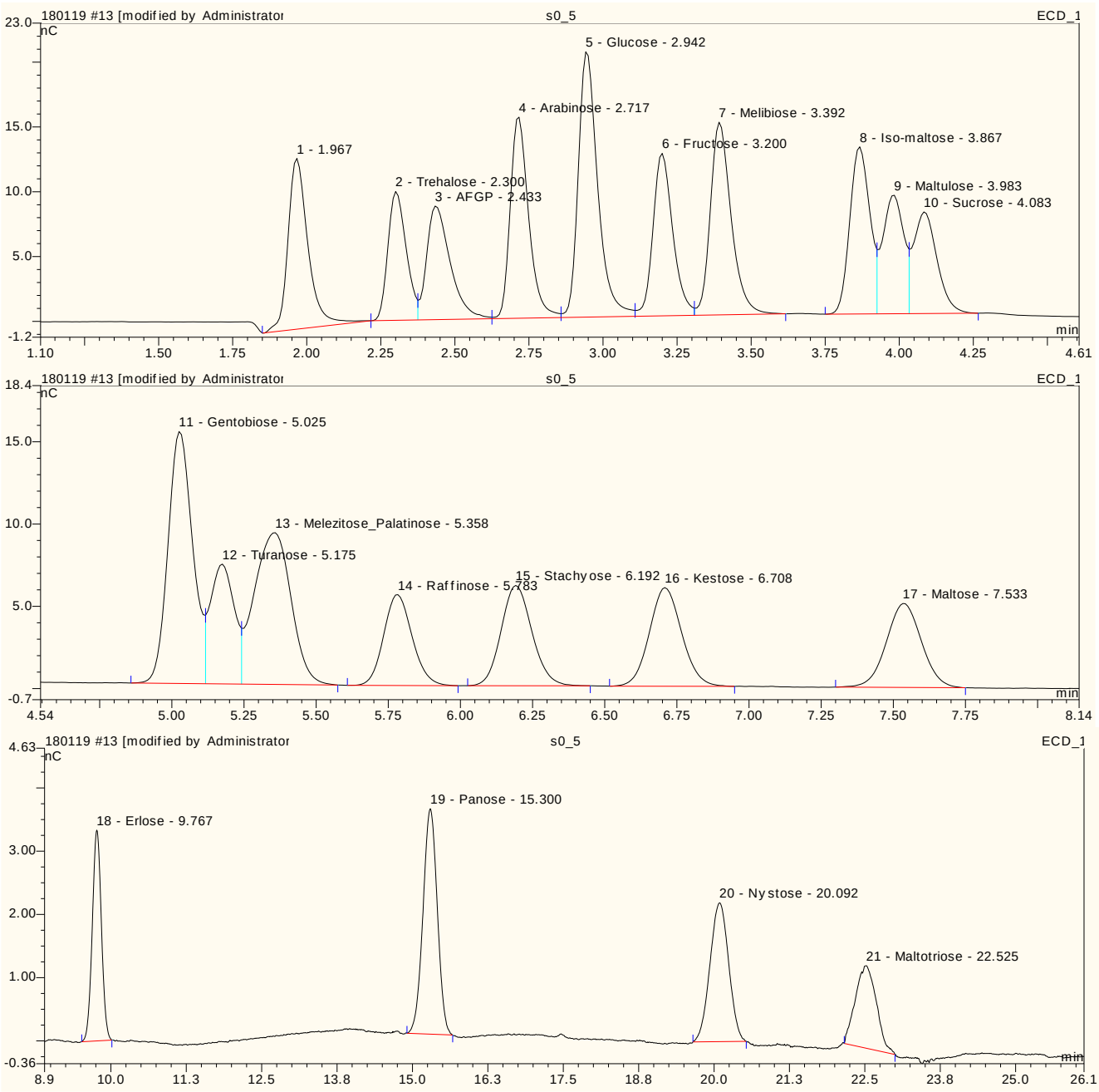

Figure 7 *Enlarged HPAE-PAD chromatogram of a standard solution of carbohydrates at a concentration of 0,5 ng/ml*

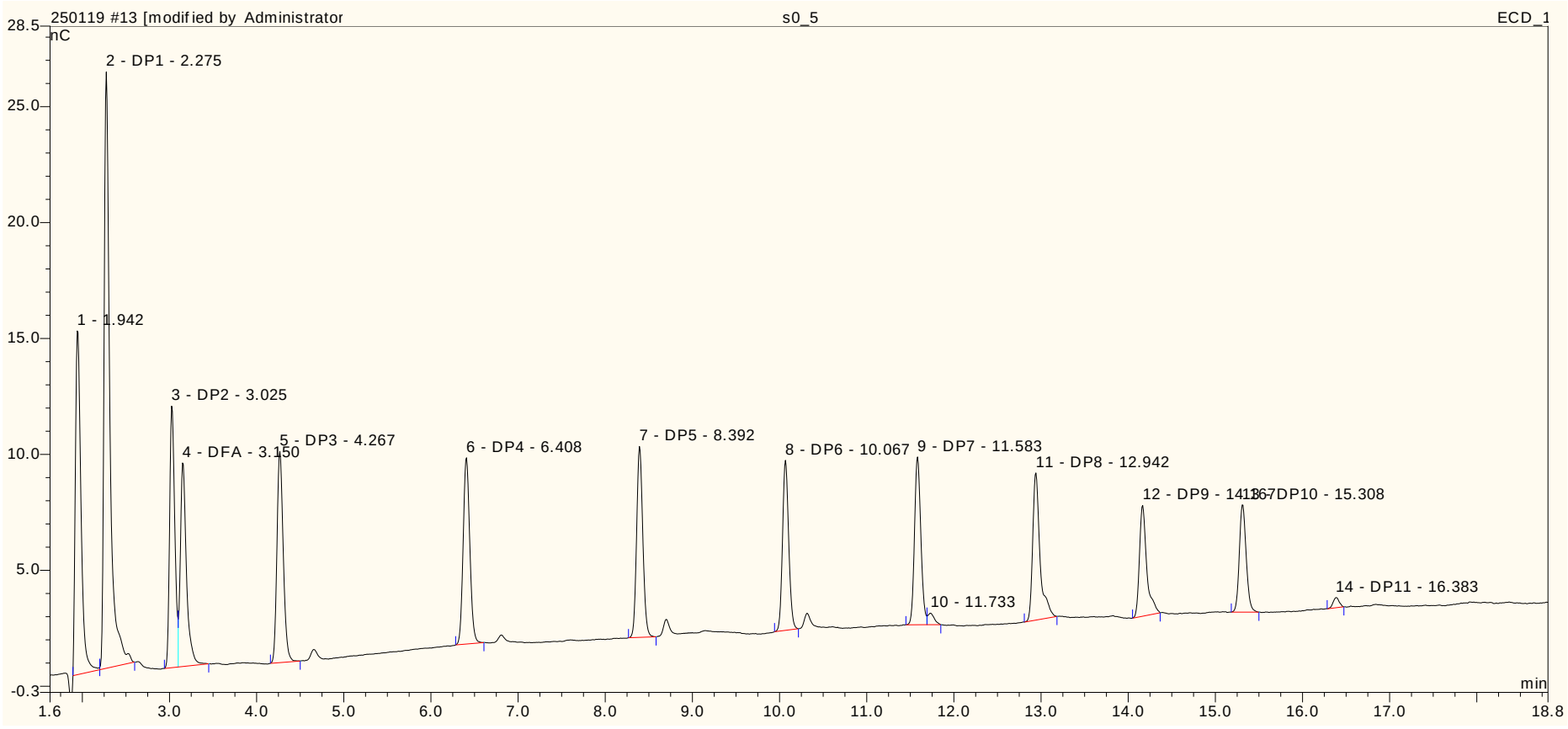

Figure 8 *HPAE-PAD chromatogram of a standard solution of maltodextrins at a concentration of 0,5 ng/ml*

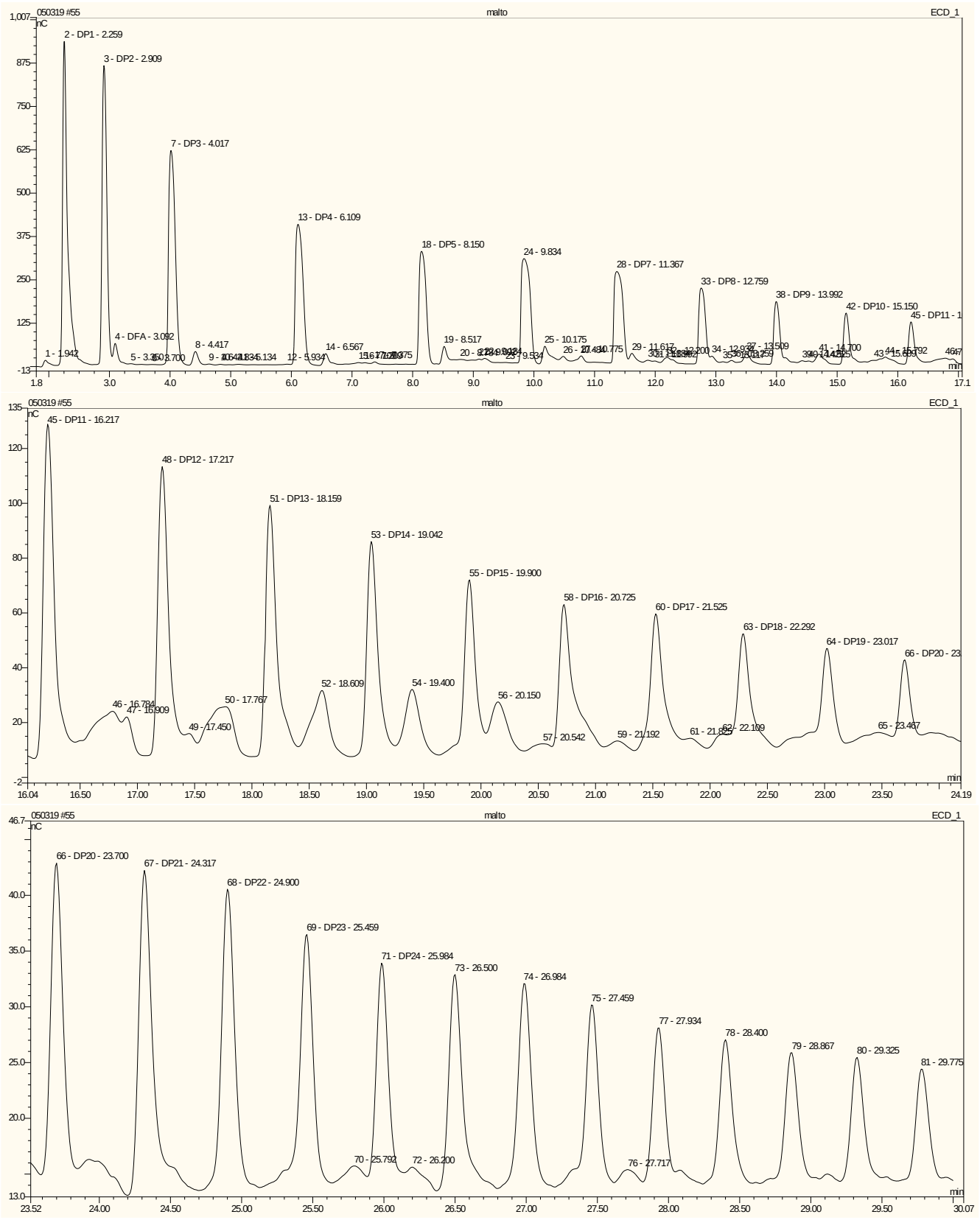

Figure 9 *HPAE-PAD chromatogram of DP 1 to 24 mixture based on maltodextrins with dextrose equivalent to 4,0-7,0, 13,0-17,0, and 16,5-19,5*

3.5 Results and Discussion

3.5.1 Optimization of sample preparation

Following the development of the method for the determination of carbohydrates and oligo-/polysaccharides in standard solutions, optimization in real honey samples was assessed. However, as most techniques of analysis require, pre-treatment of honey samples is necessary to enable them to be introduced into the chromatographic system. The purpose of the pre-treatment is to modify the sample matrix in order to be suitable for introducing the sample into the analytical column, to make the solvent suitable for the analytical technique, to extend the life of the instrument, to purify the sample in order to increase the recovery and reducing the matrix effect on the instrument, enriching and concentrating the sample in order to improve the sensitivity of the method as well as the isolation and selective collection of the components of interest. A pre-treatment technique is ideal when: a) it is simple, b) it is rapid, c) it is economical, d) it has high recovery and good repeatability, e) it is selective, f) the amount of solvent used is small and g) when it can be automated.

The sample preparation that was evaluated was dilution of honey samples, without any solid phase extraction to achieve a fast sample-throughput. The following dilutions for detecting carbohydrates and maltodextrins in honey were tested: 100, 1 000, 3 000, 5 000, 10 000, 30 000, 50 000. As the stability of our compounds in acetonitrile/water (50/50, v/v) was satisfactory the same solvent mixture was used for the dilutions. The evaluation of the results revealed that the dilution of honey with a ratio 1:100 was appropriate for detecting maltodextrins under gradient conditions, 1:50 000 for detecting glucose, fructose and sucrose under isocratic conditions and 1:5 000 for detecting trehalose, arabinose, melibiose, iso-maltose, maltulose, gentobiose, turanose, melezitose, palatinose, raffinose, stachyose, kestose, maltose, erlose, panose, nystose and maltotriose. The high dilution for glucose and fructose was necessary as they are present in dominant amounts in honey. A smaller dilution for the rest of the carbohydrates was reasonable as they are present in honey in much lower concentrations.
For maltodextrins the dilution of ratio 1:10 was too high causing saturation of the detector and significant matrix interference. The goal was to achieve as much as possible the detection of oligo-/polysaccharides at trace levels, so the dilution of 1:100 was selected even if DP 1 to 3 were saturating the detector. DP 1 to 3 were already been detected by the analysis of the sugar profile (glucose, maltose and maltotriose), so the analysis of higher than DP4 for maltodextrins was adequate for the method development. Overall, these dilutions were necessary to achieve satisfactory sensitivity and separation of the compounds from the matrix components.

In order to assess the validity of the applied sample preparation authentic honey was adulterated with rice syrup (RS) - a C3 plant and with a mixture of syrups composed of equal amounts from ten different types of syrups (sugar cane, sugar beet, HFCS, agave, maple, wheat maltose, wheat glucose, palm, spelt and rice syrup). All spiking was done at different percentages corresponding to 5, 10, 50 % and 100 % (w/w). At 5 % of adulteration DP up to 17 were observed for RS and up to 14 for the mixture of different type of syrups.

The final sample preparation protocol is described as follows: 1 g of honey sample was dissolved in 10 ml of acetonitrile/water (50/50, v/v) and mixed for 5 min on the roto-shaker. The extract was filtered through a 0,22 µm syringe nylon filter 25 mm and after applying the appriopriate dilution it was transfered to an injection vial for further analysis on the HPAE-PAD system (**Figure 10**).

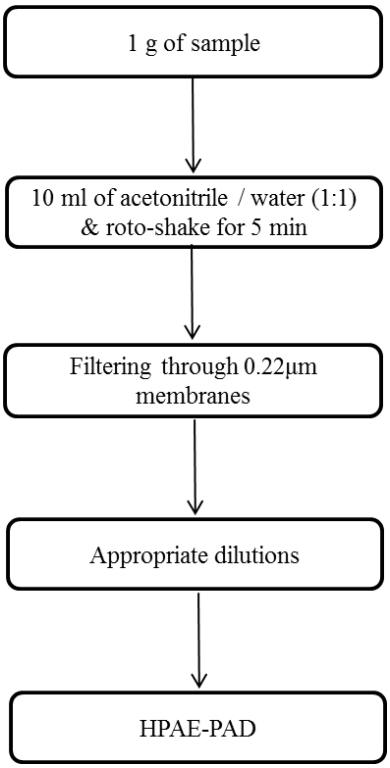

Figure 10 *Sample preparation scheme*

HPAE-PAD chromatograms of carbohydrate profiles and maltodextrins in an authentic honey are shown in the following figures: **Figure 11**, **Figure 12**, **Figure 13**, **Figure 14**, **Figure 15**.

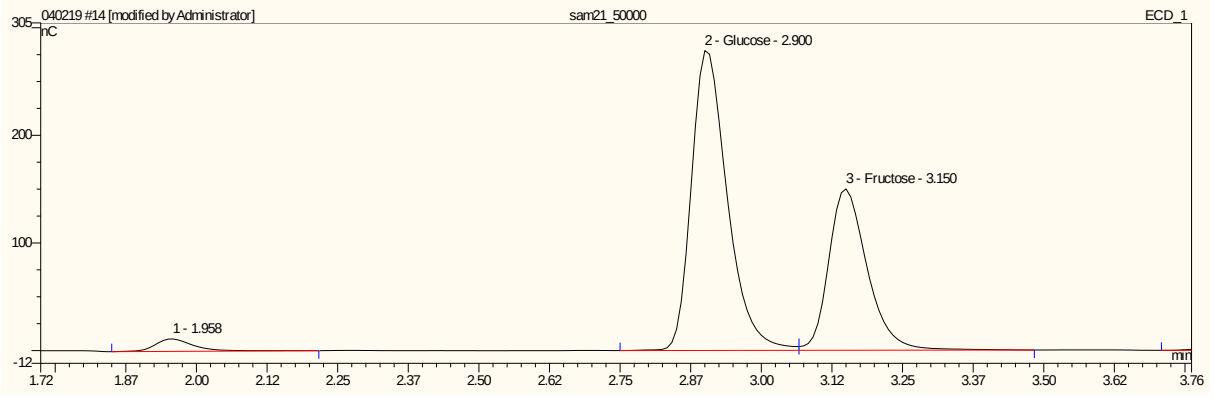

Figure 11 *Enlarged HPAE-PAD carbohydrate profile for glucose and fructose of an authentic honey sample at a 50 000 dilution*

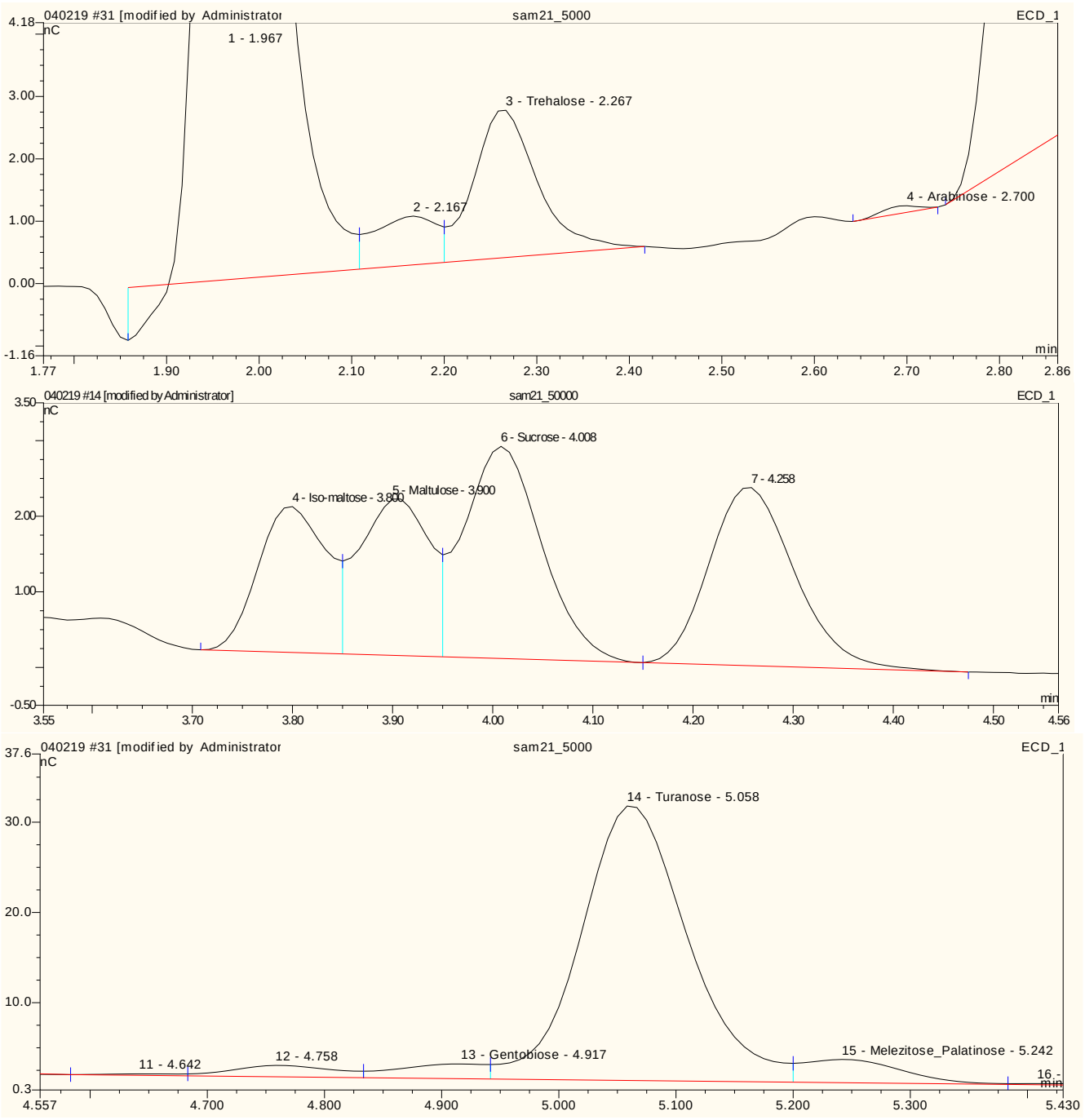

Figure 12 *Enlarged HPAE-PAD carbohydrate profile for trehalose, arabinose, isomaltose, maltulose, sucrose, gentobiose, turanose, melezitose and palatinose of an authentic honey sample at a 5 000 dilution.*

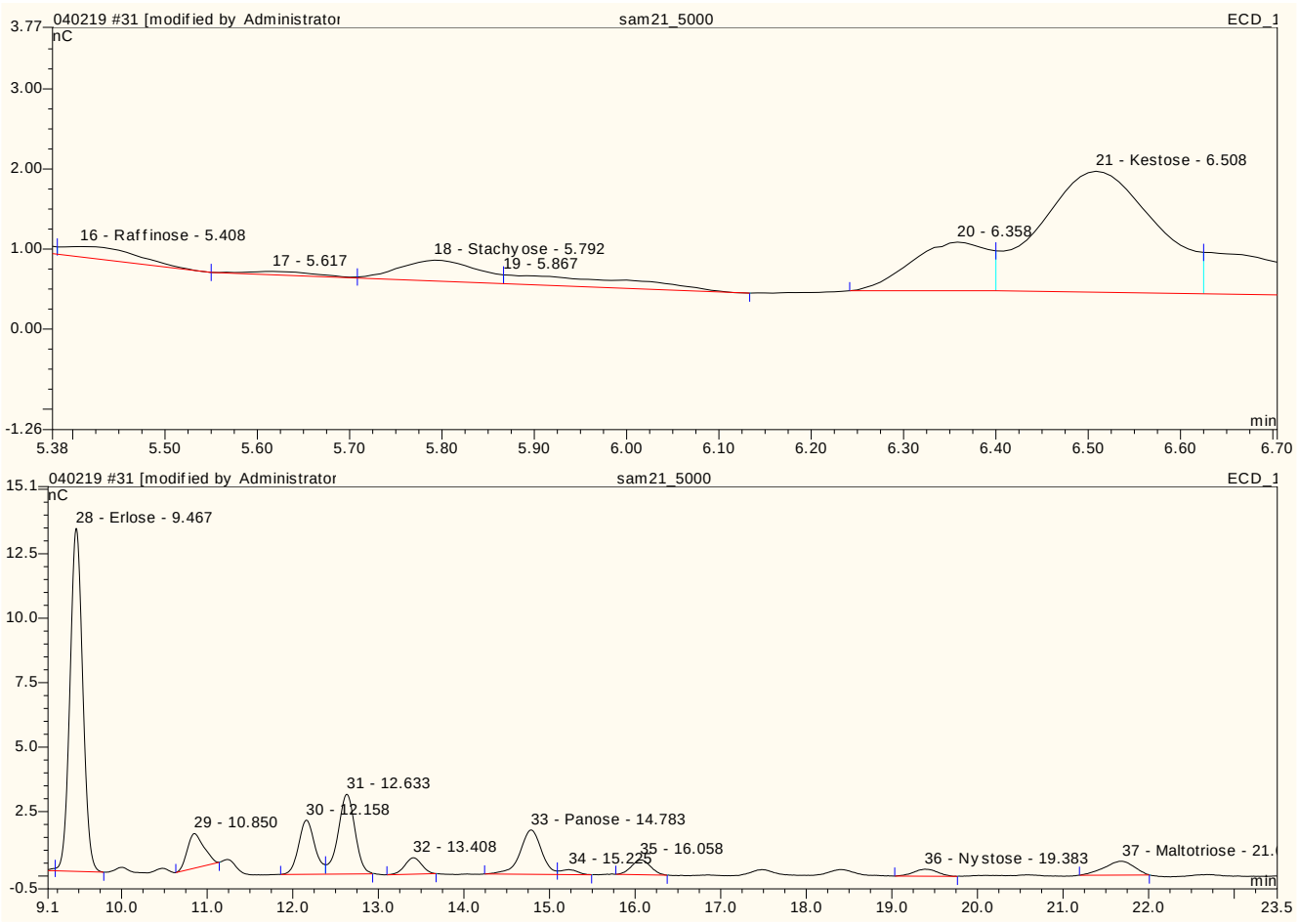

Figure 13 *Enlarged HPAE-PAD carbohydrate profile for raffinose, stachyose, kestose, maltose, erlose, panose, nystose, and maltotriose of an authentic honey sample at a 5 000 dilution.*

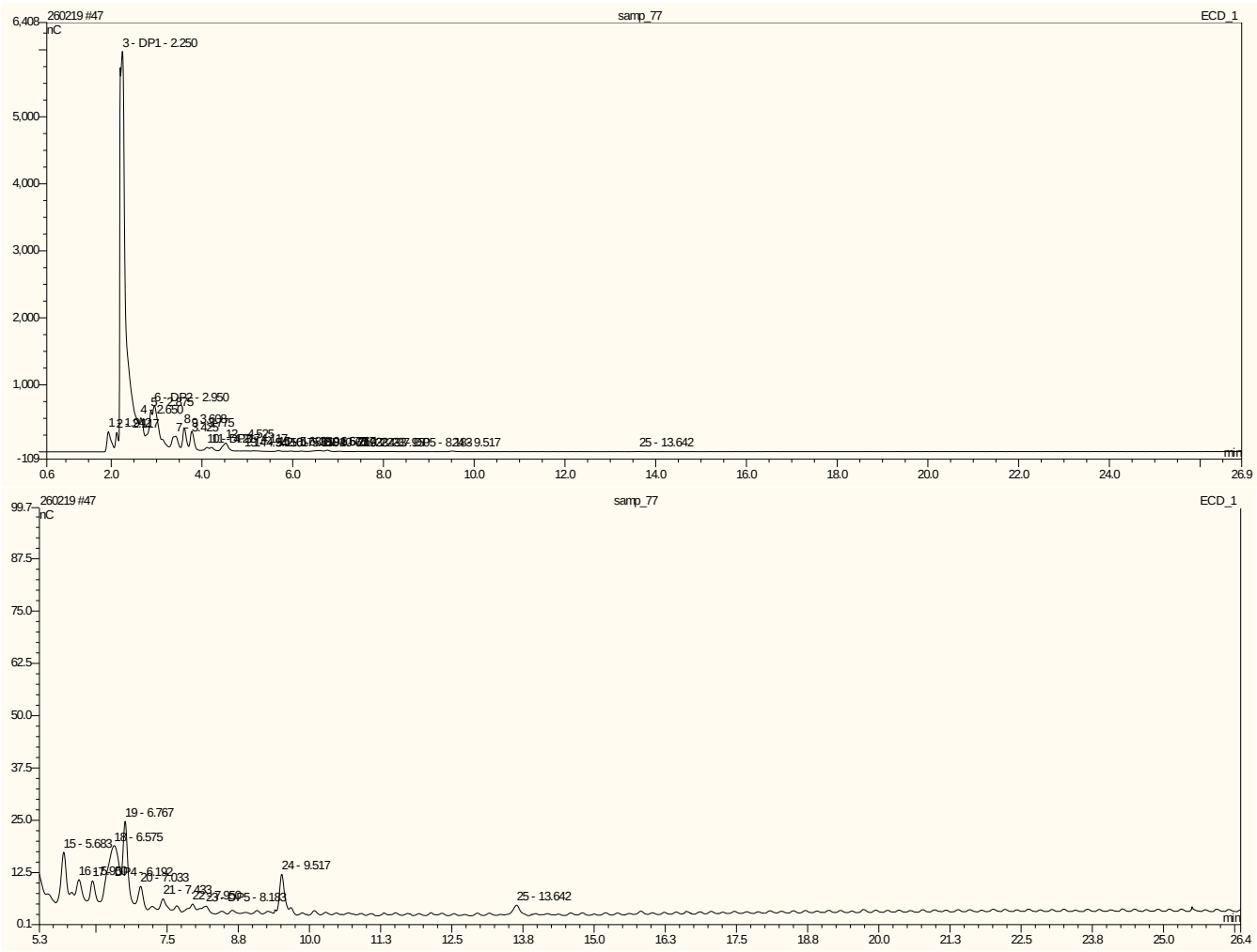

Figure 14 *HPAE-PAD maltodextrins profile of an authentic honey sample at a 100 dilution*

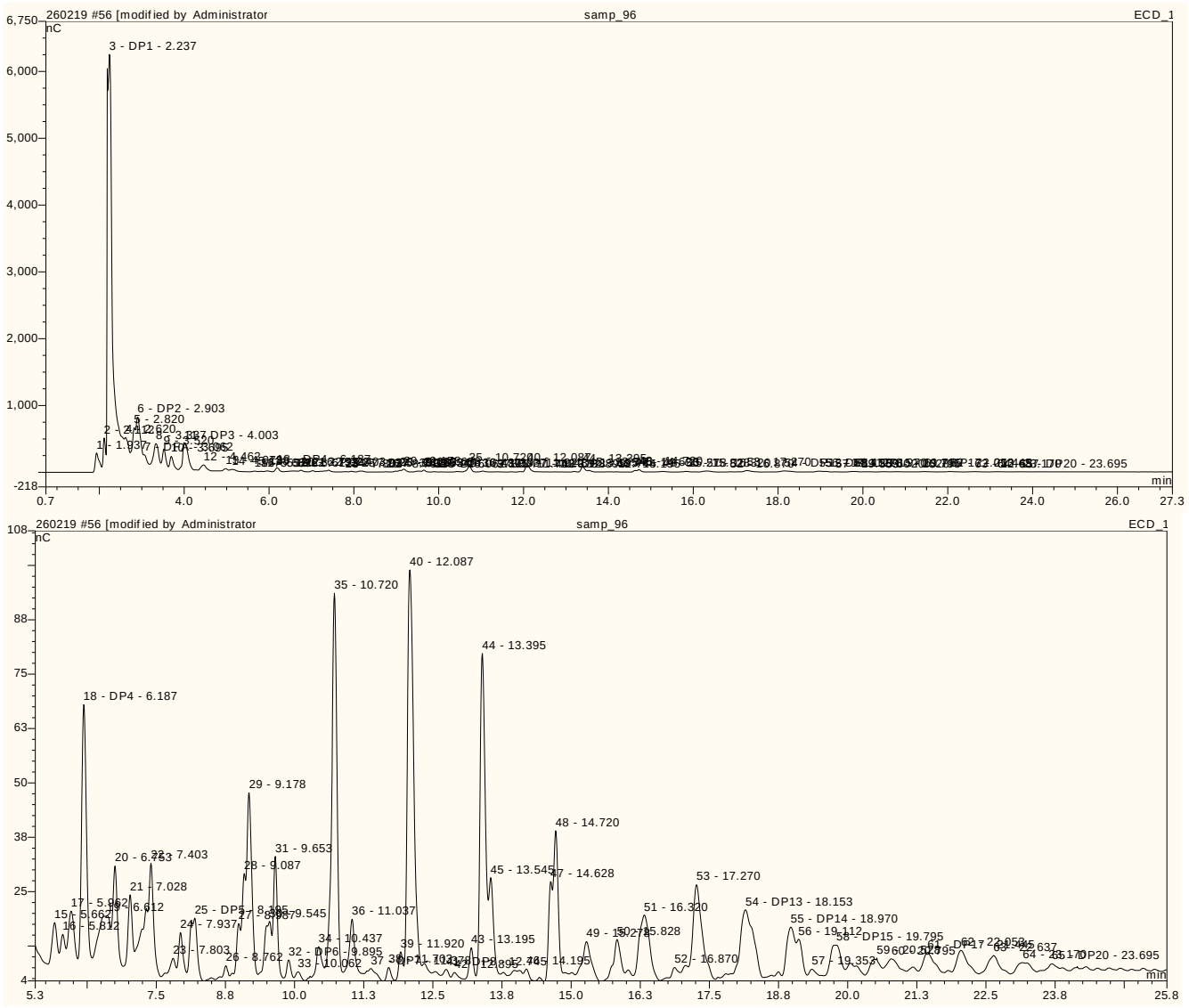

Figure 15 *HPAE-PAD maltodextrins profile of an adulterated honey sample at a 100 dilution*

3.5.2 Method validation

Validation experiments were carried out on three separate days consisting of 3 replicates for each concentration level. Each extract was injected three times, obtaining 27 results for each analyte and concentration. Quantification was based on external standard calibration curve. For the external standard calibration curve four concentration levels were tested 0,5; 1; 2 and 5 mg/kg, by spiking honey samples with different volumes of mix standard stock solutions, in order to evaluate the performance of the method. The dilution used for honey sample were 1:50 000 for sugar profile validation and 1:1 000 for maltodextrin validation. As a corresponding blank honey sample is not possible to be obtained, a honey sample with the presence of the least carbohydrates and DPs was selected. Hence, the honey sample used for spiking did not contain any DPs higher than 3 but did contain higher amounts of glucose, fructose, sucrose and traces of iso-maltose, maltulose, turanose, maltose and erlose. The method

validation data were evaluated statistically with the statistical software Analysis of Variance (ANOVA).

3.5.2.1 Linearity, sensitivity

The determination of linearity and the working concentration range of the method validation are important. Nine points were tested for the calibration curves, covering a dynamic range from 0,1 to 10 µg/kg. The prepared stock solution of carbohydrates mixture and maltodextrin mixture DP1-11 were mixed with acetonitrile/water (50/50, v/v) in appropriate ratios, as showed in **Table 7** for obtaining concentration levels at 0; 0,1; 0,2; 0,5, 1; 2; 4; 5 and 10 µg/kg for preparation of calibration curve. The calibration curve was made freshly each time.

Table 7 *Preparation of calibration curve*

Concentration (µg/kg)	**Volume of standard stock solution (µl)**	**Volume of acetonitrile/water (50/50, v/v) (µl)**
0	0 (10 µg/ml)	1000
0,1	10 (10 µg/ml)	990
0,2	20 (10 µg/ml)	980
0,5	50 (10 µg/ml)	950
1	100 (10 µg/ml)	900
2	200 (10 µg/ml)	800
4	400 (10 µg/ml)	600
5	500 (10 µg/ml)	500
10	1000 (10 µg/ml)	0

The data were evaluated by carrying out linear regression to establish the coefficient of determination (*r2*) which was between 0,9917 and 1,000 indicating acceptable linearity in the concentration range studied. **Table 8** and **Table 9** display the dynamic range, linearity and slope for all compound. Linearity was also tested including lower calibration levels at 0,02 and 0,05 µg/kg, showing still adequate coefficient of determination (r^2) higher than 0,9900.

Table 8 *Dynamic range, linearity and slope for carbohydrates*

Compound	Dynamic range (µg/kg)	r^2	Slope
Trehalose	0,1-10	0,9989	1,363
Arabinose	0,1-10	0,9988	2,116
Glucose	0,1-10	0,9993	2,861
Fructose	0,1-10	0,9973	1,718
Melibiose	0,1-10	0,9997	2,338
Iso-maltose	0,1-10	0,9998	2,023
Maltulose	0,1-5	0,9997	1,482
Sucrose	0,1-5	0,9979	1,295
Gentobiose	0,1-10	0,9994	2,941
Turanose	0,1-5	0,9982	1,417
Melezitose/Palatinose	0,1-5	1,0000	2,719
Raffinose	0,1-5	0,9999	1,257
Stachyose	0,1-5	0,9995	1,447
Kestose	0,1-10	0,9986	1,477
Maltose	0,1-10	0,9998	1,455
Erlose	0,1-10	0,9992	1,129
Panose	0,1-10	0,9998	1,971
Nystose	0,1-10	0,9998	1,582
Maltotriose	0,1-10	0,9999	1,127

Table 9 *Dynamic range, linearity and slope for maltodextrines*

Compound	Dynamic range (µg/kg)	r^2	Slope
DP1	0,1-5	99,8296	3,398
DP2	0,1-5	99,9875	1,543
DP3	0,1-5	99,9817	1,446
DP4	0,1-5	99,9697	1,490
DP5	0,1-5	99,9456	1,378
DP6	0,1-5	99,9538	1,306
DP7	0,1-5	99,9538	1,148
DP8	0,1-5	99,9448	1,160
DP9	0,1-5	99,9520	1,192
DP10	0,1-5	99,9814	0,950
DP11	0,1-5	99,9397	0,806

The limit of detection (LOD) and limit of quantification (LOQ) were calculated from the external calibration curves based on the following equations (2) and (3).

$$LOD = 3 \, x \, S_b / slope \quad (3.2)$$

$$LOQ = 10 \, x \, S_b / slope \quad (3.3)$$

where S_b is the standard deviation of the intercept.

The values obtained for LOD and LOQ for all analytes were low indicating the high sensitivity of the method as shown in **Table 10** and **Table 11**.

Table 10 *Analytical limits and response for carbohydrates*

Compound	**LOD (µg/kg)**	**LOQ (µg/kg)**
Trehalose	0,01	0,04
Arabinose	0,01	0,04
Glucose	0,02	0,07
Fructose	0,03	0,09
Melibiose	0,01	0,03
Iso-maltose	0,02	0,06
Maltulose	0,01	0,03
Sucrose	0,03	0,09
Gentobiose	0,02	0,06
Turanose	0,04	0,13
Melezitose/Palatinose	0,01	0,02
Raffinose	0,01	0,02
Stachyose	0,01	0,05
Kestose	0,03	0,10
Maltose	0,03	0,09
Erlose	0,02	0,08
Panose	0,02	0,08
Nystose	0,01	0,04
Maltotriose	0,02	0,07

Table 11 *Analytical limits and response for maltodextrins*

Compound	LOD (µg/kg)	LOQ (µg/kg)
DP4	0,04	0,14
DP5	0,04	0,13
DP6	0,04	0,13
DP7	0,04	0,14
DP8	0,04	0,13
DP9	0,03	0,09
DP10	0,04	0,15
DP11	0,11	0,39

3.5.2.2 Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained under predetermined conditions and it involves a combination of random error. The precision was expressed in terms of repeatability and intermediate precision (within-laboratory reproducibility). Repeatability expresses the precision and involved replicate measurements carried out under the same operating conditions over a short interval of time. Intermediate precision expresses within-laboratory variations and involved replicate measurements on 3 different days, under conditions which reflect, as far as possible, the conditions of routine use of the method. A total of 9 replicates at each concentration level over the 3 separate days, 3 replicates from each day, were used for the determination of the precision. Each aliquot was injected 3 times. Therefore, the experiment is a 3 level-nested experimental design. A nested design is recommended for studying the effect of sources of variability that manifest themselves over time.

In order to evaluate the performance characteristics of the method in terms of accuracy and precision, one-way ANOVA statistical analysis was applied. The parameters considered in this analysis are the repeatability relative standard deviation (RSD_r) and the reproducibility relative standard deviation RSD_R.

The precision results for all concentration levels of carbohydrates are shown in **Table 12**. The relative standard deviation for repeatability (RSDr) ranged between 0,26 % and 14,96 %, and the relative standard deviation of reproducibility (RSD_R) ranged between 1,09 % and 17,12 %, indicating that the precision of the method is sufficient. Due to the dominant presence of glucose, fructose and sucrose in the blank honey sample compared to the other carbohydrates the precision and trueness for these three compounds was omitted.

Table 12 *Precision and trueness for carbohydrates obtained from the analysis of spiked honey samples*

Compounds	**Target value (µg/kg)**	**Mean value (µg/kg)**	**RSD_r[a] (%)**	**RSD_R[b] (%)**	**S_{IP}[c]**	***U*[d]**	**RR[e] (%)**
Trehalose	0,5	0,57	0,98	3,70	0,021	0,042	113,1
Trehalose	1,0	1,14	0,52	2,49	0,028	0,057	114,3
Trehalose	2,0	2,24	0,26	1,27	0,028	0,057	112,0
Trehalose	5,0	5,31	0,81	10,22	0,543	1,085	106,2
Arabinose	0,5	0,52	0,99	5,02	0,026	0,053	104,5
Arabinose	1,0	1,07	0,90	5,21	0,056	0,112	107,1
Arabinose	2,0	2,07	0,62	7,46	0,155	0,310	103,7
Arabinose	5,0	5,04	0,76	10,46	0,526	1,053	100,7
Melibiose	0,5	0,58	1,23	3,30	0,019	0,038	115,5
Melibiose	1,0	1,13	0,89	3,77	0,043	0,085	112,8
Melibiose	2,0	2,14	0,93	4,95	0,106	0,211	106,9
Melibiose	5,0	5,20	1,10	6,82	0,354	0,709	104,0
Iso-maltose	0,5	0,53	1,27	4,51	0,024	0,047	105,2
Iso-maltose	1,0	1,08	1,93	3,83	0,041	0,082	107,7
Iso-maltose	2,0	2,09	0,95	5,71	0,119	0,238	104,3
Iso-maltose	5,0	5,12	1,44	7,87	0,403	0,806	102,4
Maltulose	0,5	0,51	2,79	2,92	0,015	0,030	102,0
Maltulose	1,0	1,01	3,11	8,92	0,091	0,181	101,5
Maltulose	2,0	2,03	3,67	8,96	0,182	0,365	101,7
Maltulose	5,0	4,50	14,97	16,79	0,755	1,510	89,9
Gentobiose	0,5	0,55	0,48	4,11	0,023	0,045	110,4
Gentobiose	1,0	1,10	0,58	4,69	0,051	0,103	109,6
Gentobiose	2,0	2,12	0,67	5,90	0,125	0,250	105,9
Gentobiose	5,0	5,17	0,97	7,84	0,405	0,811	103,4
Turanose	0,5	0,54	2,55	3,05	0,017	0,033	108,7
Turanose	1,0	1,08	3,27	8,34	0,090	0,180	107,9
Turanose	2,0	2,06	0,30	12,31	0,253	0,507	102,9
Turanose	5,0	4,80	3,22	17,12	0,822	1,644	96,0
Melezitose/Palatinose	0,5	0,55	0,94	1,88	0,010	0,021	109,0
Melezitose/Palatinose	1,0	1,10	0,96	1,92	0,021	0,042	109,7
Melezitose/Palatinose	2,0	2,11	0,68	5,99	0,126	0,252	105,3
Melezitose/Palatinose	5,0	5,03	1,43	10,57	0,531	1,062	100,5
Raffinose	0,5	0,54	1,21	1,82	0,010	0,020	108,0
Raffinose	1,0	1,08	0,80	1,09	0,012	0,024	108,0
Raffinose	2,0	2,07	0,35	5,56	0,115	0,231	103,7

Raffinose	5,0	4,94	2,68	12,72	0,629	1,257	98,8
Stachyose	0,5	0,55	0,91	0,96	0,005	0,011	110,8
Stachyose	1,0	1,10	0,89	1,50	0,017	0,033	110,3
Stachyose	2,0	2,10	0,43	5,39	0,113	0,227	105,1
Stachyose	5,0	4,90	0,77	9,62	0,472	0,943	98,0
Kestose	0,5	0,57	0,86	5,60	0,032	0,064	113,9
Kestose	1,0	1,14	0,94	5,07	0,058	0,116	114,2
Kestose	2,0	2,20	0,49	5,20	0,114	0,228	109,9
Kestose	5,0	5,20	0,69	5,65	0,294	0,598	104,0
Maltose	0,5	0,52	2,09	4,04	0,021	0,042	104,2
Maltose	1,0	1,06	0,88	4,22	0,045	0,090	106,4
Maltose	2,0	2,07	0,39	6,88	0,143	0,285	103,6
Maltose	5,0	5,09	0,94	9,27	0,471	0,942	101,7
Erlose	0,5	0,55	2,16	4,46	0,024	0,049	109,6
Erlose	1,0	1,12	1,24	3,32	0,037	0,074	111,9
Erlose	2,0	2,15	0,80	6,17	0,133	0,266	107,7
Erlose	5,0	5,15	0,76	7,64	0,393	0,786	103,0
Panose	0,5	0,55	1,18	1,29	0,007	0,014	110,2
Panose	1,0	1,10	0,80	2,81	0,031	0,062	110,4
Panose	2,0	2,13	0,63	5,78	0,123	0,246	106,5
Panose	5,0	5,18	0,97	7,75	0,401	0,803	103,6
Nystose	0,5	0,52	1,92	3,26	0,017	0,034	103,7
Nystose	1,0	1,07	1,21	2,29	0,025	0,049	106,9
Nystose	2,0	2,09	1,01	4,86	0,102	0,203	104,4
Nystose	5,0	5,14	0,74	5,04	0,259	0,518	102,8
Maltotriose	0,5	0,52	1,85	1,89	0,010	0,020	104,6
Maltotriose	1,0	1,08	0,98	3,96	0,043	0,085	107,6
Maltotriose	2,0	2,10	1,18	6,54	0,137	0,275	105,0
Maltotriose	5,0	5,17	0,92	8,49	0,439	0,878	103,3

Italics: compounds contained in blank honey sample at trace levels

[a]**RSD_r:** relative standard deviation for repeatability

[b]**RSD_R:** relative standard deviation of reproducibility

[c]**S_{IP}:** intermediate precision

[d]***U*:** measurement uncertainty

[e]**RR:** relative percentage recovery rate

The precision results for all concentration levels of maltodextrines are shown in **Table 13**. The relative standard deviation for repeatability (RSD_r) ranged between 0,68 % and 8,28 %, and the relative standard deviation of reproducibility (RSD_R) ranged between 0,98 % and 8,50 %, indicating that the precision of the method is adequate.

Table 13 *Precision and trueness for maltodextrins obtained from the analysis of spiked honey samples*

Compounds	**Target value (µg/kg)**	**Mean value (µg/kg)**	**RSD_r[a] (%)**	**RSD_R[b] (%)**	**S_{IP}[c]**	***U*[d]**	**RR[e] (%)**
DP3	0,5	0,49	4,36	4,98	0,024	0,049	97,9
DP3	1,0	1,01	4,96	7,70	0,078	0,156	101,1
DP3	2,0	1,98	1,04	4,24	0,084	0,168	99,2
DP3	5,0	4,84	8,28	8,50	0,412	0,823	96,9
DP4	0,5	0,56	1,26	2,07	0,011	0,023	111,2
DP4	1,0	1,08	3,11	3,59	0,039	0,077	107,8
DP4	2,0	2,06	0,68	1,40	0,029	0,058	103,0
DP4	5,0	4,89	0,83	3,51	0,172	0,343	97,8
DP5	0,5	0,55	1,51	3,98	0,022	0,044	110,5
DP5	1,0	1,08	3,71	4,27	0,046	0,092	107,9
DP5	2,0	2,08	0,88	1,23	0,026	0,051	103,9
DP5	5,0	4,88	0,93	3,28	0,160	0,321	97,7
DP6	0,5	0,51	1,79	2,85	0,015	0,029	102,7
DP6	1,0	1,03	2,60	2,89	0,030	0,060	103,2
DP6	2,0	2,02	0,98	1,23	0,025	0,050	101,0
DP6	5,0	4,86	1,25	3,12	0,151	0,303	97,1
DP7	0,5	0,52	1,75	2,54	0,013	0,026	104,3
DP7	1,0	1,03	2,54	2,58	0,027	0,053	102,6
DP7	2,0	2,03	0,87	0,99	0,020	0,040	101,3
DP7	5,0	4,85	0,93	3,42	0,166	0,332	97,1
DP8	0,5	0,56	1,16	1,27	0,007	0,014	111,4
DP8	1,0	1,08	2,21	2,29	0,025	0,049	107,6
DP8	2,0	2,04	0,79	1,30	0,027	0,053	102,1
DP8	5,0	4,87	0,81	3,46	0,168	0,337	97,4
DP9	0,5	0,51	3,33	3,66	0,018	0,037	101,0
DP9	1,0	1,03	1,70	1,83	0,019	0,038	102,8
DP9	2,0	1,99	0,81	0,98	0,020	0,039	99,3
DP9	5,0	4,87	1,03	3,60	0,175	0,351	97,5
DP10	0,5	0,52	2,78	3,22	0,017	0,033	103,7
DP10	1,0	1,04	1,89	2,43	0,025	0,051	104,4
DP10	2,0	2,00	1,05	1,47	0,029	0,059	100,0
DP10	5,0	4,85	0,84	3,43	0,162	0,332	97,0
DP11	0,5	0,47	6,17	7,43	0,035	0,070	94,4
DP11	1,0	1,00	2,14	4,87	0,049	0,097	99,6
DP11	2,0	1,96	1,28	2,32	0,046	0,091	98,1
DP11	5,0	4,92	1,09	2,19	0,108	0,215	98,5

Italics: compounds contained in blank honey sample at trace levels

[a]**RSD_r:** relative standard deviation for repeatability
[b]**RSD_R:** relative standard deviation of reproducibility
[c]**S_{IP}:** intermediate precision
[d]***U*:** measurement uncertainty
[e]**RR:** relative percentage recovery rate

3.5.2.3 Trueness

Accuracy is one of the key parameters to be assessed for assay validation and involves common systematic errors (bias). It is determined by determining trueness and precision. The trueness was determined as the mean value of the results from the three experiments and expressed in terms of the relative percentage recovery rate (RR%). The results of the recovery studies are shown in **Table 12**,**Table 13**. Recoveries were satisfactory ranging from 89,9 % to 115,5 % for carbohydrates and from 96,9% to 111,4 % for maltodextrins. If the carbohydrates that were already present in honey at trace levels are removed the recoveries ranged from 98,8 % to 115,5 %.

3.5.2.4 Measurement uncertainty

The uncertainty of measurement (*U*) for all compounds was calculated. The term uncertainty can be defined as a parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand. The expanded uncertainty (*U*) provides an interval within the value of the measurement and is believed to lie with a higher level of confidence. It is obtained by multiplying the combined standard uncertainty u_c (y) with a coverage factor k. The choice of the factor k is based on the level of confidence desired. For a confidence level of 95 % the k is 2. The uncertainty on the result may arise from many possible sources including contamination, sample inhomogeneity, reading errors, weighing- pipetting handling errors, purity of standards, construction of calibration curve, interferences etc. The most important variables contributing to the uncertainty of measurement were the intermediate precision (S_{ip}). The calculated expanded uncertainties for all compounds are shown in **Table 12** and **Table 13**.

3.5.3 Application in real samples

Honey samples were collected by competent authorities of the EU Member States (MS) through the European Commission JRC-Geel site (Belgium), sourced by various points of the production and supply chain, including mostly retailers but also border inspection, distributors, importers, packaging companies, processors, producers, storage companies and wholesalers of different variety and geographical origin (**Table 14**). The samples were used to evaluate authenticity and detect adulteration in honey. The analysis was applied at the facilities of the food fraud laboratories of JRC-Geel.

Table 14 *Description of samples*

No.	Sample ID	Honey botanical origin	Floral composition
1	HS-00001	Monofloral	Rosemary
2	HS-00003	Monofloral	Heather
3	HS-00005	Monofloral	Manuka
4	HS-00007	Monofloral	Heather
5	HS-00009	Polyfloral	Thyme
6	HS-00011	Polyfloral	Heather
7	HS-00012	Polyfloral	Rhododendron
8	HS-00013	Monofloral	Eucalyptus
9	HS-00014	Monofloral	Rosemary
10	HS-00015	Monofloral	Acacia
11	HS-00018	Polyfloral	Thyme
12	HS-00019	Monofloral	Thyme
13	HS-00020	Monofloral	Chestnut
14	HS-00021	Monofloral	Lavender
15	HS-00022	Polyfloral	Rhododendron
16	HS-00029	Monofloral	Thyme
17	HS-00031	Monofloral	Robinia
18	HS-00034	Monofloral	Acacia
19	HS-00040	Monofloral	Acacia
20	HS-00077	Monofloral	Acacia
21	HS-00081	Monofloral	Orange
22	HS-00089	Monofloral	Acacia
23	HS-00091	Monofloral	Acacia
24	HS-00094	Monofloral	Linden
25	HS-00095	Monofloral	Acacia
26	HS-00096	Monofloral	Acacia
27	HS-00102	Monofloral	Linden
28	HS-00122	Monofloral	Orange
29	HS-00125	Monofloral	Acacia
30	HS-00128	Monofloral	Orange
31	HS-00143	Monofloral	Acacia
32	HS-00154	Polyfloral	Spruce
33	HS-00179	Monofloral	Linden
34	HS-00197	Monofloral	Thyme
35	HS-00205	Monofloral	Thyme
36	HS-00208	Monofloral	Chestnut
37	HS-00209	Monofloral	Chestnut
38	HS-00221	Monofloral	Robinia
39	HS-00232	Monofloral	Buckwheat
40	HS-00233	Monofloral	Linden
41	HS-00253	Monofloral	Acacia
42	HS-00259	Monofloral	Linden
43	HS-00263	Monofloral	Acacia

44	HS-00265	Monofloral	Buckwheat
45	HS-00266	Monofloral	Acacia
46	HS-00268	Monofloral	Linden
47	HS-00271	Monofloral	Linden
48	HS-00293	Monofloral	Linden
49	HS-00298	Polyfloral	Forest
50	HS-00302	Monofloral	Linden
51	HS-00303	Monofloral	Lavender
52	HS-00304	Monofloral	Acacia
53	HS-00307	Monofloral	Acacia
54	HS-00311	Monofloral	Orange
55	HS-00317	Monofloral	Robinia
56	HS-00323	Monofloral	Acacia
57	HS-00325	Monofloral	Acacia
58	HS-00327	Monofloral	Orange
59	HS-00372	Monofloral	Robinia
60	HS-00375	Unknown	Robinia
61	HS-00377	Monofloral	Robinia
62	HS-00379	Monofloral	Orange
63	HS-00384	Monofloral	Heather
64	HS-00388	Monofloral	Rosemary
65	HS-00389	Monofloral	Rosemary
66	HS-00399	Monofloral	Chestnut
67	HS-00402	Monofloral	Manuka
68	HS-00403	Polyfloral	Fir
69	HS-00416	Polyfloral	Sunflower
70	HS-00417	Monofloral	Linden
71	HS-00418	Polyfloral	Echium
72	HS-00420	Monofloral	Linden
73	HS-00423	Monofloral	Buckwheat
74	HS-00426	Monofloral	Linden
75	HS-00435	Monofloral	Eucalyptus
76	HS-00439	Monofloral	Acacia
77	HS-00442	Monofloral	Linden
78	HS-00447	Monofloral	Rosemary
79	HS-00449	Monofloral	Acacia
80	HS-00451	Monofloral	Linden
81	HS-00452	Monofloral	Acacia
82	HS-00463	Monofloral	Acacia
83	HS-00464	Monofloral	Linden
84	HS-00465	Monofloral	Acacia
85	HS-00466	Monofloral	Acacia
86	HS-00478	Monofloral	Acacia
87	HS-00480	Monofloral	Heather
88	HS-00496	Monofloral	Eucalyptus
89	HS-00497	Monofloral	Thyme

90	HS-00498	Monofloral	Lavender
91	HS-00500	Monofloral	Linden
92	HS-00504	Monofloral	Linden
93	HS-00506	Monofloral	Linden
94	HS-00507	Monofloral	Buckwheat
95	HS-00508	Monofloral	Buckwheat
96	HS-00509	Monofloral	Linden
97	HS-00513	Monofloral	Buckwheat
98	HS-00515	Monofloral	Linden
99	HS-00518	Monofloral	Linden
100	HS-00519	Monofloral	Linden
101	HS-00526	Polyfloral	Forest
102	HS-00533	Polyfloral	Forest
103	HS-00551	Monofloral	Manuka
104	HS-00563	Monofloral	Acacia
105	HS-00564	Monofloral	Acacia
106	HS-00565	Monofloral	Acacia
107	HS-00567	Monofloral	Acacia
108	HS-00571	Monofloral	Acacia
109	HS-00575	Monofloral	Acacia
110	HS-00590	Monofloral	Acacia
111	HS-00592	Monofloral	Acacia
112	HS-00609	Polyfloral	Chestnut
113	HS-00612	Polyfloral	Thyme+
114	HS-00619	Monofloral	Orange
115	HS-00620	Monofloral	Orange
116	HS-00621	Monofloral	Chestnut
117	HS-00628	Monofloral	Orange
118	HS-00630	Monofloral	Acacia
119	HS-00637	Monofloral	Orange
120	HS-00641	Monofloral	Orange
121	HS-00657	Monofloral	Acacia
122	HS-00659	Monofloral	Heather
123	HS-00660	Monofloral	Eucalyptus
124	HS-00665	Monofloral	Acacia
125	HS-00674	Monofloral	Acacia
126	HS-00691	Monofloral	Acacia
127	HS-00693	Monofloral	Robinia
128	HS-00695	Monofloral	Rosemary
129	HS-00699	Monofloral	Manuka
130	HS-00706	Monofloral	Orange
131	HS-00710	Polyfloral	Buckwheat
132	HS-00711	Monofloral	Linden
133	HS-00729	Monofloral	Acacia
134	HS-00730	Monofloral	Acacia
135	HS-00732	Monofloral	Eucalyptus

136	HS-00737	Monofloral	Orange
137	HS-00738	Monofloral	Eucalyptus
138	HS-00739	Monofloral	Rosemary
139	HS-00740	Monofloral	Eucalyptus
140	HS-00742	Monofloral	Acacia
141	HS-00745	Polyfloral	Heather
142	HS-00748	Monofloral	Linden
143	HS-00760	Monofloral	Buckwheat
144	HS-00764	Monofloral	Linden
145	HS-00825	Monofloral	Orange
146	HS-00838	Monofloral	Linden
147	HS-00839	Polyfloral	Meadow
148	HS-00844	Polyfloral	Manuka
149	HS-00845	Polyfloral	Manuka
150	HS-00848	Monofloral	Manuka
151	HS-00868	Monofloral	Manuka
152	HS-00892	Polyfloral	Fir
153	HS-00938	Monofloral	Acacia
154	HS-00943	Monofloral	Acacia
155	HS-00952	Monofloral	Acacia
156	HS-00959	Monofloral	Acacia
157	HS-01075	Monofloral	Chestnut

3.5.4 Determination of adulteration

In order to assess honey adulteration, the 157 honey samples collected were analysed for carbohydrate profiles and oligo-/polysaccharides based on the developed methods. These samples had been analysed and characterized as adulterated or authentic by EA-IRMS and LC-IRMS (JRC-Geel) based on established compliance criteria [42].

Markers that were taken into account are the presences of polysaccharides, the concentration range for carbohydrates of individual honey varieties, the sum of fructose and glucose (F+G) and the following possible ratios: fructose/glucose (F/G), sucrose/turanose (S/T), sucrose/maltose (S/M), maltotriose/turanose (Mtriose/T), maltose/gentobiose (M/Gent), maltotriose/gentobiose (Mtriose/Gent), panose/gentobios (Pan/Gent) and raffinose/gentobiose (Raf/G) (Annex 1-3). Annex 4 provides an overview of the concentration ranges and ratios of individual sugars for different floral composition of honey samples. From the total amount of 157 honey samples 40 were non-compliant. **Table 15** provides a summary of the compliant (C) and non-compliant (NC) honey samples.

Table 15 *Summary of the compliant and non-compliant honey samples based on the varieties*

No.	Variety	Number of compliant and non-compliant honey samples
1	Acacia	34 C / 10 NC
2	Buckweat	6C / 1 NC
3	Buckweat+*	1 C
4	Chestnut	4 C / 2 NC
5	Chestnut+	1 C
6	Echium+	1 C
7	Eucalyptus	5 C / 2 NC
8	Fir+	1 C / 1 NC
9	Forest+	1 C / 2 NC
10	Heather	5 C
11	Heather+	1 C / 1 NC
12	Lavender	3 C
13	Linden	20 C / 6 NC
14	Manuka	3 C / 3 NC
15	Manuka+	2 NC
16	Meadow+	1 NC
17	Orange	13 C / 1 NC
18	Rhododendron+	1 C / 1 NC
19	Robinia	5 C / 2 NC
20	Rosemary	7 C
21	Spruce+	1 NC
22	Sunflower+	1 C
23	Thyme	4 C / 1 NC
24	Thyme+	3 NC

*+ Polyfloral honey

IRMS measurements confirmed 34 samples as non-compliant (85,0 %) and 6 samples were not confirmed (15,0 %). Compared to PAD analysis, taking into account the above markers, 29 non-compliant samples were confirmed (72,5 %) and 11 were not confirmed (27,5 %). This comparison is shown in **Table 16**.

Table 16 *Comparison of non-compliant honey samples between HPAE-PAD and EA/LC-IRMS analysis*

Samples		**PAD**		**IRMS**	
Compliant	117	**Confirmed**	**Not-confirmed**	**Confirmed**	**Not-confirmed**
Non-compliant	40	29	11	34	6
Total samples	157	72,5 %	27,5 %	85,0 %	15,0 %

The total agreement between the two techniques is adequate. From the 11 samples which were not confirmed by the PAD, 2 samples were considered as adulterated based on C4

adulteration and 9 samples based on combination of C3/C4. These results can be linked to no possible variations to the specific markers selected. However, C4 adulteration is well detected by the reference method of EA-IRMS at 7 % of adulteration. The LC-IRMS can detect adulteration much lower than the EA-IRMS technique, 1% for detecting adulteration with C4 sugars but 10 % for C3 sugars. HPAE-PAD can detect adulteration with C3 exogenous sugars even lower. By applying LC-IRMS a clear separation between glucose, fructose, disaccharides and trisaccharides is achievable but the oligosaccharide peak is not detected with high sensitivity and no separation is possible between the DPs. Among the non-compliant honey samples, HPAE-PAD did detect 6 samples as adulterated, which were not confirmed by LC-IRMS analysis. Additionally, different type of honeys can be blended with syrups (C3 sugars or a mixture C4/C3 sugars) in order to achieve similar isotopic patterns. Hence, HPAE-PAD is an effective analytical method providing a specific and sensitive detection of oligo- and polysaccharides in determining non-compliance in honey samples.

Further on, SIMCA-P software (Version 14, Umetrics, Umea, Sweden) was used to perform multivariate statistical analysis on the qualitative and quantitative data of ratios and the sugar/maltodetrines profiles (Annex 1-3). Orthogonal Partial Least Square Discriminant Analysis (OPLS-DA) was applied to build descriptive and predictive models. The validity and robustness of the models were evaluated by R2cum (goodness of fit) and Q2cum (predictability) parameters. A clear discrimination between adulterated and authentic honey samples was achieved (**Figure 16**).

The OPLS-DA tool of SIMCA-P was used to find features markers contributing to group discrimination. Tentative markers were considered with a variable important in projection higher than 1,2 (VIP>1,2) and centred and scaled coefficients between -0,002 and 0,002 (-0,002 > CoeffCS > 0,002). Metabolites that were highlighted revealed DP13, DP14, DP7, DP15, DP10, DP4, DP12, fructose, DP16, DP17, DP11, DP9, maltotriose, DP5 and F+G significantly contributing to the discrimination between adulterated and non-adulterated honey samples.

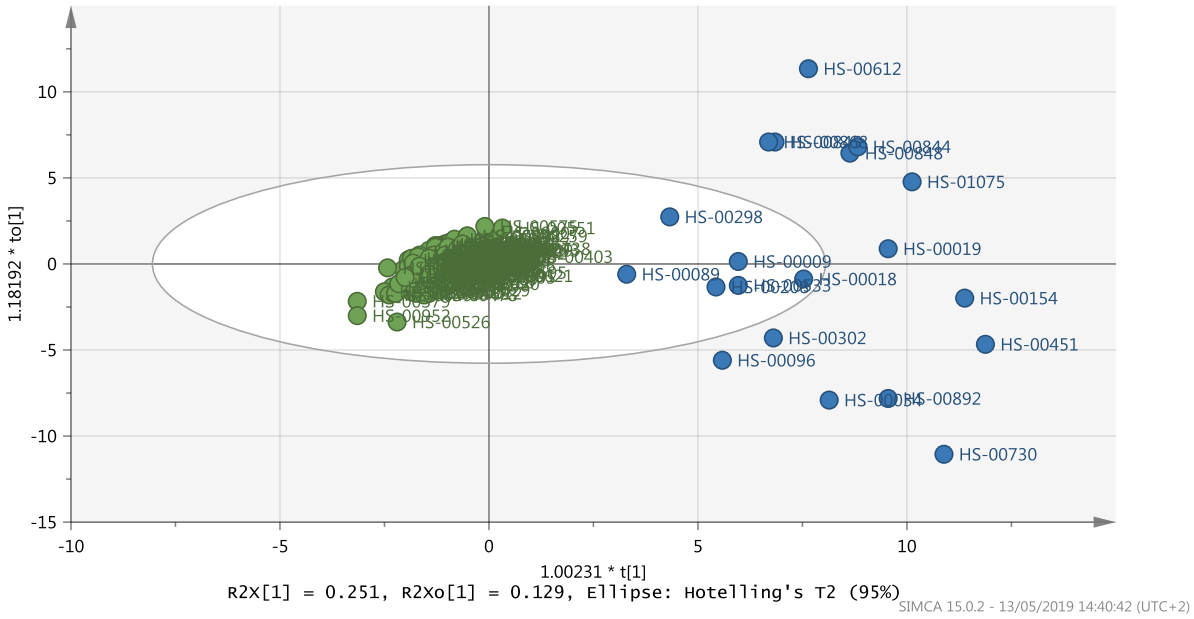

Figure 16 *OPLS-DA classification model between adulterated (blue) and authentic honey samples (green) with goodness of fit 0,9 and goodness of prediction 0,811*

3.5.5 Determination of authenticity

Honey authenticity was evaluated by calculating the range of the sum (F+G), the ratios between specific sugars and the concentration levels of the sugar profile for the individual monofloral and polyfloral samples. From the 157 honey samples, 137 samples were monofloral and 20 samples polyfloral. As it can be observed in Annex 4 a clear discrimination between the varieties based on individual sugars is not obvious, as the ranges in many cases do overlap. In order to better identify differences between the varieties, multivariate statistical analysis based on SIMCA-P was applied taking into account as variables the concentration levels of the 18 carbohydrates. Melibiose and arabinose were removed as variables as they were mostly not detected in the honey samples. As in the previous chapter Orthogonal Partial Least Square Discriminant Analysis (OPLS-DA) was applied to build descriptive and predictive models for different combinations of honey varieties. A clear discrimination was achieved between acacia and orange (**Figure 17**). By observing the loadings plot correlations between variables can be identified. Comparing the loadings plot to the scores plot enables to understand how the variables relate to the observations. By analysing the loading plot of the OPLS-DA model for acacia and orange the variables mostly contributing to the discrimination are kestose, sucrose, maltose and fructose (**Figure 18**). The same can be observed by a VIP graph as show in **Figure 19**.

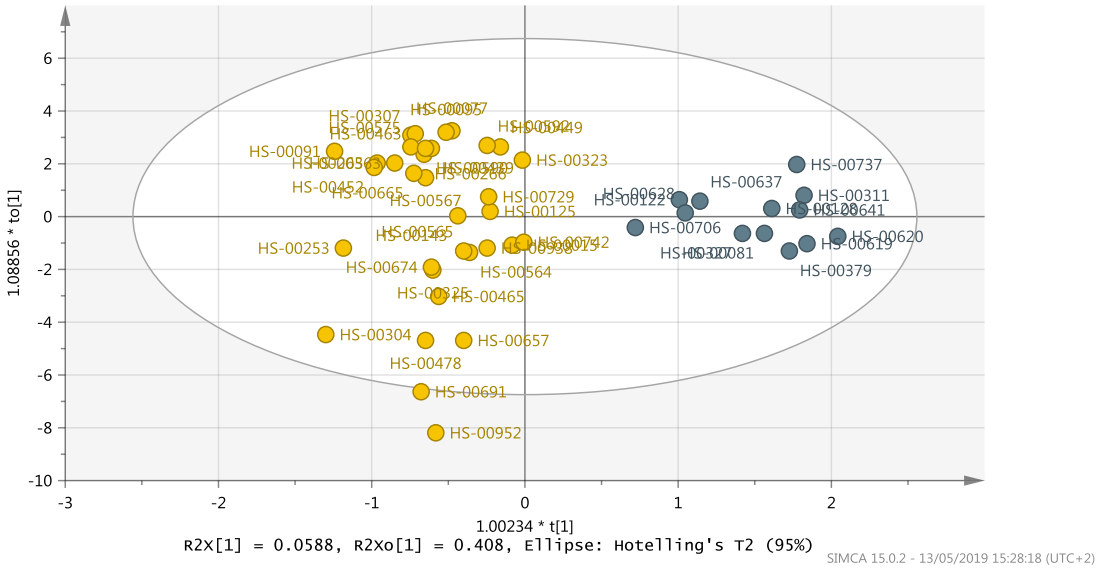

Figure 17 *OPLS-DA classification model between acacia (mustard yellow) and orange (grey) with goodness of fit 0,882 and goodness of prediction 0,634 (47 samples)*

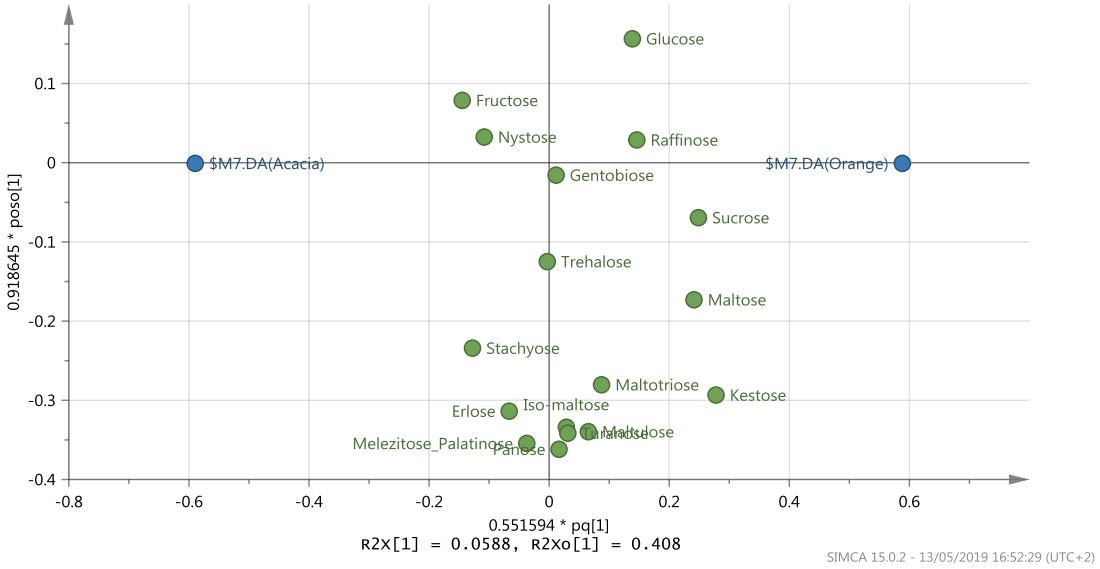

Figure 18 *OPLS-DA loading plot between acacia and orange (X in green, Y in blue)*

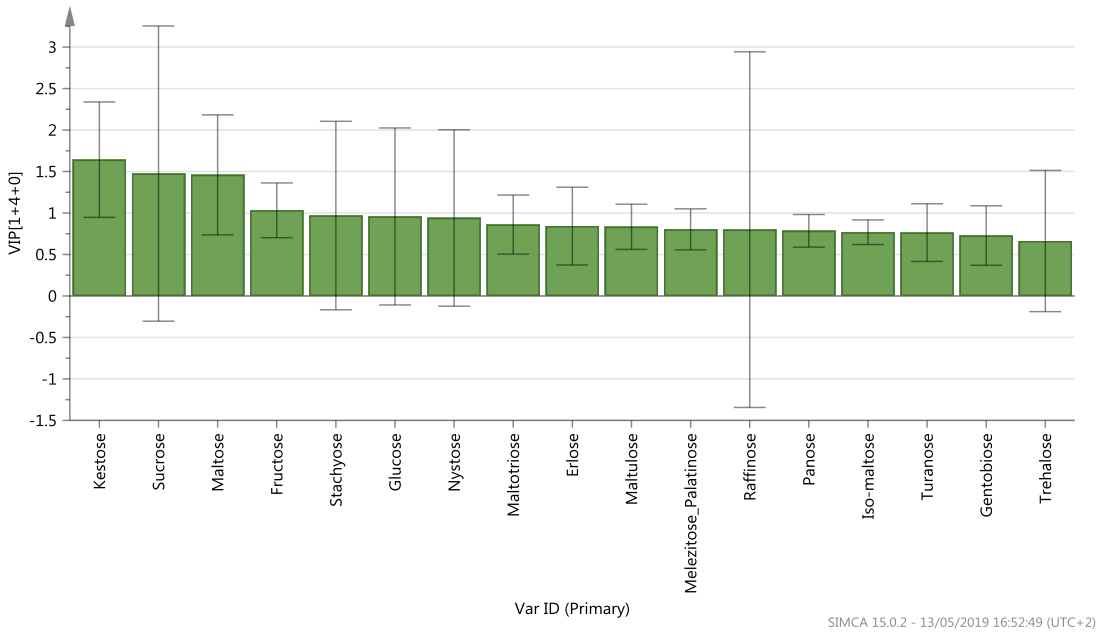

Figure 19 *VIP plot for acacia and orange*

OPLS-DA discrimination model for orange and linden revealed as discriminating markers erlose, maltotriose, maltose and gentobiose. The classification model is shown in **Figure 20**.

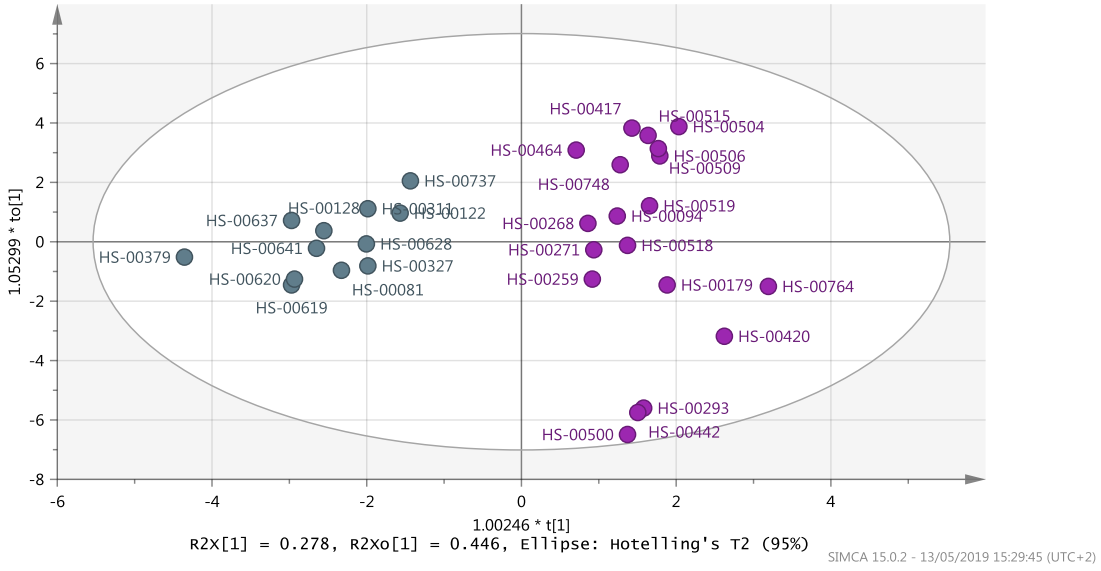

Figure 20 *OPLS-DA classification model between orange (yellow) and linden (linden) with goodness of fit 0,9 and goodness of prediction 0,873 (31 samples)*

OPLS-DA discrimination model between rosemary, chestnut, orange and linden revealed as discriminating markers panose, maltose, maltotriose, erlose and trehalose. The classification model is shown in **Figure 21**.

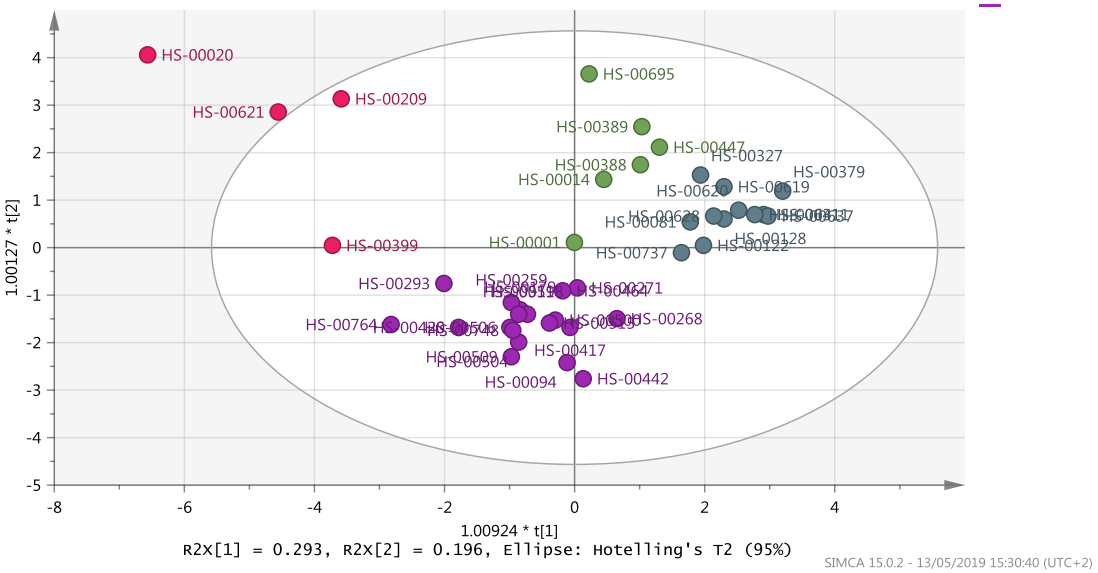

Figure 21 *OPLS-DA classification model between rosemary (green), chestnut (red), orange (grey) and linden (purple) with goodness of fit 0,821 and goodness of prediction 0,642 (41 samples)*

OPLS-DA discrimination model between rosemary, orange and linden revealed as discriminating markers maltose, maltotriose, erlose, panose, iso-maltose and gentobiose. The classification model is shown in **Figure 22**.

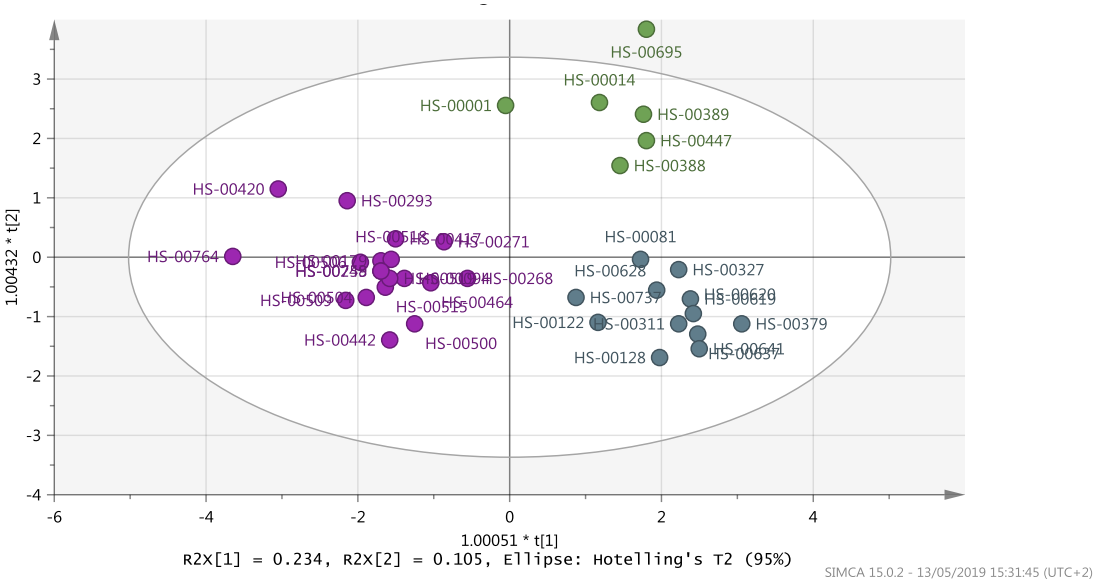

Figure 22 *OPLS-DA classification model between rosemary (green), orange (grey) and linden (purple) with goodness of fit 0,817 and goodness of prediction 0,846 (37 samples)*

OPLS-DA discrimination model between manuka, orange and linden revealed as discriminating markers erlose, maltotriose, maltose, melezitose/palatinose, stachyose and trehalose. The classification model is shown in **Figure 23**.

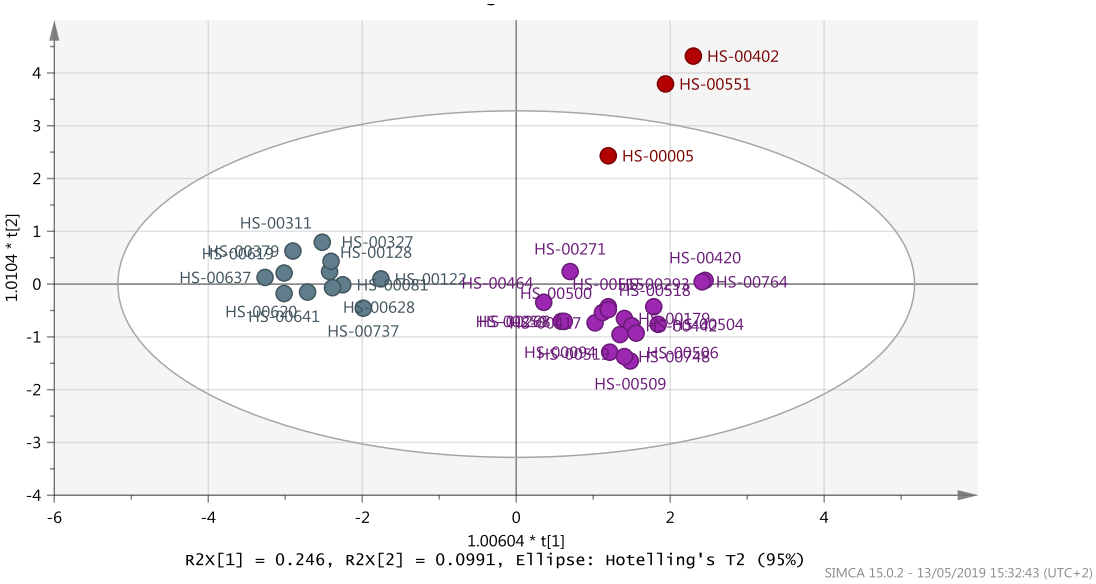

Figure 23 *OPLS-DA classification model between manuka (red), orange (grey) and linden (purple) with goodness of fit 0,907 and goodness of prediction 0,692 (34 samples)*

OPLS-DA discrimination model between manuka, lavender, orange and linden revealed as discriminating markers trehalose, erlose, melezitose/palatinose, maltotriose, maltose and stachyose. The classification model is shown in **Figure 24**.

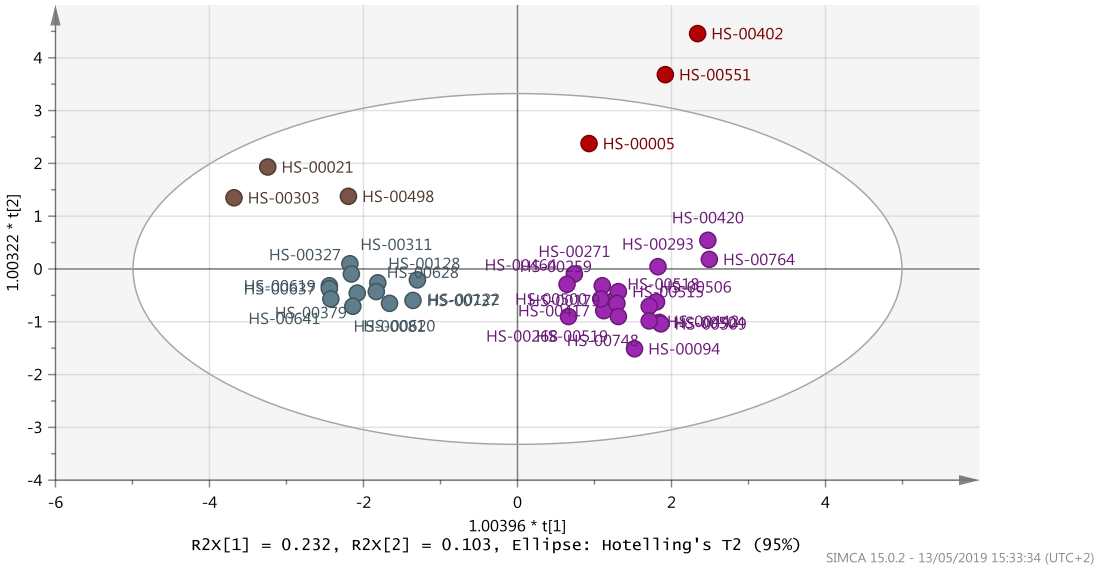

Figure 24 *OPLS-DA classification model between manuka (red), lavender (brown), orange (grey) and linden (purple) with goodness of fit 0,821 and goodness of prediction 0,529 (37 samples*

OPLS-DA discrimination model between orange and buckwheat revealed as discriminating markers erlose, maltotriose, maltose, kestose, maltulose and fructose. The classification model is shown in **Figure 25**.

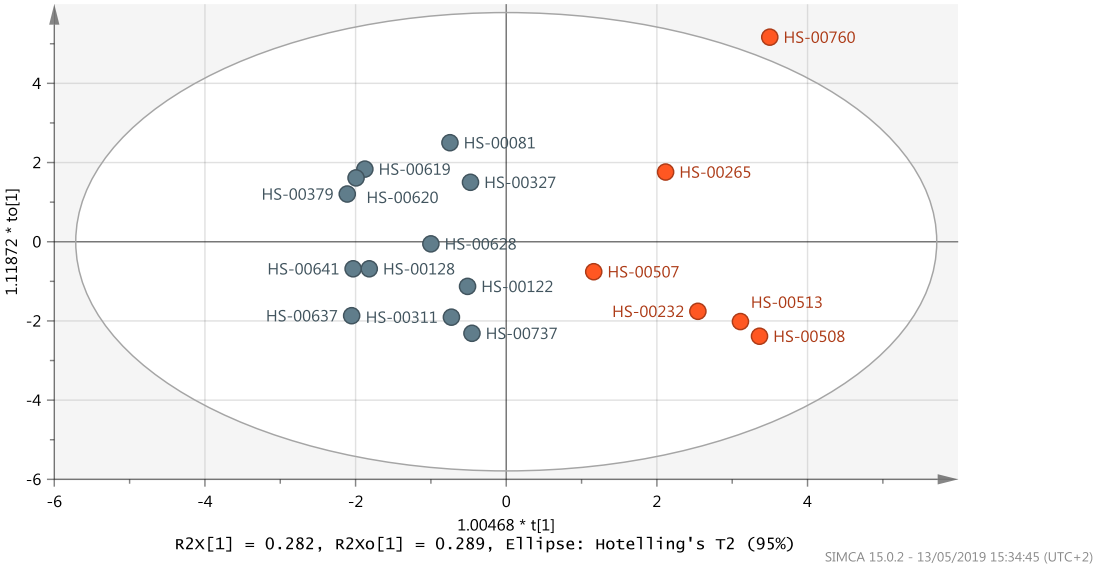

Figure 25 *OPLS-DA classification model between orange (grey) and buckwheat (mustard yellow) with goodness of fit 0,868 and goodness of prediction 0,678 (18 samples)*

OPLS-DA discrimination model between robinia, orange and buckwheat revealed as discriminating markers erlose, maltose, maltotriose, glucose and kestose. The classification model is shown in **Figure 26**.

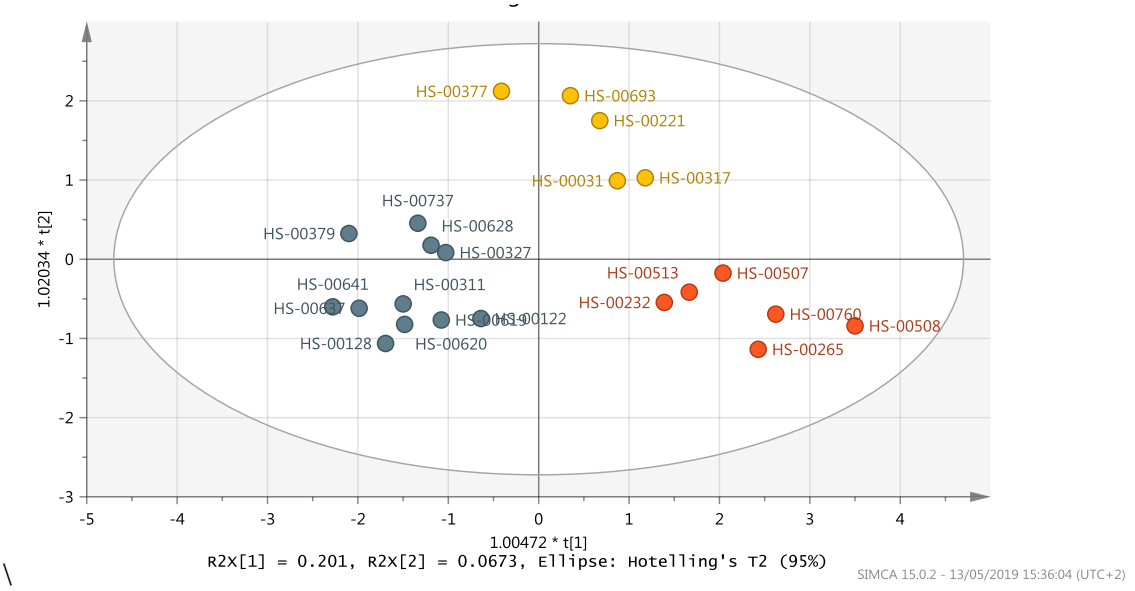

Figure 26 *OPLS-DA classification model between robinia (yellow), orange (grey), buckwheat (mustard yellow) with goodness of fit 0,846 and goodness of prediction 0,59 (22 samples)*

OPLS-DA discrimination model between thyme, orange and linden revealed as discriminating markers erlose, maltotriose, maltose, iso-maltose, maltulose and stachyose. The classification model is shown in **Figure 27**.

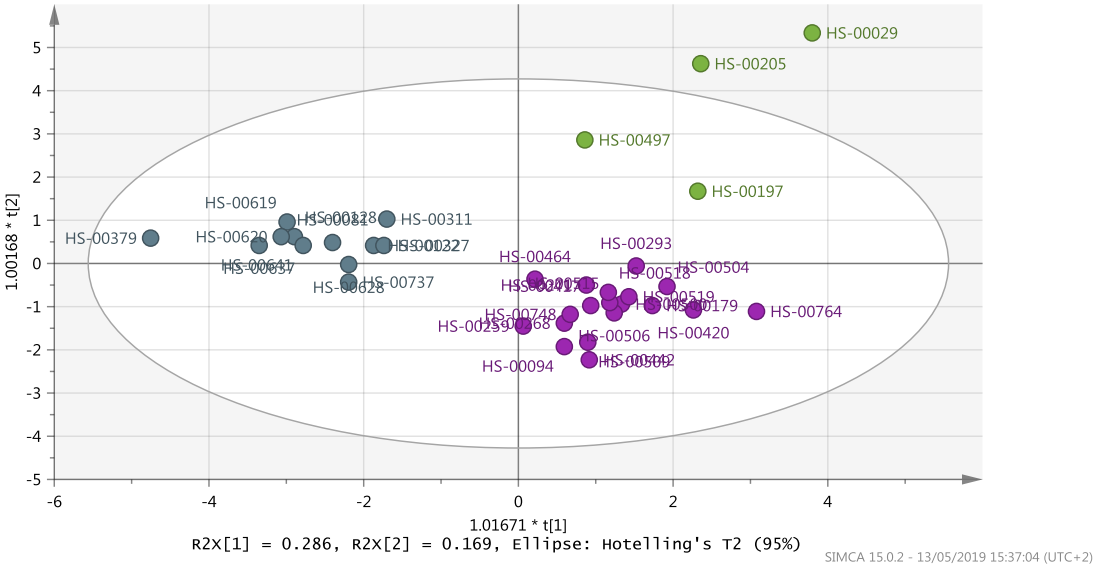

Figure 27 *OPLS-DA classification model between thyme (green), orange (grey) and linden (purple) with goodness of fit 0,854 and goodness of prediction 0,716 (35 samples)*

OPLS-DA discrimination model between chestnut, orange and linden revealed as discriminating markers erlose, maltose, maltotriose, melezitose/palatinose, stachyose and isomaltose. The classification model is shown in **Figure 28**.

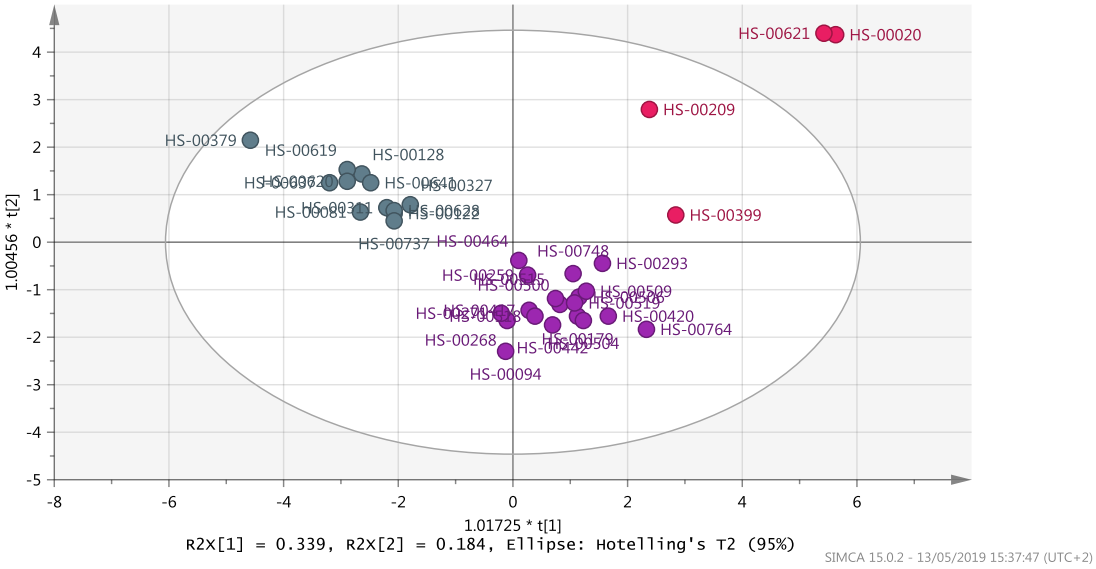

Figure 28 *OPLS-DA classification model between chestnut (red), orange (grey) and linden (purple) with goodness of fit 0,857 and goodness of prediction 0,808 (35 samples)*

OPLS-DA discrimination model between chestnut, orange and linden revealed as discriminating markers trehalose, erlose, maltotriose, maltose and gentobiose. The classification model is shown in **Figure 29**.

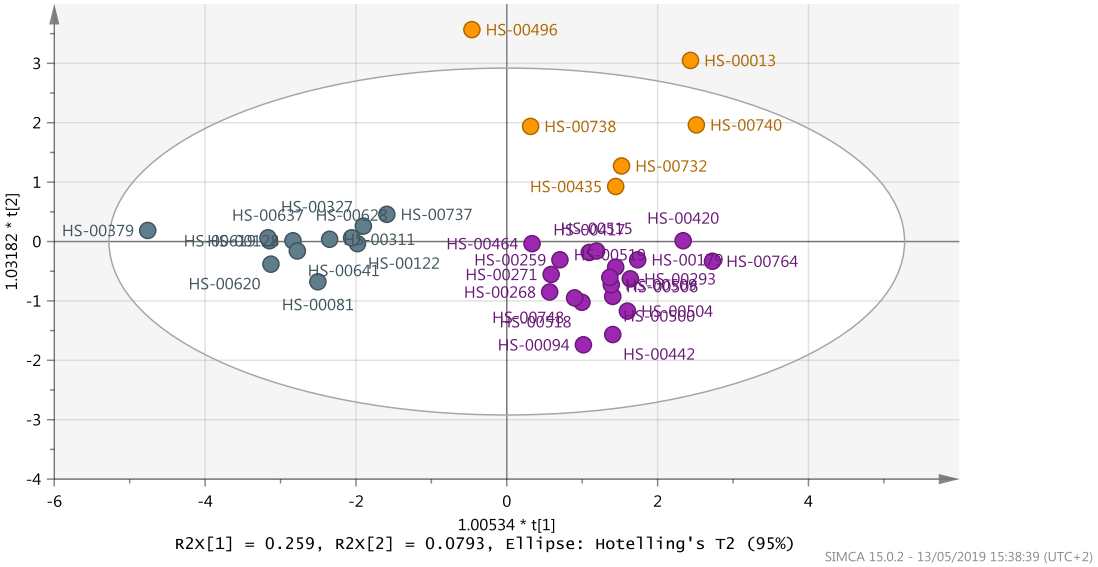

Figure 29 *OPLS-DA classification model between eucalyptus (mustard yellow), orange (grey) and linden (purple) with goodness of fit 0,816 and goodness of prediction 0,751 (37 samples)*

As observed from the above results successful classification models were able to be built for the discrimination of different honey varieties. However, in order to create robust models and come to solid conclusions, the sample size needs to be enlarged including higher sample variability.

To establish a reliable statistical model to differentiate samples based on varietal origin and perform classification of unknown samples, needs also a large number of authentic samples. However, the statistical models cannot take into account all variability of samples that is why the final sample classification in such a workflow will always have some probability of incorrect classification. This can be evaluated through diagnostic measures, such as, the classification error based on the ratio of the number of misclassified samples relative to the samples in the classes, or the values of specificity and sensitivity of the model. This is analogous to targeted analysis in which the concentration of a specific analyte is reported along with the uncertainty of the measurement. Therefore, the collection of authentic samples is crucial not only for honey authenticity studies but also for detection of honey adulteration.

4. CONCLUSIONS

The aim of the thesis was to introduce an HPAE-PAD method suitable for detecting adulteration and determinate authenticity in honey. Two methods were successfully developed for the analysis of carbohydrates and oligo-/polysaccharides using a Dionex CarboPac PA200 advanced chromatographic column. The analytical column allowed efficient separation of a significant number of sugars (trehalose, arabinose, glucose, fructose, melibiose, iso-maltose, maltulose, sucrose, gentobiose, melezitose + palatinose, turanose, raffinose, stachyose, kestose, maltose, erlsoe, panose, nystose and maltotriose) and maltodextrins with degree of polymerization from 1 to 24. The determination of 20 carbohydrates and DP1-11 in honey samples were for the first time successfully quantified. Concerning sugar profiling, methods cited in the literature quantified a few sugars and most of the analysed sugars were calculated as relative percentages. Regarding the analysis of matltodextrins, extracts and mixtures of maltodextrines were used to asses adulteration in honey samples in a qualitative approach without providing quantitative results. The pulsed amperometric detector is sensitive, thus allowed not only the determination of carbohydrates at trace levels but also provided the possibility to detect major sugar content at high concentration. The developed methods are simple, rapid, selective, cost-effective with high sample throughput.

The methods were efficiently validated in terms of linearity, dynamic range, analytical limits, precision, accuracy and measurement uncertainty. Regarding sugar profiling repeatability (RSD_r) ranged between 0,26 % and 14,96 % and reproducibility (RSD_R) between 1,09 % and 17,12 %, indicating that the precision of the method is sufficient. Moreover, the repeatability (RSD_r) and reproducibility (RSD_R) for maltodextrins profiling ranged between 0,68 % and 8,28 %, and between 0,98 % and 8,50 %, respectively. Recoveries were satisfactory ranging from 89,9 % to 115,5 % for carbohydrates and from 96,96 % to 111,4 % for maltodextrins analysis.

In order to determinate honey adulteration, 157 honey samples were analysed for carbohydrate profiles and oligo-/polysaccharides based on the developed methods. Markers that were taken into account were the presence of polysaccharides, the concentration range for carbohydrates of individual honey varieties, and different ratios between sugars. In comparison to methods already established in identifying honey adulteration, EA/LC-IRMS measurements confirmed 34 samples as non-compliant (85,0 %) and 6 samples were not confirmed (15,0 %). Compared to PAD analysis, taking into account the above markers, 29 non-compliant samples were confirmed (72,5 %) and 11 were not confirmed (27,5 %). The agreement between the two methods was acceptable. From the 11 samples which were not confirmed by the PAD analysis, 2 samples were considered as adulterated based on C4 adulteration and 9 samples based on combination of C3/C4. These results can be linked to no possible variations to the specific markers selected. On the other hand, PAD analysis succeeded to detect adulteration in 6 samples based on the presence of polysaccharides, which were not confirmed by IRMS analysis. Furthermore, by applying multivariate statistical analysis (SIMCA-P), a discrimination between adulterated and authentic honey samples was achieved.

Honey authenticity was assessed by calculating the range of the sum (F+G), the ratios between specific sugars and the concentration levels of the sugar profile for the individual floral composition of the honey samples. Multivariate statistical analysis was applied taking into account as variables the concentration levels of the carbohydrates. Sufficient discriminating models were established for different combination of honey varieties and possible markers were identified. The results were promising, but in order to create robust models and come to solid conclusions, the sample size needs to be enlarged including higher sample variability.

Overall the developed methods proved their potential and capability in determining the quality and composition of honey related to botanical origin and in identifying honey adulteration.

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6. LIST OF ABBREVIATIONS

5-HMF	5-hydroxymethylfurfal
AFGP	2-acetylfuran-3-glucopyranoside
ANOVA	Analysis of Variance
C	Compliant
CoeffCS	Centred and scaled coefficients
DFAs	Difructose anhydrides
DP	Degree of polymerization
EA-IRMS	Isotope ratio mass spectroscopy based on elemental analysis
FAO	Food and Agriculture Organization
GC	Gas chromatography
GC/MS	Gas chromatography coupled with mass spectrometry
HFCS	High fructose corn syrup
HFIS	High fructose inulin syrup
HPAEC	High-performance anion-exchange chromatography
HPAE-PAD	High-performance anion-exchange chromatography coupled with pulsed amperometric detection
HPLC	High-performance liquid chromatography
HPLC-DAD	High-performance liquid chromatography coupled with diode array detection
HPTLC	High performance thin-layer chromatography
IR	Infrared-based spectroscopy
IRMS	Isotope ratio mass spectroscopy
LC-IRMS	Isotope ratio mass spectroscopy coupled with liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MIR	Mid-infrared region
MS	Member States
NaOAC	Sodium acetate
NaOH	Sodium hydroxide
NC	Non-compliant
NIR	Near-infrared region
NMR	Nuclear magnetic resonance
OPLS-DA	Orthogonal Partial Least Square Discriminant Analysis
PAD	Pulsed amperometric detection
r^2	Coefficient of determination
RI	Refractive index
RID	Refractive index detector
RR%	Relative percentage recovery rate
RS	Rice syrup
RSD_r	Standard deviation for repeatability
RSD_R	Standard deviation of reproducibility

RT	Retention time
RUBISCO	Ribulose bisphosphate carboxylase/oxygenase
S_b	Standard deviation of the intercept
S_{ip}	Intermediate precision
TLC	Thin-layer chromatography
U	Uncertainty of measurement
UPLC-Q-TOF MS	Ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry
UV	Ultraviolet
VIP	Variable important in projection

7. LIST OF ANNEXES

ANNEXES

Annex 1 *Overview of adulteration decisions and sugar ratios for the honey samples*

ID sample	Variety	EA-IRMS	LC-IRMS	LC-IRMS with U	IRMS Adulteration	HPAE-PAD Adulteration	Comments	Fru+Glu	Fru/Glu	Suc/Tur	Suc/Maltose	Mtriose/Tur	Maltose/Gent	Mtriose/Gent	Pan/Gent	Raf/Gent
HS-00001	Rosemary	Compliant	Compliant	Compliant	N*	N		59,38	1,05	0,31	0,73	0,01	4,51	0,14	1,65	ND***
HS-00003	Heather	Compliant	Compliant	Compliant	N	N		56,37	1,39	0,47	1,08	0,01	1,72	0,04	0,35	ND
HS-00005	Manuka	Compliant	Compliant	Compliant	N	N		65,39	1,41	0,24	0,65	0,02	4,11	0,28	1,31	0,16
HS-00007	Heather	Compliant	Compliant	Compliant	N	N		61,68	0,96	0,24	0,16	0,24	7,13	1,14	0,17	ND
HS-00009	Thyme+	Compliant	Compliant	Compliant	N	Y**	Not from others	54,34	1,25	0,52	1,19	0,05	6,55	0,71	3,13	5,20
HS-00011	Heather+	Compliant	Non Compliant	Compliant	N	N		55,05	1,65	0,27	0,71	0,01	4,40	0,16	1,44	ND
HS-00012	Rhododendron+	Compliant	Compliant	Compliant	N	N		61,39	1,70	0,23	0,47	0,02	7,13	0,26	2,05	ND
HS-00013	Eucalyptus	Compliant	Compliant	Compliant	N	N		69,51	1,53	0,26	0,70	ND	7,00	ND	1,53	ND
HS-00014	Rosemary	Compliant	Non Compliant	Compliant	N	N		63,75	1,28	0,24	0,64	0,01	11,69	0,39	3,66	ND
HS-00015	Acacia	Compliant	Compliant	Compliant	N	N		64,47	1,36	0,18	0,15	0,06	28,09	1,27	2,15	ND
HS-00018	Thyme+	Compliant	Non Compliant	Compliant	N	Y	Not from others	53,48	1,68	0,32	0,80	0,05	6,71	0,80	3,21	5,13
HS-00019	Thyme	Compliant	Non Compliant	Compliant	N	Y	Not from others	54,53	1,12	0,52	1,06	0,14	5,52	1,53	3,83	9,12
HS-00020	Chestnut	Compliant	Compliant	Compliant	N	N		50,95	1,81	0,51	1,17	0,01	4,80	0,13	1,43	ND
HS-00021	Lavender	Compliant	Compliant	Compliant	N	N		70,73	0,93	0,89	0,51	0,09	77,78	3,99	5,25	1,89
HS-00022	Rhododendron+	Non compliant	Non Compliant	Non compliant	Y	Y	Yes by ratio M/Gent, Gent high	56,53	1,97	0,38	1,04	0,01	0,97	0,02	0,39	ND
HS-00029	Thyme	Compliant	Compliant	Compliant	N	N		55,79	1,29	0,27	0,44	ND	10,42	ND	2,61	ND
HS-00031	Robinia	Compliant	Compliant	Compliant	N	N		66,02	1,80	0,15	0,17	0,02	10,20	0,28	0,90	ND
HS-00034	Acacia	Compliant	Non Compliant	Non compliant	Y	Y		59,79	1,28	0,15	0,14	0,62	17,35	9,83	3,43	ND
HS-00040	Acacia	Non compliant	Compliant	Compliant	Y	N		67,81	1,28	0,25	0,15	0,03	10,73	0,22	0,52	ND
HS-00077	Acacia	Compliant	Compliant	Compliant	N	N		62,67	1,45	0,23	0,09	0,03	65,50	0,73	2,22	ND
HS-00081	Orange	Compliant	Compliant	Compliant	N	N		59,24	1,02	0,86	0,59	0,06	15,20	0,67	1,46	0,16
HS-00089	Acacia	Compliant	Compliant	Compliant	N	Y	Not from others	59,24	1,29	1,00	0,24	0,91	16,80	3,67	0,67	ND
HS-00091	Acacia	Compliant	Compliant	Compliant	N	N		77,95	1,60	0,24	0,13	0,02	10,15	0,13	0,59	ND
HS-00094	Linden	Compliant	Compliant	Compliant	N	N		60,44	0,95	0,23	0,36	0,03	3,08	0,14	0,54	ND
HS-00095	Acacia	Compliant	Non Compliant	Compliant	N	N		63,01	1,27	0,24	0,10	0,03	14,85	0,21	0,71	ND
HS-00096	Acacia	Non compliant	Non Compliant	Non compliant	Y	Y		57,29	0,98	0,64	0,23	0,83	49,85	14,78	5,06	ND
HS-00102	Linden	Compliant	Compliant	Compliant	N	N		62,48	1,01	0,18	0,35	0,03	4,64	0,26	0,96	ND
HS-00122	Orange	Compliant	Compliant	Compliant	N	N		60,03	1,04	0,68	0,30	0,05	26,89	0,61	1,09	ND
HS-00125	Acacia	Compliant	Compliant	Compliant	N	N		63,63	1,27	0,24	0,20	0,04	15,20	0,45	1,20	ND
HS-00128	Orange	Compliant	Compliant	Compliant	N	N		57,89	1,05	1,25	0,51	0,09	31,65	1,13	1,21	ND
HS-00143	Acacia	Compliant	Compliant	Compliant	N	N		60,89	1,18	0,83	0,55	0,06	18,17	0,69	1,07	ND
HS-00154	Spruce+	Compliant	Non Compliant	Non compliant	Y	Y		41,97	1,20	0,45	0,66	0,25	9,01	3,30	5,18	3,19
HS-00179	Linden	Compliant	Compliant	Compliant	N	N		60,74	1,49	0,23	0,51	0,01	3,49	0,04	0,68	ND
HS-00197	Thyme	Compliant	Compliant	Compliant	N	N		62,42	1,00	0,30	1,12	0,01	4,26	0,13	1,58	ND
HS-00205	Thyme	Compliant	Compliant	Compliant	N	N		52,36	1,22	0,42	0,58	0,01	8,47	0,12	1,64	ND
HS-00208	Chestnut	Compliant	Non Compliant	Non compliant	Y	Y		57,37	1,02	0,18	0,15	0,21	9,20	1,59	2,23	0,79
HS-00209	Chestnut	Compliant	Non Compliant	Compliant	N	N		56,65	1,47	0,29	0,44	0,05	7,88	0,59	1,35	0,09
HS-00221	Robinia	Compliant	Compliant	Compliant	N	N		60,09	1,30	0,27	0,30	0,06	25,80	1,69	3,78	ND
HS-00232	Buckwheat	Compliant	Compliant	Compliant	N	N		62,52	1,48	0,40	0,14	0,08	11,56	0,32	0,29	ND
HS-00233	Linden	Compliant	Non Compliant	Non compliant	Y	Y	Yes by raffiniose	60,71	1,38	0,34	0,20	0,05	9,84	0,31	0,60	0,21
HS-00253	Acacia	Compliant	Compliant	Compliant	N	N		57,16	1,07	0,66	0,47	0,08	34,09	1,93	2,39	0,39
HS-00259	Linden	Compliant	Compliant	Compliant	N	N		47,12	1,03	0,16	0,26	0,04	3,91	0,22	0,61	ND
HS-00263	Acacia	Compliant	Compliant	Compliant	N	N		69,75	1,32	0,77	0,24	0,48	12,24	1,80	0,45	ND
HS-00265	Buckwheat	Compliant	Non Compliant	Compliant	N	N		61,16	1,11	0,13	0,18	0,04	21,15	1,07	2,23	0,79
HS-00266	Acacia	Compliant	Compliant	Compliant	N	N		71,91	1,46	0,36	0,20	0,04	11,29	0,24	0,45	ND
HS-00268	Linden	Compliant	Compliant	Compliant	N	N		64,18	1,27	0,19	0,26	0,02	5,03	0,15	0,65	ND
HS-00271	Linden	Compliant	Non Compliant	Compliant	N	N		66,86	1,00	0,20	0,31	0,03	4,36	0,18	0,66	ND
HS-00293	Linden	Compliant	Non Compliant	Compliant	N	N		43,08	1,25	0,30	0,65	0,01	3,27	0,05	1,03	ND
HS-00298	Forest+	Compliant	Non Compliant	Non compliant	Y	Y	Yes by ratios	40,38	1,03	0,27	0,55	0,12	10,16	2,44	6,26	ND
HS-00302	Linden	Non compliant	Non Compliant	Non compliant	Y	Y		45,61	0,73	0,29	0,05	1,69	32,66	10,12	2,20	ND
HS-00303	Lavender	Compliant	Non Compliant	Compliant	N	N		42,92	1,15	2,82	0,86	0,18	85,30	4,61	2,91	ND
HS-00304	Acacia	Compliant	Compliant	Compliant	N	N		40,41	1,25	0,19	0,34	0,04	ND	ND	ND	ND
HS-00307	Acacia	Compliant	Compliant	Compliant	N	N		65,99	1,34	0,08	0,03	0,04	11,00	0,17	0,55	ND
HS-00311	Orange	Compliant	Compliant	Compliant	N	N		69,32	1,20	0,03	0,01	0,06	35,63	1,04	1,43	ND
HS-00317	Robinia	Compliant	Compliant	Compliant	N	N		64,69	1,34	0,38	0,22	0,06	12,88	0,46	0,75	ND
HS-00323	Acacia	Compliant	Compliant	Compliant	N	N		63,32	1,03	0,23	0,15	0,03	21,76	0,46	1,37	ND
HS-00325	Acacia	Compliant	Compliant	Compliant	N	N		58,48	1,35	0,18	0,24	0,03	18,76	0,81	3,08	ND
HS-00327	Orange	Compliant	Compliant	Compliant	N	N		62,01	1,45	0,17	0,18	0,07	20,45	1,47	2,64	ND
HS-00372	Robinia		Non Compliant	Compliant	N	N		63,67	1,25	0,35	0,15	0,06	14,41	0,41	0,45	ND
HS-00375	Robinia		Compliant	Compliant	N	N		54,90	1,29	0,17	0,24	0,03	19,88	0,90	3,14	ND
HS-00377	Robinia		Non Compliant	Non compliant	Y	N		57,53	1,29	0,22	0,24	0,05	25,15	1,44	3,07	ND
HS-00379	Orange	Compliant	Compliant	Compliant	N	N		66,53	1,29	3,40	2,09	0,08	21,00	1,00	1,44	ND
HS-00384	Heather	Compliant	Compliant	Compliant	N	N		63,70	1,00	0,51	0,17	0,41	56,30	7,78	3,37	ND
HS-00388	Rosemary	Compliant	Compliant	Compliant	N	N		57,34	1,09	2,48	1,49	0,07	18,95	0,85	2,11	ND
HS-00389	Rosemary	Compliant	Compliant	Compliant	N	N		57,95	1,06	0,48	0,34	0,07	18,95	0,89	2,55	ND
HS-00399	Chestnut	Compliant	Compliant	Compliant	N	N		47,65	1,46	0,28	0,62	0,01	4,45	0,09	0,90	ND
HS-00402	Manuka	Compliant	Compliant	Compliant	N	N		65,19	1,03	0,26	0,27	0,07	10,05	0,76	0,80	0,59
HS-00403	Fir+	Compliant	Compliant	Compliant	N	N		52,15	1,12	0,26	0,31	0,09	10,66	1,19	2,38	1,11
HS-00416	Sunflower+	Compliant	Compliant	Compliant	N	N		71,32	0,86	0,82	0,14	0,09	13,86	0,21	1,26	ND
HS-00417	Linden	Compliant	Compliant	Compliant	N	N		69,15	1,36	0,79	0,14	0,09	13,87	0,21	1,26	ND
HS-00418	Echium+	Compliant	Compliant	Compliant	N	N		73,04	1,44	0,26	0,14	0,03	9,45	0,17	0,46	ND
HS-00420	Linden	Compliant	Compliant	Compliant	N	N		56,25	1,34	0,34	0,71	ND	2,06	ND	0,66	ND
HS-00423	Buckwheat	Compliant	Compliant	Compliant	N	N		58,74	1,23	0,21	0,19	0,03	10,47	0,27	0,98	ND
HS-00426	Linden	Non compliant	Non Compliant	Compliant	Y	N		71,55	1,14	0,18	0,39	0,01	2,11	0,07	0,54	ND
HS-00435	Eucalyptus	Compliant	Compliant	Compliant	N	N		57,58	1,28	0,19	0,28	0,02	18,89	0,58	1,69	ND
HS-00439	Acacia	Compliant	Compliant	Compliant	N	N		56,93	1,50	0,20	0,10	0,04	17,74	0,35	0,69	ND
HS-00442	Linden	Compliant	Compliant	Compliant	N	N		49,74	1,33	0,25	0,38	0,01	2,57	0,05	0,45	ND
HS-00447	Rosemary	Compliant	Compliant	Compliant	N	N		57,94	1,35	0,30	0,29	0,05	17,07	0,84	2,69	ND
HS-00449	Acacia	Compliant	Compliant	Compliant	N	N		61,37	1,26	0,23	0,10	0,04	20,78	0,33	0,64	ND
HS-00451	Linden	Compliant	Non Compliant	Non compliant	Y	Y		49,99	0,81	0,25	0,34	0,08	3,57	0,37	2,24	ND
HS-00452	Acacia	Compliant	Compliant	Compliant	N	N		63,09	1,28	0,32	0,15	ND	10,20	ND	0,33	ND
HS-00463	Acacia	Compliant	Compliant	Compliant	N	N		62,52	1,46	0,31	0,14	0,03	13,23	0,15	0,38	ND
HS-00464	Linden	Compliant	Compliant	Compliant	N	N		62,59	1,21	0,33	0,15	0,06	21,45	0,56	0,90	ND
HS-00465	Acacia	Compliant	Compliant	Compliant	N	N		54,62	1,53	0,18	0,37	0,03	41,24	2,67	7,77	ND
HS-00466	Acacia	Compliant	Non Compliant	Non compliant	Y	N		58,81	1,11	0,20	0,37	0,06	23,22	1,03	1,68	ND
HS-00478	Acacia	Compliant	Compliant	Compliant	N	N		54,74	1,43	0,16	0,30	0,04	24,38	1,72	4,84	ND
HS-00480	Heather	Compliant	Compliant	Compliant	N	N		56,68	1,26	0,13	0,14	0,02	21,31	0,54	2,06	ND
HS-00496	Eucalyptus	Compliant	Compliant	Compliant	N	N		64,72	1,51	0,16	0,19	0,05	10,94	0,60	1,72	0,46
HS-00497	Thyme	Compliant	Compliant	Compliant	N	N		68,56	1,59	0,22	0,24	0,03	16,66	0,52	2,13	ND
HS-00498	Lavender	Compliant	Compliant	Compliant	N	N		67,35	1,41	0,38	0,47	0,03	9,79	0,37	1,60	ND
HS-00500	Linden	Compliant	Compliant	Compliant	N	N		52,79	1,41	0,25	0,49	0,01	3,11	0,05	0,66	ND
HS-00504	Linden	Compliant	Compliant	Compliant	N	N		73,27	1,24	0,20	0,19	0,01	8,82	0,08	0,51	ND
HS-00506	Linden	Compliant	Compliant	Compliant	N	N		60,87	1,35	0,20	0,22	0,02	7,30	0,15	0,53	ND
HS-00507	Buckwheat	Non compliant	Non Compliant	Non compliant	Y	Y	Yes by Erlose	61,80	1,04	0,15	0,13	0,05	16,74	0,72	2,12	ND
HS-00508	Buckwheat	Compliant	Compliant	Compliant	N	N		69,93	1,15	0,39	0,23	ND	14,99	ND	0,57	0,15
HS-00509	Linden	Compliant	Compliant	Compliant	N	N		57,66	1,57	0,26	0,16	0,03	6,94	0,11	0,31	ND
HS-00513	Buckwheat	Compliant	Non Compliant	Compliant	N	N		64,79	1,32	0,29	0,16	0,02	10,14	0,13	0,27	ND
HS-00515	Linden	Compliant	Compliant	Compliant	N	N		68,89	1,36	0,28	0,19	0,03	9,74	0,18	0,42	ND
HS-00518	Linden	Compliant	Compliant	Compliant	N	N		61,89	0,97	0,21	0,34	0,01	4,21	0,10	0,61	ND
HS-00519	Linden	Compliant	Compliant	Compliant	N	N		61,37	1,34	0,17	0,30	0,01	6,23	0,08	0,68	ND
HS-00526	Forest+	Compliant	Compliant	Compliant	N	N		78,68	1,28	0,52	0,81	0,03	10,02	0,41	2,36	0,45
HS-00533	Forest+	Compliant	Non Compliant	Compliant	N	Y	Not from others	49,55	1,13	0,21	0,25	0,10	15,48	1,90	2,77	1,09
HS-00551	Manuka	Compliant	Compliant	Compliant	N	N		57,93	1,31	0,18	0,77	ND	4,63	ND	0,19	1,24
HS-00563	Acacia	Compliant	Compliant	Compliant	N	N		66,37	1,49	0,26	0,17	0,03	14,91	0,33	0,64	ND
HS-00564	Acacia	Compliant	Compliant	Compliant	N	N		63,30	1,23	0,65	0,41	0,09	33,21	1,86	1,89	ND
HS-00565	Acacia		Compliant	Compliant	N	N		61,50	1,49	0,20	0,38	0,03	32,42	2,09	5,85	ND
HS-00567	Acacia	Compliant	Compliant	Compliant	N	N		64,83	1,46	0,32	0,20	0,04	20,60	0,56	0,86	ND
HS-00571	Acacia	Compliant	Non Compliant	Non compliant	Y	N		54,22	1,16	0,43	0,21	0,07	20,92	0,74	0,99	ND
HS-00575	Acacia	Compliant	Compliant	Compliant	N	N		47,80	1,31	0,33	0,18	0,03	9,96	0,16	0,41	ND
HS-00590	Acacia	Compliant	Compliant	Compliant	N	N		55,04	1,41	0,85	0,53	0,04	7,64	0,17	0,41	ND
HS-00592	Acacia	Compliant	Compliant	Compliant	N	N		54,24	1,17	0,22	0,11	0,02	16,28	0,19	0,69	ND
HS-00609	Chestnut+	Compliant	Compliant	Compliant	N	N		50,16	1,59	0,30	0,37	0,05	9,71	0,64	1,14	ND
HS-00612	Thyme+	Non compliant	Non Compliant	Non compliant	Y	Y	Yes by ratios and erlose	49,14	1,01	0,16	0,17	0,06	36,18	2,32	3,79	ND
HS-00619	Orange	Compliant	Compliant	Compliant	N	N		55,58	1,10	0,80	0,49	0,06	26,60	1,01	1,89	ND
HS-00620	Orange	Compliant	Compliant	Compliant	N	N		54,15	1,09	0,93	0,61	0,05	25,32	0,87	1,59	ND
HS-00621	Chestnut	Compliant	Compliant	Compliant	N	N		43,90	1,69	0,36	0,91	0,01	4,69	0,09	1,54	0,11
HS-00628	Orange	Compliant	Non Compliant	Compliant	N	N		55,31	1,26	0,16	0,17	0,05	21,76	1,17	1,58	ND
HS-00630	Acacia	Compliant	Non Compliant	Non compliant	Y	N		56,45	1,26	0,37	0,27	0,06	29,20	1,37	1,89	ND
HS-00637	Orange	Compliant	Compliant	Compliant	N	N		56,60	1,06	0,48	0,27	0,07	41,80	1,62	1,53	ND
HS-00641	Orange	Compliant	Compliant	Compliant	N	N		54,61	1,25	0,22	0,13	0,06	39,85	1,38	1,87	ND
HS-00657	Acacia	Compliant	Compliant	Compliant	N	N		52,03	1,48	0,15	0,28	0,05	19,84	1,76	3,26	0,25
HS-00659	Heather	Compliant	Compliant	Compliant	N	N		52,77	1,38	0,12	0,24	0,03	6,65	0,44	1,14	ND
HS-00660	Eucalyptus	Compliant	Non Compliant	Non compliant	Y	Y	Yes by ratios	55,23	1,07	0,13	0,18	0,07	32,07	2,77	3,25	1,25
HS-00665	Acacia		Compliant	Compliant	N	N		54,98	1,58	0,21	0,07	0,07	34,43	0,85	1,14	ND
HS-00674	Acacia	Compliant	Compliant	Compliant	N	N		63,59	1,53	0,15	0,18	0,04	16,53	0,85	2,13	ND
HS-00691	Acacia	Compliant	Compliant	Compliant	N	N		48,86	1,19	0,14	0,16	0,04	26,27	1,37	5,03	ND
HS-00693	Robinia	Compliant	Non Compliant	Non compliant	Y	N		52,12	1,27	0,58	0,31	0,06	20,66	0,67	0,90	ND
HS-00695	Rosemary	Compliant	Compliant	Compliant	N	N		44,46	1,10	0,40	0,46	0,04	26,27	1,37	5,03	ND
HS-00699	Manuka	Non compliant	Compliant	Compliant	Y	Y	Yes by ratio low F	51,31	1,22	0,19	0,15	0,03	11,91	0,30	0,91	ND
HS-00706	Orange	Compliant	Compliant	Compliant	N	N		60,58	1,43	0,18	0,38	0,01	8,68	0,18	1,56	ND
HS-00710	Buckwheat+	Compliant	Compliant	Compliant	N	N		60,45	1,31	0,28	0,16	0,06	10,85	0,35	0,50	ND
HS-00711	Linden	Compliant	Compliant	Compliant	N	Y	Not from others	68,74	1,76	0,18	0,40	0,02	3,42	0,16	1,01	ND
HS-00729	Acacia	Compliant	Compliant	Compliant	N	N		62,84	1,22	0,15	0,12	0,03	12,54	0,26	0,65	ND
HS-00730	Acacia	Compliant	Non Compliant	Non compliant	Y	Y		52,88	1,24	0,22	0,04	4,29	21,60	16,21	0,77	ND
HS-00732	Eucalyptus	Compliant	Compliant	Compliant	N	N		57,54	1,47	0,27	0,41	0,02	10,50	0,39	1,31	ND
HS-00737	Orange	Compliant	Compliant	Compliant	N	N		56,65	1,26	0,66	0,29	0,07	31,95	0,99	1,36	2,91
HS-00738	Eucalyptus	Compliant	Compliant	Compliant	N	N		55,40	1,13	0,26	0,41	0,10	11,07	1,69	2,06	1,55
HS-00739	Rosemary	Compliant	Compliant	Compliant	N	N		53,98	1,39	0,27	0,34	0,04	5,75	0,32	0,89	ND
HS-00740	Eucalyptus	Compliant	Non Compliant	Non compliant	Y	N		53,43	1,46	0,36	0,74	ND	14,40	ND	1,89	ND
HS-00742	Acacia	Compliant	Compliant	Compliant	N	N		50,78	1,74	0,20	0,38	0,03	16,61	0,85	3,37	ND
HS-00745	Heather+	Compliant	Non Compliant	Non compliant	Y	N		62,12	1,61	0,28	0,44	0,01	3,21	0,06	0,65	ND
HS-00748	Linden	Compliant	Compliant	Compliant	N	N		53,96	1,32	0,34	0,25	0,03	11,16	0,22	0,77	ND
HS-00760	Buckwheat	Compliant	Compliant	Compliant	N	N		67,18	1,56	0,21	0,23	0,02	8,08	0,21	1,09	ND
HS-00764	Linden	Compliant	Compliant	Compliant	N	N		69,30	1,39	0,17	ND	0,01	ND	0,06	0,51	ND
HS-00825	Orange		Non Compliant	Non compliant	Y	N		51,75	1,39	0,54	0,23	0,07	23,46	0,66	0,99	ND
HS-00838	Linden	Compliant	Non Compliant	Non compliant	Y	Y	Yes by maltose	70,81	1,81	0,21	0,17	0,03	10,73	0,21	0,85	ND
HS-00839	Meadow+	Compliant	Non Compliant	Non compliant	Y	Y	Yes by F/G high	69,96	2,30	0,54	0,27	0,04	15,99	0,31	0,88	0,22
HS-00892	Fir+	Non compliant	Non Compliant	Non compliant	Y	Y		43,13	0,83	0,29	0,22	1,23	25,53	23,99	8,62	2,08
HS-00938	Acacia	Compliant	Compliant	Compliant	N	N		51,53	1,29	0,25	0,24	0,09	27,85	2,37	3,20	ND
HS-00943	Acacia	Compliant	Non Compliant	Non compliant	Y	Y		68,07	1,57	0,23	0,22	0,05	19,49	0,86	2,13	ND
HS-00952	Acacia	Compliant	Compliant	Compliant	N	N		67,82	1,87	0,09	0,06	0,08	21,70	1,21	1,86	ND
HS-00959	Acacia	Compliant	Non Compliant	Non compliant	Y	N		69,16	1,47	0,20	0,18	0,07	22,29	1,30	2,27	ND
*no	**yes	***not detected														

Annex 2 *Results of sugar profile for honey samples*

	Sugar profile (g/100g)																		
ID sample	Trehalose	Arabinose	Glucose	Fructose	Melibiose	Iso-maltose	Maltulose	Sucrose	Gentobiose	Turanose	Melezitose_Palatinose	Raffinose	Stachyose	Kestose	Maltose	Erlose	Panose	Nystose	Maltotriose
HS-00001	ND *	ND	28,98	30,39	ND	1,09	1,47	0,45	0,14	1,42	0,17	ND	0,02	0,19	0,62	0,15	0,23	0,02	0,02
HS-00003	0,03	0,01	23,58	32,78	ND	1,69	2,12	0,74	0,40	1,57	0,36	ND	0,03	0,17	0,69	ND	0,14	0,01	0,02
HS-00005	0,11	ND	27,18	38,22	ND	2,05	1,91	0,47	0,18	1,96	0,25	0,03	0,04	0,11	0,72	0,06	0,23	ND	0,05
HS-00007	ND	ND	31,46	30,22	ND	0,29	0,26	0,13	0,11	0,55	0,05	ND	ND	0,10	0,81	0,18	0,02	0,07	0,13
HS-00009	0,13	ND	24,12	30,22	ND	1,74	1,92	0,69	0,09	1,33	0,82	0,46	0,04	0,33	0,58	0,05	0,28	ND	0,06
HS-00011	0,10	0,01	20,78	34,27	ND	2,10	2,43	0,52	0,16	1,88	0,42	ND	0,06	0,34	0,72	0,02	0,24	0,01	0,03
HS-00012	0,52	0,03	22,72	38,67	ND	1,91	1,89	0,45	0,13	1,94	0,27	ND	0,02	0,16	0,95	0,04	0,27	0,01	0,03
HS-00013	0,16	ND	27,43	42,08	ND	1,02	1,63	0,47	0,10	1,80	0,14	ND	0,01	0,05	0,67	ND	0,15	0,02	ND
HS-00014	ND	0,02	27,99	35,76	ND	0,99	1,50	0,43	0,06	1,80	0,25	ND	ND	0,24	0,68	0,05	0,21	0,02	0,02
HS-00015	0,01	ND	27,36	37,11	ND	0,57	0,70	0,27	0,07	1,49	0,09	ND	ND	0,10	1,88	0,83	0,14	0,02	0,08
HS-00018	0,16	0,01	19,99	33,49	ND	2,13	2,20	0,55	0,10	1,71	0,99	0,52	0,05	0,38	0,68	0,07	0,33	ND	0,08
HS-00019	0,18	0,01	25,74	28,79	ND	2,36	2,07	0,59	0,10	1,13	1,41	0,92	0,06	0,34	0,56	0,13	0,39	ND	0,15
HS-00020	0,27	0,01	18,15	32,80	ND	2,42	2,29	0,77	0,14	1,51	0,41	ND	0,07	0,34	0,66	ND	0,20	ND	0,02
HS-00021	0,07	ND	36,73	33,99	ND	0,34	0,56	0,97	0,02	1,09	0,05	0,05	0,01	0,08	1,89	1,04	0,13	0,03	0,10
HS-00022	0,15	ND	19,01	37,52	ND	3,90	3,19	0,71	0,70	1,87	0,69	ND	0,21	0,59	0,68	0,02	0,28	ND	0,01
HS-00029	0,18	0,01	24,32	31,48	ND	2,37	2,49	0,58	0,13	2,11	0,30	ND	0,06	0,22	1,30	0,07	0,33	ND	ND
HS-00031	ND	ND	23,54	42,47	ND	0,60	0,59	0,18	0,10	1,20	0,09	ND	ND	0,05	1,01	0,02	0,09	0,02	0,03
HS-00034	0,01	ND	26,25	33,54	ND	0,78	0,89	0,24	0,10	1,58	0,13	ND	ND	0,08	1,73	0,47	0,34	ND	0,98
HS-00040	0,01	ND	29,79	38,02	ND	0,38	0,51	0,25	0,15	1,01	0,07	ND	ND	0,05	1,64	0,16	0,08	0,02	0,03
HS-00077	ND	ND	25,57	37,10	ND	0,15	0,21	0,08	0,01	0,35	0,03	ND	ND	0,02	0,91	0,13	0,03	0,03	0,01
HS-00081	ND	ND	29,33	29,91	ND	0,71	0,83	0,97	0,11	1,12	0,10	0,02	ND	0,16	1,64	0,54	0,16	0,01	0,07
HS-00089	ND	ND	25,85	33,39	ND	0,18	0,25	0,42	0,10	0,42	0,04	ND	ND	0,03	1,75	0,09	0,07	0,02	0,38
HS-00091	ND	ND	29,99	47,95	ND	0,27	0,29	0,13	0,10	0,54	0,04	ND	ND	0,02	0,99	0,12	0,06	0,03	0,01
HS-00094	ND	ND	30,97	29,47	ND	0,62	0,66	0,23	0,21	1,02	0,10	ND	ND	0,07	0,65	0,05	0,11	0,01	0,03
HS-00095	ND	ND	27,75	35,26	ND	0,20	0,18	0,08	0,06	0,33	0,03	ND	ND	0,02	0,82	0,12	0,04	0,02	0,01
HS-00096	ND	ND	28,89	28,40	ND	0,53	0,48	0,54	0,05	0,84	0,07	ND	ND	0,04	2,36	0,34	0,24	0,01	0,70
HS-00102	0,03	ND	31,03	31,45	ND	0,67	0,74	0,28	0,17	1,50	0,11	ND	ND	0,18	0,79	0,40	0,16	0,01	0,04
HS-00122	0,03	ND	29,38	30,65	ND	0,34	0,51	0,64	0,08	0,94	0,06	ND	ND	0,11	2,14	0,40	0,09	0,02	0,05
HS-00125	0,01	ND	28,01	35,63	ND	0,37	0,63	0,31	0,10	1,28	0,07	ND	ND	0,08	1,56	0,50	0,12	0,02	0,05
HS-00128	0,02	ND	28,27	29,62	ND	0,33	0,77	1,11	0,07	0,89	0,06	ND	ND	0,13	2,19	0,45	0,08	0,01	0,08
HS-00143	0,01	ND	27,99	32,90	ND	0,36	0,65	0,91	0,09	1,11	0,07	ND	ND	0,06	1,66	0,74	0,10	0,02	0,06
HS-00154	0,45	ND	19,06	22,90	ND	2,67	1,67	0,60	0,10	1,33	0,38	0,32	0,20	0,68	0,91	0,26	0,52	0,05	0,33
HS-00179	0,03	ND	24,37	36,37	ND	0,99	1,24	0,37	0,20	1,62	0,16	ND	0,01	0,09	0,71	ND	0,14	0,01	0,01
HS-00197	0,02	ND	31,16	31,26	ND	1,18	1,50	0,42	0,09	1,41	0,21	ND	0,02	0,16	0,37	0,02	0,14	ND	0,01
HS-00205	0,04	ND	23,56	28,79	ND	3,00	2,33	0,69	0,14	1,64	0,42	ND	0,08	0,44	1,19	0,01	0,23	ND	0,02
HS-00208	0,36	0,01	28,38	28,99	ND	1,68	1,20	0,26	0,18	1,40	0,25	0,15	0,02	0,21	1,69	0,27	0,41	0,01	0,29
HS-00209	0,18	ND	22,90	33,75	ND	1,85	1,60	0,48	0,14	1,67	0,25	0,01	0,03	0,16	1,09	0,07	0,19	ND	0,08
HS-00221	ND	ND	26,07	34,02	ND	0,59	0,69	0,35	0,04	1,27	0,10	ND	ND	0,12	1,13	0,97	0,17	0,01	0,07
HS-00232	ND	ND	25,17	37,35	ND	0,27	0,25	0,17	0,10	0,43	0,04	ND	ND	0,03	1,19	0,03	0,03	0,01	0,03
HS-00233	0,12	ND	25,55	35,16	ND	0,49	0,50	0,24	0,12	0,72	ND	0,03	0,01	0,14	1,21	0,11	0,07	0,01	0,04
HS-00253	0,01	ND	27,60	29,56	ND	0,39	0,57	0,74	0,05	1,13	0,07	0,02	ND	0,05	1,58	1,77	0,11	0,01	0,09
HS-00259	0,06	ND	23,22	23,90	ND	0,76	0,79	0,21	0,20	1,27	0,11	ND	0,01	0,09	0,79	0,11	0,12	0,01	0,04
HS-00263	ND	ND	30,09	39,65	ND	0,10	0,17	0,23	0,08	0,30	0,03	ND	ND	0,01	0,97	0,24	0,04	0,02	0,14
HS-00265	0,07	ND	28,98	32,18	ND	0,80	0,69	0,23	0,06	1,76	ND	0,05	ND	0,08	1,27	0,05	0,13	0,02	0,06
HS-00266	ND	ND	29,28	42,63	ND	0,19	0,36	0,22	0,10	0,61	0,04	ND	ND	0,04	1,10	0,15	0,04	0,03	0,02
HS-00268	0,02	ND	28,31	35,87	ND	0,62	0,68	0,23	0,18	1,24	0,09	ND	ND	0,12	0,90	0,05	0,12	0,02	0,03
HS-00271	0,06	ND	33,41	33,44	ND	0,77	0,89	0,27	0,20	1,36	0,12	ND	ND	0,12	0,87	0,09	0,13	0,01	0,04
HS-00293	0,03	ND	19,16	23,91	ND	1,71	1,53	0,46	0,22	1,55	0,26	ND	0,04	0,18	0,70	ND	0,22	ND	0,01
HS-00298	0,26	ND	19,90	20,48	ND	2,11	1,32	0,37	0,07	1,35	0,46	0,58	0,07	0,37	0,66	0,18	0,41	0,03	0,16
HS-00302	ND	ND	26,35	19,26	ND	0,61	0,51	0,17	0,10	0,58	0,06	ND	0,01	0,02	3,15	0,02	0,21	0,01	0,98
HS-00303	0,07	ND	19,94	22,98	ND	0,32	0,27	2,18	0,03	0,77	0,03	ND	ND	0,11	2,53	1,07	0,09	0,02	0,14
HS-00304	ND	ND	17,92	22,48	ND	0,89	1,13	0,39	ND	2,01	0,20	ND	0,01	0,13	1,14	1,75	0,20	ND	0,08
HS-00307	ND	ND	28,15	37,83	ND	0,21	0,23	0,03	0,08	0,35	0,03	ND	ND	0,02	0,86	0,09	0,04	0,02	0,01
HS-00311	0,02	ND	31,48	37,83	ND	0,33	0,52	0,03	0,06	1,03	0,04	ND	ND	0,18	2,06	0,47	0,08	0,02	0,06
HS-00317	0,01	ND	27,69	37,00	ND	0,24	0,32	0,21	0,07	0,55	0,04	ND	ND	0,03	0,94	0,38	0,06	0,02	0,03
HS-00323	0,01	ND	31,13	32,18	ND	0,34	0,40	0,18	0,06	0,79	0,05	ND	ND	0,03	1,26	0,16	0,08	0,02	0,03
HS-00325	0,01	ND	24,89	33,59	ND	0,73	0,97	0,30	0,07	1,66	0,14	ND	0,01	0,10	1,27	0,69	0,21	0,01	0,06
HS-00327	0,01	0,01	25,35	36,66	ND	0,60	0,71	0,24	0,07	1,36	0,09	ND	ND	0,18	1,34	0,28	0,17	0,02	0,10
HS-00372	ND	ND	28,35	35,32	ND	0,10	0,20	0,12	0,06	0,35	0,02	ND	ND	0,03	0,81	0,29	0,03	0,02	0,02
HS-00375	0,01	ND	24,02	30,87	ND	0,95	1,30	0,32	0,07	1,89	0,19	ND	0,01	0,12	1,36	0,57	0,21	0,01	0,06
HS-00377	0,01	0,01	25,15	32,38	ND	0,67	0,85	0,34	0,06	1,52	0,13	ND	0,00	0,13	1,45	1,07	0,18	0,01	0,08
HS-00379	ND	ND	29,03	37,50	ND	0,59	0,67	4,03	0,09	1,18	0,08	ND	ND	0,19	1,93	0,73	0,13	0,01	0,09
HS-00384	0,06	ND	31,90	31,80	ND	0,31	0,32	0,25	0,03	0,49	0,04	ND	ND	0,03	1,45	0,22	0,09	0,01	0,20
HS-00388	0,04	ND	27,50	29,85	ND	0,73	0,71	2,51	0,09	1,01	0,09	ND	ND	0,15	1,68	0,89	0,19	0,01	0,08
HS-00389	0,05	ND	28,17	29,79	ND	1,00	0,99	0,60	0,09	1,24	0,13	ND	ND	0,15	1,78	0,80	0,24	0,01	0,08
HS-00399	0,10	0,01	19,40	28,25	ND	1,03	1,38	0,34	0,12	1,23	0,17	ND	0,02	0,10	0,55	0,01	0,11	ND	0,01
HS-00402	0,07	0,01	32,16	33,03	ND	0,38	0,52	0,19	0,07	0,74	0,55	0,04	0,02	0,07	0,70	0,15	0,06	0,01	0,05
HS-00403	0,27	ND	24,63	27,52	ND	1,96	1,51	0,40	0,12	1,51	0,24	0,13	0,05	0,51	1,28	0,26	0,29	0,04	0,14
HS-00416	ND	ND	38,31	33,01	ND	0,31	0,21	0,17	0,09	0,21	0,03	ND	0,01	ND	1,19	0,02	0,11	0,02	0,02
HS-00417	ND	ND	29,28	39,87	ND	0,31	0,21	0,17	0,09	0,21	0,03	ND	0,01	ND	1,19	0,02	0,11	0,02	0,02
HS-00418	0,16	ND	29,88	43,16	ND	0,36	0,46	0,20	0,15	0,75	0,05	ND	ND	0,07	1,45	0,12	0,07	0,02	0,03
HS-00420	0,08	ND	23,99	32,26	ND	1,32	1,27	0,42	0,29	1,25	0,21	ND	0,03	0,13	0,59	ND	0,19	0,01	ND
HS-00423	ND	0,01	26,34	32,40	ND	0,83	0,80	0,26	0,13	1,26	0,10	ND	ND	0,05	1,39	0,03	0,13	0,02	0,04
HS-00426	0,12	ND	33,48	38,07	ND	1,28	1,24	0,30	0,37	1,66	0,15	ND	ND	0,13	0,77	0,02	0,20	ND	0,02
HS-00435	0,07	ND	25,27	32,31	ND	0,42	0,68	0,18	0,03	0,95	0,06	ND	0,01	0,05	0,66	0,02	0,06	0,01	0,02
HS-00439	0,01	ND	22,80	34,13	ND	0,18	0,26	0,10	0,05	0,47	0,04	ND	ND	0,03	0,97	0,11	0,04	0,02	0,02
HS-00442	0,04	ND	21,37	28,37	ND	1,75	1,65	0,40	0,41	1,62	0,27	ND	0,03	0,22	1,05	0,01	0,18	0,01	0,02
HS-00447	0,03	ND	24,68	33,26	ND	0,92	1,05	0,43	0,09	1,45	0,10	ND	0,01	0,16	1,50	0,63	0,24	0,02	0,07
HS-00449	ND	ND	27,11	34,25	ND	0,24	0,30	0,13	0,06	0,58	0,04	ND	ND	0,03	1,26	0,13	0,04	0,01	0,02
HS-00451	0,06	0,01	27,66	22,33	ND	1,15	0,79	0,29	0,24	1,16	0,13	ND	0,01	0,12	0,85	0,02	0,54	0,01	0,09
HS-00452	0,05	ND	27,62	35,47	ND	0,21	0,35	0,20	0,13	0,64	0,04	ND	ND	0,03	1,33	0,15	0,04	0,02	ND
HS-00463	ND	ND	25,45	37,07	ND	0,12	0,20	0,13	0,07	0,40	0,02	ND	ND	0,02	0,92	0,13	0,03	0,02	0,01
HS-00464	0,03	ND	28,31	34,28	ND	0,23	0,30	0,18	0,06	0,56	0,04	ND	ND	0,03	1,25	0,15	0,05	0,02	0,03
HS-00465	ND	ND	21,61	33,01	ND	0,75	1,25	0,39	0,03	2,24	0,13	ND	0,01	0,13	1,05	1,11	0,20	0,01	0,07
HS-00466	ND	ND	27,94	30,87	ND	0,40	0,53	0,21	0,07	1,07	0,06	ND	ND	0,05	1,51	0,58	0,11	0,02	0,07
HS-00478	ND	ND	22,54	32,20	ND	0,96	1,52	0,41	0,06	2,62	0,18	ND	0,01	0,13	1,34	1,03	0,27	0,01	0,09
HS-00480	0,08	ND	25,06	31,62	ND	0,83	0,99	0,23	0,07	1,79	0,11	ND	ND	0,13	1,59	0,16	0,15	0,02	0,04
HS-00496	0,14	ND	25,81	38,91	ND	1,38	1,36	0,32	0,16	1,98	0,16	0,07	0,04	0,08	1,73	0,41	0,27	0,01	0,09
HS-00497	0,18	ND	26,48	42,08	ND	1,14	1,29	0,40	0,10	1,79	0,12	ND	ND	0,08	1,68	0,40	0,22	0,01	0,05
HS-00498	0,12	ND	27,94	39,40	ND	1,16	1,54	0,71	0,16	1,87	0,13	ND	ND	0,12	1,52	0,52	0,25	0,02	0,06
HS-00500	0,03	ND	21,95	30,84	ND	1,72	1,91	0,52	0,34	2,05	0,25	ND	0,03	0,21	1,05	ND	0,22	ND	0,02
HS-00504	ND	ND	32,64	40,63	ND	0,23	0,29	0,12	0,07	0,62	0,03	ND	ND	0,05	0,64	0,02	0,04	0,01	0,01
HS-00506	0,02	ND	25,88	34,98	ND	0,34	0,40	0,14	0,09	0,70	0,05	ND	ND	0,04	0,64	0,02	0,05	0,01	0,01
HS-00507	ND	ND	30,34	31,46	ND	0,46	0,38	0,12	0,06	0,79	0,06	ND	ND	0,07	0,95	0,41	0,12	0,02	0,04
HS-00508	0,02	ND	32,58	37,35	ND	0,21	0,25	0,17	0,05	0,44	0,03	0,01	ND	0,07	0,73	0,01	0,03	0,01	ND
HS-00509	0,01	ND	22,47	35,19	ND	0,24	0,24	0,12	0,11	0,45	0,04	ND	ND	0,05	0,74	0,06	0,03	0,01	0,01
HS-00513	ND	ND	27,96	36,84	ND	0,28	0,31	0,16	0,10	0,55	0,04	ND	ND	0,03	1,03	0,01	0,03	0,02	0,01
HS-00515	0,03	ND	29,19	39,70	ND	0,26	0,32	0,18	0,10	0,64	0,04	ND	ND	0,03	0,94	0,06	0,04	0,02	0,02
HS-00518	0,05	ND	31,37	30,52	ND	0,69	0,90	0,26	0,19	1,29	0,10	ND	ND	0,11	0,78	0,08	0,11	0,01	0,02
HS-00519	0,02	ND	26,25	35,12	ND	0,52	0,78	0,21	0,11	1,20	0,08	ND	ND	0,07	0,69	0,01	0,08	0,01	0,01
HS-00526	0,60	0,04	34,54	44,14	ND	2,69	2,73	1,01	0,12	1,92	0,32	0,06	0,13	0,31	1,24	0,16	0,29	ND	0,05
HS-00533	0,29	ND	23,22	26,33	ND	1,49	1,38	0,38	0,10	1,80	0,30	0,11	0,05	0,27	1,52	0,26	0,27	0,03	0,19
HS-00551	0,06	ND	25,08	32,85	ND	0,55	0,71	0,21	0,06	1,18	0,17	0,07	0,09	0,77	0,27	0,10	0,01	0,05	ND
HS-00563	ND	ND	26,69	39,69	ND	0,20	0,37	0,20	0,08	0,76	0,04	ND	ND	0,03	1,17	0,25	0,05	0,03	0,03
HS-00564	ND	ND	28,36	34,94	ND	0,38	0,67	0,87	0,06	1,35	0,07	ND	ND	0,08	2,13	1,28	0,12	0,02	0,12
HS-00565	ND	ND	24,73	36,77	ND	0,10	0,13	0,43	0,04	2,13	0,15	ND	0,01	0,14	1,15	0,55	0,21	0,01	0,07
HS-00567	ND	ND	26,34	38,49	ND	0,25	0,42	0,28	0,07	0,88	0,05	ND	ND	0,03	1,45	0,40	0,06	0,02	0,04
HS-00571	ND	ND	25,04	29,17	ND	0,25	0,34	0,27	0,06	0,64	0,03	ND	ND	0,07	1,30	0,57	0,06	0,02	0,05
HS-00575	ND	ND	20,69	27,11	ND	0,13	0,25	0,14	0,08	0,43	0,03	ND	ND	0,04	0,81	0,26	0,03	0,02	0,01
HS-00590	ND	ND	22,81	32,23	ND	0,15	0,22	0,37	0,09	0,44	0,03	ND	ND	0,05	0,71	0,24	0,04	0,02	0,02
HS-00592	ND	ND	24,94	29,30	ND	0,18	0,27	0,12	0,07	0,54	0,03	ND	ND	0,04	1,08	0,20	0,05	0,01	0,01
HS-00609	0,07	ND	19,35	30,81	ND	0,63	0,75	0,23	0,06	0,77	0,09	ND	ND	0,07	0,62	0,09	0,07	0,01	0,04
HS-00612	0,17	ND	24,40	24,73	ND	0,57	0,86	0,29	0,05	1,77	0,09	ND	ND	0,12	1,71	1,86	0,18	0,01	0,11
HS-00619	0,02	ND	26,50	29,08	ND	0,66	0,89	1,02	0,08	1,28	0,08	ND	ND	0,18	2,08	0,54	0,15	0,01	0,08
HS-00620	ND	ND	25,87	28,28	ND	0,64	0,92	1,21	0,08	1,30	0,09	ND	ND	0,18	2,00	0,53	0,12	0,01	0,07
HS-00621	0,13	ND	16,31	27,59	ND	3,24	2,79	0,56	0,13	1,54	0,51	0,01	0,15	0,42	0,61	ND	0,20	ND	0,01
HS-00628	ND	ND	24,45	30,86	ND	0,45	0,67	0,22	0,06	1,42	0,07	ND	ND	0,14	1,35	0,48	0,10	0,02	0,07
HS-00630	ND	ND	25,02	31,43	ND	0,39	0,62	0,48	0,06	1,29	0,07	ND	ND	0,10	1,77	1,27	0,11	0,01	0,08
HS-00637	ND	ND	27,43	29,17	ND	0,36	0,46	0,55	0,05	1,13	0,04	ND	ND	0,12	2,04	0,64	0,07	0,02	0,08
HS-00641	ND	ND	24,31	30,30	ND	0,42	0,62	0,27	0,05	1,22	0,06	ND	ND	0,17	2,06	0,50	0,10	0,01	0,07
HS-00657	0,05	ND	20,94	31,09	ND	0,96	1,35	0,34	0,06	2,21	0,17	0,01	0,02	0,18	1,20	1,02	0,20	0,01	0,11
HS-00659	ND	ND	22,16	30,61	ND	0,57	0,67	0,16	0,10	1,30	0,10	ND	ND	0,08	0,67	0,14	0,11	0,01	0,04
HS-00660	0,03	ND	26,74	28,50	ND	0,58	0,66	0,20	0,04	1,53	0,07	0,05	0,02	0,11	1,16	0,78	0,12	0,01	0,10
HS-00665	0,03	ND	21,34	33,64	ND	0,26	0,33	0,13	0,05	0,62	0,03	ND	ND	0,01	1,78	0,11	0,06	0,02	0,04
HS-00674	ND	ND	25,12	38,47	ND	0,72	0,85	0,30	0,10	2,01	0,09	ND	ND	0,08	1,60	0,71	0,21	0,02	0,08
HS-00691	0,03	ND	22,34	26,52	ND	2,00	1,76	0,22	0,05	1,56	0,19	ND	0,04	0,26	1,33	1,08	0,26	0,01	0,07
HS-00693	0,07	ND	22,99	29,13	ND	0,22	0,31	0,42	0,07	0,73	0,03	ND	ND	0,05	1,36	0,61	0,06	0,02	0,04
HS-00695	0,03	ND	21,14	23,32	ND	2,00	1,76	0,62	0,05	1,56	0,19	ND	0,04	0,26	1,33	1,08	0,26	0,01	0,07
HS-00699	ND	ND	23,15	28,17	ND	0,47	0,53	0,16	0,09	0,84	0,06	ND	ND	0,05	1,03	0,15	0,08	0,01	0,03
HS-00706	0,02	ND	24,92	35,65	ND	0,63	1,15	0,30	0,09	1,70	0,12	ND	ND	0,14	0,79	0,15	0,14	0,02	0,02
HS-00710	0,02	ND	26,20	34,25	ND	0,39	0,46	0,19	0,11	0,68	0,05	ND	ND	0,03	1,23	0,05	0,06	0,02	0,04
HS-00711	0,07	ND	24,91	43,83	ND	0,91	1,04	0,28	0,20	1,56	0,13	ND	0,01	0,08	0,70	0,03	0,21	0,02	0,03
HS-00729	0,02	ND	28,29	34,56	ND	0,38	0,59	0,19	0,13	1,26	0,06	ND	ND	0,06	1,58	0,28	0,08	0,01	0,03
HS-00730	ND	ND	23,64	29,24	ND	0,23	0,27	0,09	0,11	0,42	0,03	ND	ND	0,02	2,40	0,02	0,09	0,02	1,80
HS-00732	0,09	ND	23,33	34,21	ND	0,51	0,84	0,27	0,06	0,99	0,10	ND	0,01	0,06	0,66	0,02	0,08	0,01	0,02
HS-00737	ND	ND	25,09	31,55	ND	0,29	0,45	0,55	0,06	0,83	0,05	0,17	ND	ND	1,88	0,46	0,08	0,02	0,06
HS-00738	0,06	ND	26,03	29,37	ND	0,57	0,72	0,27	0,06	1,07	0,16	0,09	0,04	0,08	0,67	0,15	0,12	0,01	0,10
HS-00739	0,04	ND	22,61	31,37	ND	0,58	0,71	0,26	0,13	0,93	0,10	ND	ND	0,06	0,75	0,14	0,12	0,01	0,04
HS-00740	0,15	ND	21,68	31,75	ND	0,67	1,16	0,43	0,04	1,19	0,14	ND	0,01	0,08	0,58	ND	0,08	ND	ND
HS-00742	0,01	ND	18,50	32,28	ND	0,75	0,94	0,32	0,05	1,58	0,12	ND	ND	0,10	0,85	0,31	0,17	0,02	0,04
HS-00745	0,08	ND	23,83	38,28	ND	1,67	1,87	0,50	0,35	1,77	0,20	ND	0,02	0,08	1,13	0,01	0,23	ND	0,02
HS-00748	0,01	ND	23,22	30,74	ND	0,25	0,24	0,17	0,06	0,51	0,03	ND	ND	0,10	0,69	0,03	0,05	0,01	0,01
HS-00760	ND	ND	26,29	40,89	ND	1,13	0,84	0,32	0,17	1,55	0,11	ND	ND	0,06	1,37	0,03	0,19	0,01	0,04
HS-00764	0,11	ND	28,95	40,35	ND	0,96	0,93	0,27	0,28	1,53	0,12	ND	0,01	0,13	ND	0,03	0,14	ND	0,02
HS-00825	0,04	ND	21,65	30,11	ND	0,30	0,44	0,41	0,08	0,76	0,05	ND	ND	0,11	1,80	0,33	0,08	0,02	0,05
HS-00838	0,05	ND	25,22	45,60	ND	0,68	0,74	0,27	0,15	1,27	0,10	ND	ND	0,10	1,62	0,09	0,13	0,01	0,03
HS-00839	ND	ND	21,20	48,76	ND	0,26	0,36	0,29	0,07	0,54	0,04	0,01	ND	0,03	1,08	0,07	0,06	0,03	0,02
HS-00892	0,42	0,02	23,50	19,62	ND	1,35	0,96	0,36	0,06	1,24	0,22	0,13	0,03	0,29	1,62	0,69	0,55	0,02	1,52
HS-00938	ND	ND	22,51	29,02	ND	0,58	0,65	0,32	0,05	1,31	0,09	ND	ND	0,09	1,33	0,86	0,15	0,02	0,11
HS-00943	ND	ND	26,52	41,54	ND	0,53	0,70	0,28	0,07	1,24	0,08	ND	ND	0,06	1,31	0,44	0,14	0,02	0,06
HS-00952	0,03	ND	23,59	44,23	ND	1,13	1,31	0,22	0,18	2,56	0,19	ND	ND	0,22	3,90	1,69	0,34	0,05	0,22
HS-00959	ND	ND	27,95	41,20	ND	0,70	0,80	0,35	0,09	1,71	0,09	ND	ND	0,11	1,93	0,97	0,20	0,02	0,11
*not detected																			

Annex 3 *Results of maltodextrins profiles for honey samples*

	DPs 4-11 (ppm)							Presence												
ID sample	DP4	DP5	DP6	DP7	DP8	DP9	DP10	DP11	DP12	DP13	DP14	DP15	DP16	DP17	DP18	DP19	DP20	DP21	DP22	DP23
HS-00001	50,3	43,6	ND *	ND	11,8	ND	ND	ND	N**	N	N	N	N	N	N	N	N	N	N	N
HS-00003	13,5	17,2	ND	ND	30,6	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00005	50,5	ND	8,5	14,2	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00007	57,0	64,4	23,4	ND	39,2	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00009	419,8	251,8	162,3	130,9	205,8	124,2	119,4	23,6	Y***	Y	N	N	N	N	N	N	N	N	N	N
HS-00011	19,6	36,3	37,5	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00012	94,0	90,2	ND	11,6	ND	25,0	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00013	30,1	90,4	31,2	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00014	61,3	49,3	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00015	53,0	63,2	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00018	424,8	253,0	169,8	162,8	129,7	161,2	155,0	32,4	Y	Y	Y	Y	N	N	N	N	N	N	N	N
HS-00019	1274,8	669,3	328,0	216,3	ND	166,6	138,3	34,2	Y	Y	Y	N	N	N	N	N	N	N	N	N
HS-00020	22,0	117,2	23,6	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00021	37,6	96,9	ND	12,7	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00022	49,5	ND	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00029	34,3	52,8	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00031	19,5	53,5	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00034	2157,5	2086,0	1721,2	339,2	64,0	22,3	115,9	51,4	Y	Y	Y	Y	N	Y	Y	N	N	N	N	N
HS-00040	40,5	35,6	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00077	32,7	14,9	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00081	56,1	41,8	29,4	ND	73,5	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00089	363,2	195,7	119,3	11,7	ND	ND	37,6	22,5	Y	Y	Y	Y	N	N	N	N	N	N	N	N
HS-00091	28,4	ND	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00094	32,5	34,8	12,2	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00095	29,4	16,0	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00096	445,3	138,1	30,4	31,0	12,4	14,3	194,5	118,7	Y	Y	Y	Y	Y	Y	N	Y	Y	N	N	N
HS-00102	24,1	46,9	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00122	51,0	52,4	ND	ND	22,6	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00125	48,5	29,2	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00128	36,7	53,4	24,0	ND	35,9	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00143	49,3	38,3	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00154	1879,8	854,7	548,6	369,4	156,9	159,3	110,3	27,0	Y	Y	Y	Y	Y	N	N	N	N	N	N	N
HS-00179	22,5	59,8	22,6	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00197	23,2	14,1	31,2	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00205	24,0	19,8	82,5	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00208	527,0	169,5	70,7	36,3	107,9	325,7	140,7	55,3	Y	Y	Y	N	N	N	N	N	N	N	N	N
HS-00209	174,0	87,3	35,6	16,5	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00221	43,7	23,7	10,1	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00232	31,1	57,7	ND	ND	52,5	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00233	46,6	16,4	ND	ND	13,3	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00253	40,3	21,0	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00259	63,5	43,0	ND	11,2	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00263	162,4	87,3	27,8	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00265	151,0	49,8	13,0	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00266	35,7	16,4	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00268	28,2	44,6	13,5	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00271	24,2	39,3	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00293	13,8	30,3	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00298	196,9	78,3	24,5	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00302	189,9	72,6	11,7	ND	ND	19,2	116,2	59,3	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N
HS-00303	28,3	50,5	22,2	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00304	19,5	ND	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00307	15,8	19,2	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00311	11,2	27,0	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00317	61,6	38,8	15,5	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00323	31,5	36,8	16,8	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00325	48,9	36,0	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00327	56,6	42,1	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00372	34,1	10,4	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00375	58,6	30,2	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00377	51,2	27,9	10,4	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00379	37,7	37,9	15,8	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00384	90,1	130,8	53,8	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00388	59,1	38,6	34,2	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00389	73,8	33,6	25,1	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00399	11,3	26,1	10,2	ND	16,4	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00402	58,6	49,6	17,8	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00403	106,0	92,7	38,6	11,5	ND	16,1	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00416	9,4	134,4	24,9	17,2	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00417	18,3	19,5	ND	ND	41,6	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00418	22,6	ND	12,3	5,0	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00420	11,4	ND	20,0	17,1	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00423	133,0	125,6	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00426	20,9	ND	5,8	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00435	10,9	51,6	ND	ND	51,6	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00439	36,0	ND	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00442	20,3	45,7	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00447	50,8	42,1	ND	ND	37,9	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00449	30,6	22,7	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00451	449,2	121,5	278,7	230,4	284,5	647,2	493,1	150,9	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N
HS-00452	55,6	26,0	10,1	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00463	32,7	13,6	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00464	22,8	19,0	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00465	48,1	12,9	7,6	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00466	35,4	25,0	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00478	86,2	40,5	25,2	18,1	14,5	21,6	11,1	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00480	34,1	67,3	33,7	ND	5,1	8,4	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00496	89,8	ND	33,4	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00497	98,3	ND	47,7	ND	6,7	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00498	54,8	ND	67,8	14,0	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00500	22,6	36,0	14,9	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00504	20,6	16,9	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00506	19,2	17,8	ND	ND	26,0	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00507	46,0	115,6	22,8	7,2	11,2	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00508	10,2	ND	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00509	13,9	11,5	ND	ND	30,1	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00513	50,7	ND	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00515	7,9	11,0	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00518	23,0	45,9	14,1	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00519	18,4	16,7	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00526	20,1	16,6	17,2	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00533	493,9	87,1	61,9	41,2	19,8	42,4	20,8	2,4	Y	Y	Y	Y	Y	N	Y	N	N	N	N	Y
HS-00551	13,9	20,8	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00563	15,5	12,9	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00564	29,1	34,4	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00565	27,1	13,6	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00567	24,2	8,7	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00571	61,2	35,0	6,7	6,8	6,1	8,1	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00575	27,6	14,4	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00590	34,7	16,2	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00592	26,4	12,4	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00609	96,2	13,7	32,3	24,1	65,9	6,2	7,5	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00612	50,4	44,1	ND	ND	11,2	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00619	57,8	45,5	29,0	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00620	55,7	45,1	24,0	6,2	2,5	6,2	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00621	51,2	26,2	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00628	25,1	82,1	28,6	2,0	53,5	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00630	60,3	26,4	8,0	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00637	22,9	100,5	26,1	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00641	36,3	49,3	12,8	4,7	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00657	248,2	75,0	29,1	20,1	8,3	9,5	5,9	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00659	23,6	4,5	19,6	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00660	62,8	75,4	23,6	7,4	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00665	19,4	23,3	31,9	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00674	57,6	33,8	10,3	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00691	52,6	34,7	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00693	36,6	29,7	ND	ND	13,5	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00695	65,5	18,9	ND	8,1	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00699	40,5	55,4	11,0	ND	78,5	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00706	51,8	48,7	19,6	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00710	62,1	48,1	13,1	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00711	47,3	5,3	5,2	ND	10,7	ND	24,8	13,9	Y	N	N	N	N	N	N	N	N	N	N	N
HS-00729	40,1	32,8	15,1	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00730	2669,0	2206,6	2411,3	296,8	61,6	79,1	147,2	82,2	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N
HS-00732	26,5	82,3	ND	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00737	35,5	55,5	6,3	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00738	111,6	81,7	23,0	20,3	5,2	ND	8,7	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00739	31,7	37,3	26,8	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00740	15,9	73,8	17,8	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00742	33,8	ND	12,9	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00745	20,2	16,6	15,8	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00748	17,7	28,0	6,0	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00760	146,5	37,2	ND	ND	6,2	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00764	19,9	ND	7,2	ND	ND	7,0	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00825	28,9	61,6	25,2	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00838	37,0	ND	9,0	ND	ND	6,2	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00839	20,4	ND	19,7	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00844	1480,6	503,7	210,4	161,6	45,7	60,3	76,3	3,6	N	Y	N	N	N	N	Y	N	N	N	N	Y
HS-00845	1315,1	333,7	126,5	98,9	33,6	47,6	92,6	2,2	N	N	N	N	N	N	N	N	N	N	N	N
HS-00848	2891,5	1287,2	445,9	239,6	52,2	65,0	80,3	N	N	N	N	N	N	N	N	N	N	N	N	N
HS-00868	1612,5	406,7	160,2	103,1	35,7	47,5	76,2	1,9	N	N	N	N	N	N	N	N	N	N	N	N
HS-00892	1503,9	351,0	156,2	19,0	36,5	88,6	88,1	245,2	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N
HS-00938	190,4	78,0	24,9	21,2	5,4	7,4	8,4	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00943	55,6	42,7	21,2	ND	ND	7,2	ND	ND	Y	Y	N	N	N	N	N	N	N	N	N	N
HS-00952	24,4	13,7	5,8	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-00959	50,6	ND	20,3	ND	ND	ND	ND	ND	N	N	N	N	N	N	N	N	N	N	N	N
HS-01075	358,8	93,3	53,1	46,1	20,3	207,5	22,1	ND	N	Y	Y	Y	Y	Y	Y	N	N	N	N	Y

*not detected **no ***yes

Annex 4 *Overview of concentration ranges and ratios of individual sugars for different floral composition of honey samples*

Variety	Range	Fru+Glu	Fru/Glu	Suc/Tur	Suc/Maltose	Mtriose/Tur	Maltose/Gent	Mtriose/Gent	Pan/Gent	Raf/Gent	Trehalose	Arabinose	Glucose	Fructose	Melibiose	Iso-maltose	Maltulose	Sucrose	Gentobiose	Turanose	Melezitose_Palatinose	Raffinose	Stachyose	Kestose	Maltose	Erlose	Panose	Nystose	Maltotriose
Acacia	MIN	40,41	1,03	0,08	0,03	0,02	7,64	0,13	0,33	0,25	0,01	0,00	17,92	22,48	ND*	0,10	0,13	0,03	0,01	0,30	0,02	0,01	0,01	0,01	0,71	0,09	0,03	0,01	0,01
	MAX	77,95	1,87	1,00	0,55	0,91	65,50	3,67	7,77	0,39	0,05	0,00	31,13	47,95	ND	2,00	1,76	0,91	0,18	2,62	0,20	0,02	0,04	0,26	3,90	1,77	0,34	0,05	0,38
AD**	MIN	52,88	0,98	0,15	0,04	0,03	10,73	0,22	0,52	ND	0,01	0,00	23,64	28,40	ND	0,23	0,27	0,09	0,05	0,42	0,03	ND	ND	0,02	1,30	0,02	0,06	0,01	0,03
	MAX	69,16	1,57	0,64	0,37	4,29	49,85	16,21	5,06	ND	0,01	0,00	29,79	41,54	ND	0,78	0,89	0,54	0,15	1,71	0,13	ND	ND	0,11	2,40	1,27	0,34	0,02	1,80
Buckwheat	MIN	58,74	1,11	0,13	0,14	0,02	8,08	0,13	0,27	0,15	0,02	0,01	25,17	32,18	ND	0,21	0,25	0,16	0,05	0,43	0,03	0,01	0,00	0,03	0,73	0,01	0,03	0,01	0,01
	MAX	69,93	1,56	0,40	0,23	0,08	21,15	1,07	2,23	0,79	0,07	0,01	32,58	40,89	ND	1,13	0,84	0,32	0,17	1,76	0,11	0,05	0,00	0,08	1,39	0,05	0,19	0,02	0,06
Buckwheat+	ND	60,45	1,31	0,28	0,16	0,06	10,85	0,35	0,50	ND	0,02	ND	26,20	34,25	ND	0,39	0,46	0,19	0,11	0,68	0,05	ND	ND	0,03	1,23	0,05	0,06	0,02	0,04
AD Buckwheat	ND	61,80	1,04	0,15	0,13	0,05	16,74	0,72	2,12	ND	ND	ND	30,34	31,46	ND	0,46	0,38	0,12	0,06	0,79	0,06	ND	ND	0,07	0,95	0,41	0,12	ND	ND
Chestnut	MIN	43,90	1,46	0,28	0,44	0,01	4,45	0,09	0,90	0,09	0,10	0,01	16,31	27,59	ND	1,03	1,38	0,34	0,12	1,23	0,17	0,01	0,02	0,10	0,55	0,01	0,11	0,00	0,01
	MAX	56,65	1,81	0,51	1,17	0,05	7,88	0,59	1,54	0,11	0,27	0,01	22,90	33,75	ND	3,24	2,79	0,77	0,14	1,67	0,51	0,01	0,15	0,42	1,09	0,07	0,20	0,00	0,08
Chestnut+	ND	50,16	1,59	0,30	0,37	0,05	9,71	0,64	1,14	ND	0,07	ND	19,35	30,81	ND	0,63	0,75	0,23	0,06	0,77	0,09	ND	ND	0,07	0,62	0,09	0,07	0,01	0,04
AD Chestnut	ND	57,37	1,02	0,18	0,15	0,21	9,20	1,59	2,23	0,79	0,36	0,01	28,38	28,99	ND	1,68	1,20	0,26	0,18	1,40	0,25	0,15	0,02	0,21	1,69	0,27	0,41	0,01	0,29
Echium+	ND	73,04	1,44	0,26	0,14	0,03	9,45	0,17	0,46	ND	0,16	ND	29,88	43,16	ND	0,36	0,46	0,20	0,15	0,75	0,05	ND	ND	0,07	1,45	0,12	0,07	0,02	0,03
Eucalyptus	MIN	55,40	1,13	0,16	0,19	0,02	7,00	0,39	1,31	0,46	0,06	0,00	23,33	29,37	0,00	0,42	0,68	0,18	0,03	0,95	0,06	0,07	0,01	0,05	0,66	0,02	0,06	0,01	0,02
	MAX	69,51	1,53	0,27	0,70	0,10	18,89	1,69	2,06	1,55	0,16	0,00	27,43	42,08	0,00	1,38	1,63	0,47	0,16	1,98	0,16	0,09	0,04	0,08	1,73	0,41	0,27	0,02	0,10
AD	MIN	53,43	1,07	0,13	0,18	0,07	14,40	2,77	1,89	1,25	0,03	0,00	21,68	28,50	0,00	0,58	0,66	0,20	0,04	1,19	0,07	0,05	0,01	0,08	0,58	0,78	0,08	0,01	0,10
	MAX	55,23	1,46	0,36	0,74	0,07	32,07	2,77	3,25	1,25	0,15	0,00	26,74	31,75	0,00	0,67	1,16	0,43	0,04	1,53	0,14	0,05	0,02	0,11	1,16	0,78	0,12	0,01	0,10
Fir+	ND	52,15	1,12	0,26	0,31	0,09	10,66	1,19	2,38	1,11	0,27	ND	24,63	27,52	ND	1,96	1,51	0,40	0,12	1,51	0,24	0,13	0,05	0,51	1,28	0,26	0,29	0,04	0,14
AD	ND	43,13	0,83	0,29	0,22	1,23	25,53	23,99	8,62	2,08	0,42	0,02	23,50	19,62	ND	1,35	0,96	0,36	0,06	1,24	0,22	0,13	0,03	0,29	1,62	0,69	0,55	0,02	1,52
Forest+	ND	78,68	1,28	0,52	0,81	0,03	10,02	0,41	2,36	0,45	0,60	0,04	34,54	44,14	ND	2,69	2,73	1,01	0,12	1,92	0,32	0,06	0,13	0,31	1,24	0,16	0,29	ND	0,05
AD	MIN	40,38	1,03	0,21	0,25	0,10	10,16	1,90	2,77	1,09	0,26	0,00	19,90	20,48	0,00	1,49	1,32	0,37	0,07	1,35	0,30	0,11	0,05	0,27	0,66	0,18	0,27	0,03	0,16
	MAX	49,55	1,13	0,27	0,55	0,12	15,48	2,44	6,26	8,94	0,29	0,00	23,22	26,33	0,00	2,11	1,38	0,38	0,10	1,80	0,46	0,58	0,07	0,37	1,52	0,26	0,41	0,03	0,19
Heather	MIN	52,77	0,96	0,12	0,14	0,01	1,72	0,04	0,17	ND	0,03	0,01	22,16	30,22	ND	0,29	0,26	0,13	0,03	0,49	0,04	ND	0,03	0,03	0,67	0,14	0,02	0,01	0,02
	MAX	63,70	1,39	0,51	1,08	0,41	56,30	7,78	3,37	ND	0,08	0,01	31,90	32,78	ND	1,69	2,12	0,74	0,40	1,79	0,36	ND	0,03	0,17	1,59	0,22	0,15	0,07	0,20
Heather+	ND	55,05	1,65	0,27	0,71	0,01	4,40	0,16	1,44	ND	0,10	0,01	20,78	34,27	ND	2,10	2,43	0,52	0,16	1,88	0,42	ND	0,06	0,34	0,72	0,02	0,24	0,01	0,03
AD Heather +	ND	62,12	1,61	0,28	0,44	0,01	3,21	0,06	0,65	ND	0,08	ND	23,83	38,28	ND	1,67	1,87	0,50	0,35	1,77	0,20	ND	0,02	0,08	1,13	0,01	0,23	ND	0,02
Laveer	MIN	55,05	1,39	0,27	0,44	0,01	3,21	0,06	0,65	ND	0,08	0,01	20,78	32,78	ND	1,67	1,87	0,50	0,16	1,77	0,20	ND	0,02	0,08	0,72	0,01	0,15	0,01	0,02
	MAX	63,70	1,65	0,51	1,08	0,41	56,30	7,78	3,37	ND	0,10	0,01	31,90	38,28	ND	2,10	2,43	0,74	0,40	1,88	0,42	ND	0,06	0,34	1,59	0,22	0,24	0,07	0,20
Lien	MIN	43,08	0,95	0,16	0,14	0,01	2,06	0,04	0,31	ND	0,01	ND	19,16	23,90	ND	0,23	0,21	0,12	0,06	0,21	0,03	ND	0,01	0,03	0,59	0,01	0,03	0,01	0,01
	MAX	73,27	1,57	0,79	0,71	0,09	21,45	0,56	1,26	ND	0,11	ND	33,41	40,63	ND	1,75	1,91	0,52	0,41	2,05	0,27	ND	0,04	0,22	1,25	0,40	0,22	0,02	0,04
AD	MIN	45,61	0,73	0,18	0,05	0,01	2,11	0,07	0,54	0,21	0,05	0,01	24,91	19,26	ND	0,49	0,50	0,17	0,10	0,58	0,06	0,03	0,01	0,02	0,70	0,02	0,07	0,01	0,02
	MAX	71,55	1,81	0,34	0,40	1,69	32,66	10,12	2,24	0,21	0,12	0,01	33,48	45,60	ND	1,28	1,24	0,30	0,37	1,66	0,15	0,03	0,01	0,14	3,15	0,11	0,54	0,02	0,98
Manuka	MIN	57,93	1,03	0,18	0,27	0,02	4,11	0,28	0,19	0,16	0,06	0,01	25,08	32,85	ND	0,38	0,52	0,19	0,06	0,74	0,17	0,03	0,02	0,07	0,27	0,06	0,01	0,01	0,05
	MAX	65,39	1,41	0,26	0,77	0,07	10,05	0,76	1,31	1,24	0,11	0,01	32,16	38,22	ND	2,05	1,91	0,47	0,18	1,96	0,55	0,07	0,09	0,77	0,72	0,15	0,23	0,05	0,05
AD	ND	51,31	1,22	0,19	0,15	0,03	11,91	0,30	0,91	ND	ND	ND	23,15	28,17	ND	0,47	0,53	0,16	0,09	0,84	0,06	ND	ND	0,05	1,03	0,15	0,08	0,01	0,03
AD Meadow+	ND	69,96	2,30	0,54	0,27	0,04	15,99	0,31	0,88	0,22	ND	ND	21,20	48,76	ND	0,26	0,36	0,29	0,07	0,54	0,04	0,01	ND	0,03	1,08	0,07	0,06	0,03	0,02
Orange	MIN	54,15	1,02	0,03	0,01	0,01	8,68	0,18	1,09	0,16	0,01	0,01	24,31	28,28	ND	0,29	0,45	0,03	0,05	0,83	0,04	0,02	0,00	0,11	0,79	0,15	0,07	0,01	0,02
	MAX	69,32	1,45	3,40	2,09	0,09	41,80	1,62	2,64	2,91	0,03	0,01	31,48	37,83	ND	0,71	1,15	4,03	0,11	1,70	0,12	0,17	0,00	0,19	2,19	0,73	0,17	0,02	0,10
AD	ND	51,75	1,39	0,54	0,23	0,07	23,46	0,66	0,99	ND	0,04	ND	21,65	30,11	ND	0,30	0,44	0,41	0,08	0,76	0,05	ND	ND	0,11	1,80	0,33	0,08	0,02	0,05
Rhododeron+	ND	61,39	1,70	0,23	0,47	0,02	7,13	0,26	2,05	ND	0,52	0,03	22,72	38,67	ND	1,91	1,89	0,45	0,13	1,94	0,27	ND	0,02	0,16	0,95	0,04	0,27	0,01	0,03
AD	ND	56,53	1,97	0,38	1,04	0,01	0,97	0,02	0,39	ND	0,15	ND	19,01	37,52	ND	3,90	3,19	0,71	0,70	1,87	0,69	ND	0,21	0,59	0,68	0,02	0,28	ND	0,01
Robinia	ND	54,90	1,25	0,15	0,15	0,02	10,20	0,28	0,45	ND	0,01	0,00	23,54	30,87	ND	0,10	0,20	0,12	0,04	0,35	0,02	0,00	0,01	0,03	0,81	0,02	0,03	0,01	0,02
	ND	66,02	1,80	0,38	0,30	0,06	25,80	1,69	3,78	ND	0,01	0,00	28,35	42,47	ND	0,95	1,30	0,35	0,10	1,89	0,19	0,00	0,01	0,12	1,36	0,97	0,21	0,02	0,07
AD	ND	57,53	1,29	0,22	0,24	0,05	25,15	1,44	3,07	ND	0,01	0,01	25,15	32,38	ND	0,67	0,85	0,34	0,06	1,52	0,13	ND	ND	0,13	1,45	1,07	0,18	0,01	0,08
	ND	52,12	1,27	0,58	0,31	0,06	20,66	0,67	0,90	ND	0,07	ND	22,99	29,13	ND	0,22	0,31	0,42	0,07	0,73	0,03	ND	ND	0,05	1,36	0,61	0,06	0,02	0,04
Rosemary	ND	44,46	1,05	0,24	0,29	0,01	4,51	0,14	0,89	ND	0,03	0,02	21,14	23,32	ND	0,58	0,71	0,26	0,05	0,93	0,09	ND	0,01	0,06	0,62	0,05	0,12	0,01	0,02
	ND	63,75	1,39	2,48	1,49	0,07	26,27	1,37	5,03	ND	0,05	0,02	28,98	35,76	ND	2,00	1,76	2,51	0,14	1,80	0,25	ND	0,04	0,26	1,78	1,08	0,26	0,02	0,08
Spruce+	ND	41,97	1,20	0,45	0,66	0,25	9,01	3,30	5,18	3,19	0,45	ND	19,06	22,90	ND	2,67	1,67	0,60	0,10	1,33	0,38	0,32	0,20	0,68	0,91	0,26	0,52	0,05	0,33
Sunflower+	ND	71,32	0,86	0,82	0,14	0,09	13,86	0,21	1,26	ND	ND	ND	38,31	33,01	ND	0,31	0,21	0,17	0,09	0,21	0,03	ND	0,01	ND	1,19	0,02	0,11	0,02	0,02
Thyme	ND	52,36	1,00	0,22	0,24	0,01	4,26	0,12	1,58	ND	0,02	0,01	23,56	28,79	ND	1,14	1,29	0,40	0,09	1,41	0,12	0,00	0,02	0,08	0,37	0,01	0,14	0,01	0,01
	ND	68,56	1,59	0,42	1,12	0,03	16,66	0,52	2,61	ND	0,18	0,01	31,16	42,08	ND	3,00	2,49	0,69	0,14	2,11	0,42	0,00	0,08	0,44	1,68	0,40	0,33	0,01	0,05
AD Thyme	ND	54,53	1,12	0,52	1,06	0,14	5,52	1,53	3,83	9,12	0,18	0,01	25,74	28,79	ND	2,36	2,07	0,59	0,10	1,13	1,41	0,92	0,06	0,34	0,56	0,13	0,39	ND	0,15
AD Thyme+	ND	49,14	1,01	0,16	0,17	0,05	6,55	0,71	3,13	5,13	0,13	0,01	19,99	24,73	ND	0,57	0,86	0,29	0,05	1,33	0,09	0,46	0,04	0,12	0,58	0,05	0,18	0,01	0,06
	ND	54,34	1,68	0,52	1,19	0,06	36,18	2,32	3,79	5,20	0,17	0,01	24,40	33,49	ND	2,13	2,20	0,69	0,10	1,77	0,99	0,52	0,05	0,38	1,71	1,86	0,33	0,01	0,11

*not detected | **adultarated | blue highlighted - polyfloral samples | red number - out of range