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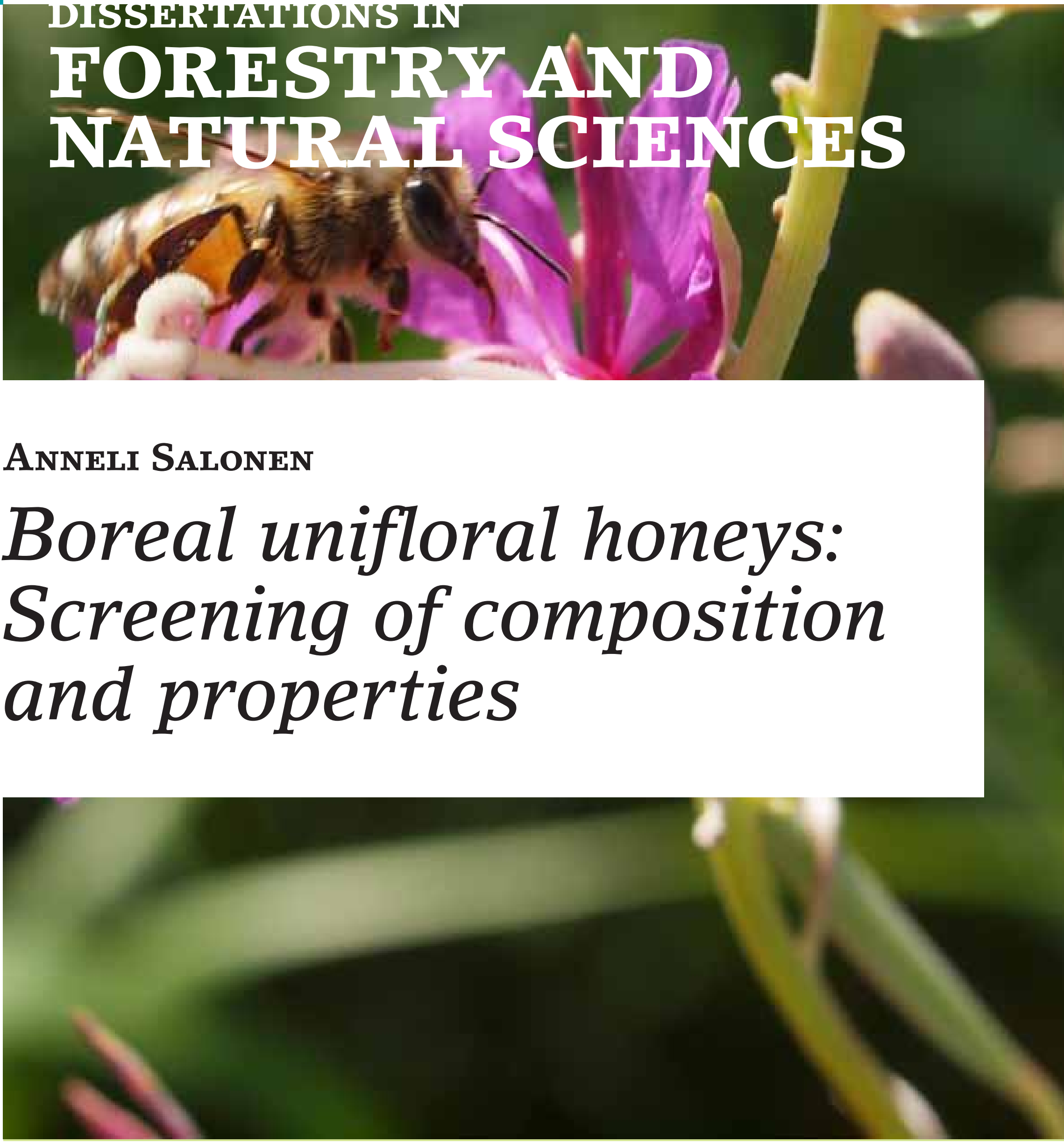

ANNELI SALONEN

Boreal unifloral honeys: Screening of composition and properties

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EASTERN FINLAND

ANNELI SALONEN

Boreal unifloral honeys: Screening of composition and properties

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ABSTRACT

In this thesis the aim was to investigate the physico-chemical, organoleptic and melissopalynological characterisations and properties of buckwheat, willow, heather, lingonberry, raspberry, fireweed, dandelion, mire and honeydew unifloral honeys collected in Finland and find tools for their process of discrimination. The main interest laid in those unifloral honeys on which almost no scientific data can be found and which are typical of the boreal coniferous zone in Scandinavia (lingonberry, raspberry, fireweed and mire honey). The composition of nectar and honey from fireweed were compared and the phenolic content of Finnish propolis was analysed, as well. Concerning the results on physico-chemical properties, the greatest variation was found in electrical conductivity. The carbohydrate content of the studied unifloral honeys was surprisingly similar, except for some variation in the di- and oligosaccharide contents. Melissopalynological analyses revealed some differences in pollen amounts compared to European unifloral honeys and some special features in the pollen content of fireweed honey, but they did not provide an answer to the question of the botanical origin of mire honey. The spectra of phenolic compounds are different in unifloral honey varieties, but a better discriminating tool is the ratio of phenolic acids to flavonoids. PCA of amino acids was also useable in distinguishing unifloral honey varieties. The analytical data obtained in this research may be useful in future studies concerning the characterisation and properties of Finnish unifloral honeys and propolis, their antimicrobial properties, their use as sources for biologically active compounds or their impact on human health.

Universal Decimal Classification: 638.162, 638.165.8

CAB Thesaurus: honey; physicochemical properties; composition; palynology; pollen; pollen analysis; propolis; nectar; sensory evaluation; characterization; phenolic compounds; electrical conductivity; carbohydrates; amino acids; liquid chromatography; HPLC; boreal forests; Finland

Yleinen suomalainen asiasanasto: hunaja; koostumus; fysikaaliset ominaisuudet; kemialliset ominaisuudet; siitepöly; siitepölyanalyysi; aistinvarainen arviointi; fenoliset yhdisteet; sähkönjohtavuus; hiilihydraatit; aminohapot; nestekromatografia; boreaalinen vyöhyke; Suomi

Preface

I am most indebted to many people for helping me to make this thesis possible. I am most grateful to Professor Riitta Julkunen-Tiitto for all her encouragement, advice, supervision and support when helping me through this long journey. Thanks are also due to the University of Joensuu and Eastern Finland for providing the facilities for my work and for financial support in 2008.

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My dear beekeeper friends around Finland have helped me to get to know the wonderful world of bees and honey. I am especially thankful for Tarja Ollikka for all the conversations, advice, photos and materials she has given me as well as the other staff of the Finnish Beekeepers Association, i.e. Heikki Vartiainen, Lauri Ruottinen and Ari Seppälä. Maritta Martikkala, thank you, you have been indispensable during past year.

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And last but not at all least my dearest Antero, Hanna and Simo; my sincere thanks to you for your love, patience and support. You are the sunshine of my life!

"Honey is a wonderful gift from
the merciful God for all mankind!"

- Dr. M. Kamaruddin

University of Malaya, Malaysia

LIST OF ABBREVIATIONS

DAD	Diode array detector
EU value	A value given to a physical or chemical parameter of honey in the Council Directive 74/409/EEC relating to honey by the Commission of the European Union
F+G	Total amount of fructose and glucose in honey
F/G ratio	Ratio of fructose to glucose content of honey
G/W ratio	Ratio of glucose to water content of honey
HMF	Hydroxymethylfurfural, a compound derived from the dehydration of sugars; its amount in honey increases if the honey is heated or stocked for a long time; an indicator of the honey's quality
HPLC	High pressure liquid chromatography
PCA	Principal Component Analysis
RID	Refractive index detector
Rt	Retention time

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles. The articles are referred to in the text by their Roman numerals, I-IV.

I Salonen A, Ollikka T, Grönlund E, Ruottinen L and Julkunen-Tiitto R. Pollen analyses of honey from Finland. *Grana 48: 281-289, 2009.*

II Salonen A, Saarnio S and Julkunen-Tiitto R. Phenolic compounds of propolis from the boreal coniferous zone. Submitted.

III Salonen A, Hiltunen J and Julkunen-Tiitto R. Composition of Unique Unifloral Honeys from the Boreal Coniferous Forest Zone: Fireweed and Raspberry Honey. *Journal of ApiProduct and ApiMedical Science 3: 128-136, 2011.*

IV Salonen A and Julkunen-Tiitto R. Characterisation of two unique unifloral honeys from the boreal coniferous zone: lingonberry and mire honeys. Submitted.

In addition to the original publications, this thesis includes unpublished results concerning buckwheat, willow, heather, dandelion and honeydew honeys as well as fireweed nectar.

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Contents

1 Introduction 11

2 Materials and methods 19
2.1 Honey, propolis and nectar samples 19
2.2 Methods 19

3 Results and discussion 25
3.1 Composition and properties of Finnish unifloral honeys 25
3.1.1 Buckwheat honey 25
3.1.2 Willow honey 28
3.1.3 Heather honey 29
3.1.4 Lingonberry honey 31
3.1.5 Raspberry honey 32
3.1.6 Fireweed honey 34
3.1.7 Dandelion honey 38
3.1.8 Mire honey 39
3.1.9 Honeydew honey 41
3.2 Phenolic compounds of propolis in unifloral honeys 43
3.3 Tools for discrimination of Finnish unifloral honeys 44

4 Concluding remarks 49

References 53

Appendix 59

1 Introduction

In Council Directive 74/409/EEC relating to honey the Commission of the European Union defines honey as follows: “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.” No additions of any other food ingredients or water are allowed in natural honey. It is not permitted to remove pollen or other specific constituents of honey from honey, excluding the pollen which is filtered out in the fine filtration of foreign inorganic and organic matter. Moreover, the criteria for fructose, glucose, sucrose, moisture, water-insoluble matter, electrical conductivity, free acid, diastase and HMF content are defined for marketed honey in the Council Directive relating to honey (European Commission, 2002).

As bees collect nectar or honeydew from the plants growing in the surrounding of their hive, the main types of honey are nectar or blossom and honeydew honeys. Honeydew honey comes mainly from the excretions of plant sucking insects (*Hemiptera*), which bees collect from the surface of the plants. Nectar or blossom honey contains bee-collected nectar from several plant species and is generally called multifloral or polyfloral honey. However, if the nectar from which the bees derive the honey is collected mainly from one specific plant species, the honey can be called as unifloral honey and named according the species (White, 2005).

Over one hundred plant species are known to give unifloral honey in Europe, but only a few of them are of any great commercial importance and are sold on major markets (Persano Oddo *et al.*, 2004). There is no absolutely pure unifloral honey, because bees always collect nectar from several plant species, although a single plant species may be the predominant one. If the unifloral honeys can be identified by their physico-chemical, organoleptical and melissopalynological properties, the European Directive for honey allows the use of specific denominations, where the simple product name "honey" can be supplemented by information on the floral, vegetable, regional, territorial or topographical origin (European Commission, 2002). Unifloral honeys may have a special taste, odour or they may consist of substances that are supposed to be beneficial to human health and are, as such, a more expensive class of honey on the honey market in Europe. In Italy, Spain and France about half of the sold honey is unifloral honey (Persano Oddo & Bogdanov, 2004).

In Finland, the total honey yield ranges from 1 to 3 million kilos per annum (Anonymous, 2009), 65 % of which is harvested in the southern and western parts of the country. In Finnish nature the growing season is very short, and many plant species bloom at the same time, so that the honey crop is collected within 5 to 6 weeks (Korpela, 2010). Finnish commercial honey is typically polyfloral nectar honey because most Finnish beekeepers harvest honey from their colonies only once in a growing season. The main honey producing plants in South-Western Finland are cultivated *Brassica* species, while in other parts of Finland the main honey crop is collected from wild plants. Where unifloral honeys are concerned, if the weather is favourable, willow (*Salix* spp), dandelion (*Taraxacum* spp.), lingonberry (*Vaccinium vitis-idaea* L.), raspberry (*Rubus idaeus* L.), fireweed (*Epilobium angustifolium* L.), clovers (*Trifolium* spp) and heather (*Calluna vulgaris* L.) have the potential to produce sufficient unifloral honeys for commercial production. Occasionally, when the summer is dry and warm, honeydew honey can be collected as well. In recent years, some Finnish beekeepers have started to collect and sell these unifloral honeys, but there is no official information on the extent of the production of or trade in these honey varieties.

Defining discriminating factors for unifloral honeys is problematic. Bees generally collect nectar from several plant species, although one species may be predominant, and therefore no references exist for totally pure unifloral honey. It is not possible to define the exact amount of nectar obtained from one plant species in unifloral honey or to observe the point where unifloral honey becomes multifloral honey. In fact, the typical organoleptical properties of a unifloral honey turn out to be more reliable discriminating factors than the exact amount of nectar of a specific plant species (Persano Oddo & Bogdanov, 2004).

The main constituents of honey are fructose and glucose, di- and oligosaccharides, organic and amino acids and enzymes originating from plants or bees, or constituents developed during the maturation of the honey. Furthermore, honey contains small amounts of vitamins and minerals as well as phenolic and volatile compounds (White, 2005). In order to protect consumers in cases where unifloral honeys are not authentic or are adulterated, the International Honey Commission has proposed reference methods and standards that can be used in the authentication of unifloral honeys (Persano Oddo & Bogdanov, 2004). In addition, Persano Oddo and Piro (2004) have presented a characterisation of 15 European unifloral honeys, which is a collaborative work of the International Honey Commission. This analytical data is a valuable tool used in honey laboratories around Europe.

The classical physico-chemical analysis of e.g. colour, electrical conductivity, pH, proline content, diastase and invertase activity, the moisture content and content and quantity of carbohydrates have widely been used in the botanical denomination of unifloral honeys. The colour of honey is a factor that strongly influences the price of a honey variety, as it is easy for the customers to perceive. In classical honey analysis colour is measured with a Pfund meter, but the colour intensity can also be determined with a spectrophotometer (Negueruela & Perez-Arquillue, 2000). Due to the presence of organic acids, the pH value of honey is usually between 3.5 and 5.5, and it varies according to the floral origin of the honey, giving some classification power for discrimination between unifloral honeys (Bogdanov *et al.,* 2004). Proline is the most abundant amino acid in honey and it is added to honey by the bees. Due to the wide variation, the proline content of the honey cannot be the only criterion for the classification of unifloral honeys. However, it is an indicator of honey ripeness (von der Ohe *et al.,* 1991). The invertase and diastase activities of honey varieties differ greatly based on the amount of daily nectar flow of the plants. This is because bees add these enzymes to nectar during foraging flights and honey processing in the hive, and if the nectar flow is high, the amount of these enzymes is lower (Persano Oddo *et al*., 1999). There is no EU value for invertase number of honey, but in Finnish commercial honey with the *Food from Finland – label of origin* it has to be over 35 (Finnish Beekeepers Association, 2011). The EU value for diastase is above 8 (Schade scale). In some honey varieties that are mentioned in the EU directive (e.g. dandelion), the diastase number is naturally low (3-8). In Finland, at least raspberry and fireweed give an abundant nectar yield during one to two weeks, and it could be possible that honey derived from these plants has a low diastase number. Invertase and diastase values have to be analysed from fresh samples, since enzyme activity generally decreases as a result of storing or heating honey (Persano Oddo *et al.,* 1999). The moisture content of honey has little importance in the characterisation of unifloral honeys, but it reveals the quality and shelf life of the samples. In some cases moisture content may also affect the viscosity and crystallisation of unifloral honeys (Bogdanov *et al.,* 2004).

Approximately 95% of the dry weight of honey consists of carbohydrates (White, 2005). The amounts of fructose and glucose, F/G and G/W ratios and sometimes the amounts of individual di- and oligosaccharides can be used in the characterisation of unifloral honeys (Bogdanov *et al.,* 2004). In order to protect customers against honey adulteration, the maximum EU-value for sucrose is set at 5 g/100 g. The EU values for the total content of F+G for nectar honey is above 60 g/100 g and for honeydew honey above 45 g/100 g (European Commission, 2002). Oligosaccharides in honey may have an important role in its nutritional and health properties (Al-Qassemi and Robinson, 2003), but only the content of melezitose and raffinose in honeydew honey has some value in the classification of unifloral honeys. The crystallisation of honey is a very complicated process and it can be affected by many factors, e.g. the amount and balance of fructose and glucose. The F/G and G/W ratios that are used in identifying the botanical origin of honey can also be used in predicting the crystallisation tendency of honey. If the G/W ratio is lower than 1.7 and the F/G ratio higher than 1.3 honey does not crystallise. On the other hand, if the G/W ratio is over 2.1 the honey will crystallise very fast (Manikis and Thrasivoulou, 2001).

Melissopalynological analyses are a routine method used in controlling the quality of honey and when verifying the origin of unifloral honeys. They are always used in conjunction with physico-chemical and sensorial analyses. Basically, a honey is considered to be a unifloral honey if a minimum of 45% of the pollen grains in it derive from one plant species. In honeydew honey the ratio of the number of honeydew elements (e.g. fungal spores, hyphae and microscopic algae) to the number of pollen grains must be over 3 (von der Ohe *et al.,* 2004). Due to the structure of flowers and the behaviour of bees during foraging flights, the pollen content of nectar does not always reveal the absolute amount of the nectar in a plant species used for the honey. The pollen grains of a plant species may be normally-represented (percentage of pollen grains correlates one-to-one with the amount nectar from which the honey is derived), over-represented (percentage of pollen grains is higher than the amount of nectar from which the honey is derived), or under-represented (the percentage of pollen grains is lower than the amount of nectar from which the honey is derived) in honey samples (Bryant & Jones, 2001). As a result, the required level of 45% of pollen grains is not always met.

Sensorial analyses are the third method used in routine unifloral honey analyses. The organoleptic properties of the honey play a very important role in the process of distinguishing unifloral honeys, while they are usually the only "analysis" that can be conducted by the ordinary beekeeper for recognising honey varieties from honey combs. Official sensory analysis consists of visual, olfactory and taste observations made by a trained panel of assessors using the harmonised terms and descriptions for odour and taste presented by the International Honey Commission in the evaluation (Piana *et al.,* 2004).

Newer methods that can be used in the denomination of unifloral honeys include analyses of free amino acids and various secondary metabolites. Their total amounts in honey are very low, but in some unifloral honey varieties they may have some discriminating value. The free amino acids in honey originate from bees (bees add enzymes to the honey) or from pollen, which also contains proteins. In many studies the spectra of the amino acids have been fairly similar, but there is a great deal of variation in the quantity of individual amino acids (Bergner and Hahn, 1972; Rebane & Herodes, 2008). It seems that on the basis of the analyses of free amino acids it might be easier to differentiate the geographical rather than the botanical origin of the honey (Davies & Harris, 1982; Cotte *et al*., 2004).

Plant-derived secondary compounds, phenolic acids and flavonoids in honey originate from nectar or propolis. Plants add phenolic compounds to nectar in order to discourage a certain nectar consumer, to reduce the number of potential visitors, to reduce growth of moulds and bacteria, or to avoid oxidation of nectar substances (Pacini & Nepi, 2007). There are also observations that bees may prefer nectar with a higher phenolic content (Liu *et al.,* 2007). The phenolic compounds in propolis originate from the resins, leaf buds, mucilages and gums of plants, which use these substances to protect themselves against microbes (Marcucci, 1995). In some cases phenolic compounds can be used as markers for the unifloral honey (e.g. Ferreres *et al*., 1994; Tomás-Barberán *et al.,* 2001). Sometimes, when the amount of an individual phenolic compound or the profile of phenolic compounds cannot be used for distinguishing the unifloral honeys, the total amount of phenolic compounds, the total amount of phenolic acids or the total amount of flavonoids or their ratios may be more useful (Yao *et al*., 2004; Al *et al*., 2009). Amiot *et al.*

(1989) observed that dark honeys contain more phenolic compounds than light honeys and that nectar honeys contain more flavonoids than honeydew honeys. Despite their low amount in honey, phenolic compounds may contribute e.g. to the antioxidant properties of the honey (Gheldof *et al.*, 2002; Socha *et al.*, 2011; Baltrusaityte *et al.*, 2007; Tuberoso *et al.*, 2011).

There are only few studies on Finnish honey. Martimo (1945) and Aarnio (1961) studied the properties and melissopalynology of Finnish honey. More extensive work was done by Varis *et al.* (1982 and 1983), consisting of physico-chemical and melissopalynological analyses on 120 honey samples from the years 1977–1978. In 2000 Varis conducted another study on the melissopalynology of Finnish honey. Since 1995 the Finnish Beekeepers Association has observed the quality of commercial Finnish honeys by analysing some one hundred honey samples annually. In all these studies the main objective has been to analyse the properties and quality of Finnish polyfloral honeys. At the moment unifloral honeys are known to exist, but there is very little analytically reliable data on them from Finland. The only more detailed work on Finnish unifloral rapeseed honeys is the study done by Ruoff (2003).

The aims of this study were:

1. to investigate the physico-chemical, organoleptic and melissopalynological characterisations and properties of buckwheat, willow, heather, lingonberry, raspberry, fireweed, dandelion, mire and honeydew unifloral honeys collected in Finland. The main interest of this study lies in those unifloral honeys on which almost no scientific data can be found and which are typical of the boreal coniferous zone in Scandinavia (lingonberry, raspberry, fireweed and mire honey)
2. to compare the composition of nectar and honey from fireweed (*Epilobium angustifolium* L.) in order to identify those compounds of fireweed honey which originate from fireweed plant
3. to study the phenolic content of Finnish propolis. Phenolic acids and flavonoids in honey originate from plants and propolis. By identifying those phenolic compounds that originate from

propolis, it may be possible to recognise the botanical markers for unifloral honeys

4. to find tools for the process of discrimination of Finnish unifloral honeys

2 Materials and methods

2.1 HONEY, PROPOLIS AND NECTAR SAMPLES

The one hundred and sixteen unifloral honey samples analysed originated from buckwheat (*Fagopyrum esculentum* Moench, number of samples 3), willow (*Salix* spp, 12), heather (*Calluna vulgaris* L., 11), lingonberry (*Vaccinium vitis-idaea* L., 7), raspberry (*Rubus idaeus* L., 22), fireweed (*E. angustifolium* L., 42), dandelion (*Taraxacum* spp, 4), flora from mire (8) and honeydew (7). The source of the honey samples and conducted analyses are presented in table 1. The samples were stored in a refrigerator (+6˚C) in the dark until analysis. Some analyses were conducted on only one honey sample (Table 1), and these preliminary results give only a tentative indication of the properties of these Finnish unifloral honeys.

The propolis samples (19 samples) were collected from beekeepers. The source and age of the propolis samples are described in article II. The samples were stored at room temperature until analysis.

The nectar samples (12 samples) were collected in the year 2009 in the immediate neighbourhood of the research apiaries. For one sample, 20 inflorescences of *E. angustifolium* were collected, and the nectar was sucked out of their flowers with capillary pipettes. The samples were stored frozen (-19˚C) until analysis.

2.2 METHODS

All physical properties and invertase and diastase activity were analysed following the analytical methods harmonised by the International Honey Commission (III and IV). A digital refractometer was used for the determination of water content directly

Table 1. *Number and source of the samples and conducted analyses*

	Buckwheat	Willow	Heather	Lingonberry	Raspberry	Fireweed	Mire honey	Dandelion	Honeydew	Total
Research apiary[1]		6	10			31			1	**48**
Finnish beekeepers asossiation	2	4		2	20	6	5	1		**40**
directly from beekeepers	1	2	1	5	2	5	3	3	6	**28**
water%	3	12	11	7	22	42	8	4	7	**116**
electrical condactivity	3	12	11	7	22	42	8	4	7	**116**
pH	3	10	11	7	22	42	8	4	7	**114**
colour					7	10				**17**
diastase	1	10	11		13	41		4	2	**82**
invertase	2	8	11	6	22	42	7	3	3	**104**
fructose	3	12	11	7	22	42	8	4	7	**116**
glucose	3	12	11	7	22	42	8	4	7	**116**
oligosaccharides	1	1	1	6	5	12	6	1	1	**34**
pheolics		1	1	5	5	7	5			**24**
amino acids			9	3	7	10	6			**35**
sensorial				1	1	1	1			**4**
melisso-palynological	3	6	2	7	22	11	6	4	6	**67**

[1] *samples were collected directly from honey cakes*

from the honey. Conductivity was measured from a 10 g/dry matter/dose of honey and dissolved in 50 ml of MilliQ water. The 10% honey-water solution was used in pH-measurements. Invertase activity was determined by the methods published by Bogdanov (2009). Invertase activity is expressed as invertase number, which indicates the amount of sucrose (g) in 100 g of honey hydrolysed for one hour by the invertase. Diastase activity was determined by the Phadebas method, and the diastase activity is expressed as a diastase number in Shade units (III).

The sugar analyses are based on a liquid chromatographic method (III). The 0.5% honey water-acetonitrile solution was eluted with isogratic aq. 75% acetonitrile elution solvent by HPLC (Agilent, Series 1100, Germany containing binary pump (G1316A), a thermostated autosampler (G1329A), thermostated column oven (G1316A) and refractive index detector (RID) (G1362A), HP Chem Station Software and Zorbax colum carbohydrate, 4.6 x 1500 mm with 5 μm particle size). For the qualification and quantification of the carbohydrates, the HPLC chromatograms of the samples were compared to those of twelve commercial standards: fructose, glucose, maltose, D-turanose, panose, erlose, melezitose, isomaltose, gentiobiose, raffinose, sucrose and trehalose (III and IV). However, it was possible to identify and quantify only eight individual carbohydrates, while the disaccharides maltose and trehalose and the oligosaccharides melezitose and erlose could not be separated (overlapped peaks) with the novel carbohydrate column used (III). These peaks are referred in the text as maltose/trehalose and melezitose/erlose.

The method presented by Piana *et al.* (2004) was used in the analyses of the visual, olfactory and taste characteristics of the honey samples. The qualitative melissopalynological characteristics of the honey samples were analysed according to Louveaux *et al.* (1978). At least 400 pollen grains were counted with microscope (Zeiss axioskop 2 plus) from a 10 gram washed and centrifuged honey sample.

Phenolic compounds were extracted and reversed phase HPLC analysed according to Tomás-Barberán *et al.* (2001) with slight modifications (III). Briefly, the phenolics in twenty-five (25) grams of honey were allowed to bind with amberlite XAD-2 resin in a separation funnel for ten minutes at room temperature, the acidified water was separated, and the amberlite was washed with neutral water. The phenolic compounds were recovered using methanol. Before HPLC analysis, 5 ml of water was added, and the samples were extracted into 5 ml diethyl ether. The ether was evaporated and the sample was dissolved in methanol (0.25 ml) and MilliQ water (0.25 ml). Each honey sample was fractionated and analysed in duplicate. Phenolic compounds were analysed using an HPLC instrument (Agilent, Series 1100, Germany, instrument containing a binary pump (G1316A), a thermostated autosampler (G1329A), a thermostated column oven (G1316A), a Diode Array Detector (DAD) (G1315B), HP Chem Station Software and the column used was Zorbax, SB-C18, 4.6 x 75 mm with 3.5 μm particle size) with 1.5% tetrahydrofuran + 0.25% ortho-phosphoric acid in water (=A) and 100% methanol (=B) as elution solvents. The HPLC runs were monitored at 220 and 320 nm. The identification of phenolic compounds was based on a comparison of retention times, HPLC/DAD spectral characteristics and HPLC/MS-identification of the MS-ions. The quantification of phenolic compounds was based on the commercial standards (III and IV).

The phenolics of the propolis samples were analysed using HPLC. Fifty (50) milligrams of propolis were extracted with 8.5 ml of methanol at room temperature for 30 minutes. The extract was filtered through a filter paper, and the volume was adjusted to 10 ml with methanol. One millilitre of this sample was mixed with 0.5 ml MilliQ water and centrifuged for 3 minutes at 13000 rpm, and the supernatant was used directly for HPLC analysis. Each propolis sample was extracted and analysed in triplicate. Phenolic compounds were analysed using HPLC (Agilent, Series 1100, Germany, instrument containing a binary pump (G1316A), a thermostated autosampler (G1329A), a thermostated column oven (G1316A), a Diode Array Detector (DAD) (G1315B), HP Chem Station Software and the column used was Zorbax, SB-C18, 4.6 x 75 mm with 3.5 μm particle size) with the elution solvents 1.5% tetrahydrofuran + 0.25% orthophosphoric acid in water (=A) and 100% methanol (=B). The HPLC runs were monitored at 220 and 320 nm for 70 min. The identification of the compounds was based on the HPLC/MS-identification or on comparison of retention times and spectral characteristics as described in article II. The compounds were quantified against commercial standards.

The procedures used in the isolation and derivatisation of amino acids are described in Rebane and Herodes (2008). Briefly: one gram of honey was mixed with 25 ml of phosphate buffer (0.03 M and pH 2.12) and this mixture was applied to a conditioned solid phase extraction cartridge (styrene-divinylbenzene polymeric strong cation exchange resin, 500 mg, Alltech Associates, Inc.) at 1.5 ml/ min flow rate. The samples were eluted and evaporated to dryness under nitrogen and redissolved with 1 ml of MilliQ water. The sample was derivatised with 30 μl of diethyl ethoxymethylenemalonate, 1.5 ml of methanol and 3.5 ml of borate buffer. The HPLC analyses were carried out with Agilent 1100 series equipment and a Hydro-RP (80A 250 mm × 4.60 mm) analytical column (Phenomenex Synergi 4μ) at 45˚C. The flow rate of the elution solvent (A= acetate buffer and B= acetonitrile) was 0.9 ml/min with the following gradients: 0-12 min, 20-25% B; 12-20 min 25% B; 20-50 min, 25-60% B. The HPLC runs were monitored at 280 nm.

The analyses of total sugar content, quantities of individual carbohydrates and content of phenolic compounds were conducted for the nectar samples. The total sugar content (brix %) was determined directly from the 0.5 ml of nectar sample using a digital refractometer (Atago 3810 PAL-1 Digital Brix Refractometer). HPLC analyses were used for determining carbohydrates and phenolics. For carbohydrate analysis 100 μl nectar and 100 μl acetonitrile were combined and mixed, centrifuged and the supernatant was analysed by HPLC. A sample for phenolic analysis was prepared by mixing 80 μl of nectar and 80 μl of methanol. The HPLC conditions for the carbohydrates and phenolic compounds analyses were the same as described in articles III and IV.

3 Results and discussion

3.1 COMPOSITION AND PROPERTIES OF FINNISH UNIFLORAL HONEYS

3.1.1 Buckwheat honey

The colour of buckwheat honey is dark brown, reddish, purple or black, and it is supposed to contain abundant minerals. Its aroma is known to be very characteristic and strong, and some people may even find it unpleasant (Crane *et al.,* 1984). The buckwheat honey samples analysed in this study fulfilled these sensorial characterisations. Their electrical conductivity values varied between 0.3 and 0.5 mS/cm (Appendix 1; Table 2), results that are somewhat higher than those measured from buckwheat honey from Poland (Szczêsna & Rybak-Chmielewska, 2004). The F/G and G/W ratios (Appendix 2) suggest that this honey crystallises slowly. Sucrose was not found in the buckwheat honey samples in this study, although it has been found in earlier studies (Crane at al., 1984), whereas all other di- and oligosaccharides were the same as those reported in earlier studies (Appendix 3).

Sawyer (1988) found that pollen of *Fagopyrum* is over-represented in buckwheat honey samples. However, in Finnish buckwheat honey samples, the *Fagopyrum* pollen was highly under-represented, accounting for only four per cent. The main pollen groups in the buckwheat honey samples were *Trifolium* and Rosaceae. Buckwheat is a cultivated plant species in Finland and it blooms in July at the same time as other plants in Finnish nature. The nectar of buckwheat gives honey strong organoleptic characteristics even if it is not the main nectar source for the honey.

Twenty one individual phenolic compounds were found (Table 4; Appendix 4), and the total amount of phenolic compounds was highest in buckwheat honey, i.e. two to three times higher

Table 2. Physico-chemical properties of buckwheat, willow, heather, dandelion and honeydew honey (mean±standard error of the mean)

Parameter	Moisture (%)	Conductivity (mS/cm)	pH	Diastase activity	Invertase activity
Buckwheat	16.5±0.9	0.39 ±0.05	4.0±0.1	22.5	132.0±23.8
n	3	3	3	1	2
Willow	16.8±0.4	0.30±0.02	4.2±0.05	8.6±1.05	86.3±16.8
n	12	22	10	10	8
Heather	18.0±0.3	0.65±0.03	4.4±0.06	14.3±0.9	67.8 9.2
n	12	12	12	11	11
Dandelion	16.4±0.3	0.27±0.09	4.3±0.1	8.4±1.4	140.4±65.2
n	4	4	4	3	3
Honeydew	15.6±0.3	0.50±0.07	4.4±0.1	5.8±3.8	79.4±3.0
n	7	7	7	2	3

Table 3. Results of melissopalynological analysis of buckwheat, willow, heather, dandelion and honeydew honeys give the percentage of a pollen group out of all counted pollen grains (mean ± s.e.)

Pollen group	Buckwheat	Willow	Heather	Dandelion	Honeydew
Salix	6.1±1.2	68.1±8.6	12.0	28.2± 9.7	8.2±2.9
Brassicaceae	11.6±6.7	8.6±5.5	1.2	1.7±1.1	9.6±5.2
Trifolium	36.9±19.4	2.3±1.2	26.7	0.5±0.3	6.6±2.5
Rosaceae	27.4±24.4	16.0±4.6	33.3	61.6±20.0	37.5±12.2
Vaccinium	2.9±2.9	0.6±0.4	21.8	0.9±0.9	
Taraxacum				0.9±0.4	
Fagopyrum	3.9±1.7				
Other pollen groups	11.0 ±1.9	4.4±1.2	4.0	6.1±2.3	6.2±1.5
Honeydew elements	0.1±0.1		1.0	0.2±0.2	31.8±13.6

than in other honey varieties (Table 5). This is in agreement with the results obtained from Polish buckwheat honey (Kaskoniene *et al.,* 2009; Socha *et al.,* 2011). The vanillic acid, p-coumaric acid and naringenin that were found have also been found in buckwheat honey from the USA and Poland (Gheldof *et al.,* 2002;

Biesaga & Pyrzynska, 2009). In Finland production of buckwheat honey is quite low, but there may be a potential demand for this unifloral honey, since it has been discovered that buckwheat honey given before bedtime provides better relief of night-time coughing and sleep difficulty in children (Paul *et al.*, 2007). Moreover it has been shown to have antioxidant properties (Gheldof *et al.*, 2002; Socha *et al.*, 2011).

Table 4. Amounts of phenolic compounds in buckwheat, willow and heather honeys (μg/g)

	Phenolic compound	**Buckwheat**	**Willow**	**Heather**
Rt	**Name**	**μg/g**	**μg/g**	**μg/g**
3.1	Cinnamic acid der 1		0.04	
4.0	Protochateuic acid	0.68	0.20	
7.5	Cinnamic acid der 2	0.56		
8.5	Vanillic acid	0.39	1.07	
10.4	Chlorogenic acid der 1	1.21	0.55	
13.3	ρ-OH-cinnamic acid der 1	2.23	1.30	1.93
13.5	Benzoic-acid	3.67	1.18	24.64
14.4	Ferulic acid		1.14	
16.2	Tetragalloylglucose	3.39	0.78	0.85
22.4	Cinnamic acid der 3		0.17	3.70
23.4	Unknown	tr	tr	tr
25.4	Cinnamic acid der 4		0.42	
27.4	Kaempferol 3-O-rhamnoside	2.21	0.46	
29.1	Rhamnetin der 1	0.70	0.13	
30.5	Luteolin	0.69		
31.3	p-OH-cinnamicacid der 2	0.24		
33.2	Galangin der 1	1.24	0.16	
33.8	Galangin der 2	0.76	0.21	
34.4	Apigenin	0.31	0.03	
34.5	Naringenin der	1.30		
34.7	Rhamnetin der 2		tr	
35.5	Cinnamic acid der 5	0.24		
38.5	Methyl-naringenin	2.22	4.09	0.90
40.1	Chlorogenic acid der 2		0.05	
43.4	Chrorogenic acid der 3	0.96		
43.6	Acacetin der	tr		
45.6	Chlorogenic acid der 4	0.15	0.04	

Table 5. Total phenolic content of seven unifloral honeys (mg/g)

	Total amount of phenolic compounds	Amount of phenolic acids	Amount of flavonoids	Ratio phenolic acids/ flavonoids
Buckwheat	23.16	13.71	9.45	1.45
Willow	12.02	6.9	5.08	1.36
Heather	32.03	31.13	0.9	34.58
Lingonberry *	11.22	11.08	0.57	19.49
Raspberry**	10.48	5.93	2.62	2.26
Fireweed**	7.58	3.56	4.01	0.89
Mire *	10.06	9.61	0.63	15.25

**Reference: article IV.*

***Reference: article III*

3.1.2 Willow honey

As suggested by Crane *et al*. (1984), beekeepers must build strong colonies in order to be able to collect willow honey, because willows bloom early in the spring. For this reason, beekeepers generally do not harvest willow honey in Finland. Unlike many other European countries, in Finland there are no observations on bees collecting honeydew honey from willows (Jerković *et al.,* 2010). The willow honey samples analysed here had the same colour and taste as described by Crane *et al.* (1984): golden yellow with a mild and distinctive flavour. Kaskoniene *et al.* (2010) have studied Lithuanian willow honeys, finding electrical conductivity of 0.39-0.89 mS/cm and an F/G ratio of 0.78-1.10. The values for electrical conductivity in the willow honey samples of this study were slightly lower (0.21-0.42 mS/cm) and for F/G ratios higher (Table 2; Appendix 2). The carbohydrate content of the willow honey samples was totally different from that of Lithuanian willow honey; Finnish samples are rich in fructose while the glucose content is lower (Appendix 2), whereas in Lithuanian willow honey this relation is reversed (Kaskoniene *et al.,* 2010). The willow honey samples in this study contained all disaccharides, the amount of turanose being the most abundant and the highest of all honey varieties (Appendix 3). Oligosaccharide melezitose/erlose and traces of raffinose and panose were found in willow honey (Appendix 3). These findings agree with the results obtained from Lithuanian willow honeys (Kaskoniene *et al.* 2010).

Melissopalynological analyses of willow honey revealed that percentage of *Salix* pollen was nearly 70% and of Rosaceae pollen 16% (Table 3). Due to the structure of the *Salix* flower, its pollen is over-represented in honey.

In one willow honey sample in this study twenty phenolic compounds were found, the most abundant ones being flavonoid naringenin derivative and vanillic, p-coumaric, benzoic and ferulic acids (Table 4). Similarly, Baltrusaityte *et al.*, (2007) found p-coumaric acid, kaempferol, chrysin and apigenin in Lithuanian willow honey. In willow honey the total amount of phenolics is only about a half of that in buckwheat or heather honeys (Table 5). In some studies willow honey has been shown to have antioxidant properties, possibly based on these phenolics (Baltrusaityte *et al.*, 2007; Tuberoso *et al.*, 2011).

3.1.3 Heather honey

Heather honey is produced abundantly all around Europe. According to Persano Oddo & Piro (2004) the most unique characteristic of heather honey is that its physical state is thixotrophy, which is caused by the colloidal proteins. The consistency of the honey is jelly-like with a multitude of tiny air bubbles trapped in the honey. It is very difficult to extract heather honey from honey combs. The colour of heather honey is normal honey colour with a reddish tone, and the taste is described as floral, fresh fruit and warm (Persano Oddo & Piro, 2004). Finnish heather honey samples were clearly darker than e.g. fireweed or raspberry honey samples. On account of the special physical composition, a higher water content is allowed in heather honey; the EU value for the maximum water content of heather honey is 23% and its electrical conductivity is permitted to be over 0.8 mS/cm, although it is not a honeydew honey (European Commission, 2002). In the heather honey samples analysed in this study the averages for these values were 18% and 0.67 mS/cm, and the pH was as high as 4.4 (Table 2). In European unifloral studies the values of water content, electrical conductivity and pH of heather honey were reported to be 18.5-18.8%, 0.73-0.83 mS/cm and 4.1-4.5, respectively (Persano Oddo & Piro, 2004; Smanalieva & Senge, 2009; Ruoff, 2006). The diastase number for heather honeys in European research ranged from 12 to 36 (Persano Oddo & Piro, 2004) and in this study from 9.2 to 17.4 (Table 2).

In Finnish heather honey samples the variation in fructose and glucose content was quite broad (Appendix 2) as was also found in Swiss heather honey samples (Ruoff, 2006). The F/G and G/W ratios (Appendix 2) suggest that this honey crystallises slowly, and when it does, it forms large crystals (Crane *et al.,* 1984).

For melissopalynology analyses we had only one heather honey sample, which contained 22% of *Vaccinium* pollen grains. Other pollen groups were Rosaceae, *Trifolium* and *Salix* (Table 3). The sample also contained some honeydew elements. In European unifloral studies, too, the amount of *Vaccinium* pollen grains in heather honey has been under 45% (Persano Oddo & Piro, 2004).

Phenolic compounds were analysed from one heather honey sample, and these data are rather unusual: the amount of benzoic acid is very high, and in addition only five other compounds were found (Table 4). In heather honey samples from Bulgaria large amounts of benzoic acid were also measured in addition to smaller amounts of vanillic, ferulic and protocatechuic acids (Dimitrova *et al.,* 2007), whereas wider spectra of other phenolic compound were found in the heather honey from Lithuania (Kaskoniene *et al.,* 2009), Portugal (Ferreres *et al.,* 1994; Andrade *et al.*, 1997) and Bulgaria (Dimitrova *et al.,* 2007).

Amino acids analyses revealed that heather honey has high amounts of glutamine and glutamic acid. The amino acid composition of Finnish heather honey (Appendix 5) resembles that of Estonian heather honey, and the quantitative differences are minor, excluding proline content, which is higher in Finnish heather honey (Rebane & Herodes, 2008). This difference in the proline content is in agreement with the statement that geographical origin may affect the amino acid content of unifloral honeys (Davis & Harris, 1982). Generally, heather honey has a well-known reputation as a remedy for many ailments, probably due to its rich mineral content (Dezmirean *et al*., 2010).

3.1.4 Lingonberry honey

Although the lingonberry blooms more or less intensively every year, it seems that their nectar production varies greatly from year to year and the yield of lingonberry honey depends greatly on the weather conditions in June. Lingonberry honey is reddish and its medium odour is described as “resinous, dry hay, toffee, fresh, orange, cedar, pungent and apricot”. Its acidic taste is medium with descriptions “toffee, citrus fruit, candied fruits, fruits and exotic fruit”. The electrical conductivity, pH and invertase activity values of lingonberry honey are markedly higher than those of other Finnish unifloral honeys excluding mire honey (Appendix 1). Lingonberry honey is rich in fructose and its F/G ratio is high and G/W ratio low (Appendix 2), indicating that this honey granulates slowly. All the samples contained disaccharides sucrose, turanose, maltose/trehalose and isomaltose and oligosaccharides melezitose/erlose (IV; Appendix 3).

Nearly half of the pollen grains in the lingonberry honey samples were from *Vaccinium* species (*V. vitis-idaea*, *V. myrtillus* L. and *V. oxycoccus* L.). Other pollen grains typical for the samples of this honey were from Rosaceae and *Trifolium* as well as *Salix,* Apiaceae species and *Geranium sylvaticum* L. (IV), whose pollen grains are found in higher amounts than in Finnish polyfloral honeys (I), since these species flower at the same time as lingonberries.

Eighteen different phenolic compounds were identified in the samples of lingonberry honey: seven cinnamic acid derivatives and flavonoids, benzoic, vanillic and protocatechuic acids and tetragalloylglucose (Appendix 4). The most abundant were vanillic and benzoic acids and the amount of vanillic acid is the highest among unifloral honey varieties (IV). The amount of flavonoids was very low, only 0.57 μg/g, and the ratio of phenolic acids to flavonoids was 19.5 (Table 5), which is much higher than in other unifloral honeys. A phenolic compound typical for lingonberry honey samples was the *p*-OH-cinnamic acid derivative 3 (Rt 23.9) which is not found in other Finnish unifloral honey samples (Appendix 4).

Generally, in the analyses of free amino acids from the unifloral honeys in this study, methionine, tryptophan and ornithine were not present, which is in agreement with the results from Estonia (Rebane & Herodes, 2008), where methionine was absent, although tryptophan and ornithine were found in very low amounts. All these amino acids have been found in French and Spanish honeys (Cotte *et al.*, 2004; Hermosin *et al.*, 2003). The spectrum of amino acids in lingonberry honey is the same as that detected in other honey samples and the total amount of amino acids is the lowest (Appendix 5). Amounts of asparagine and glutamic acid are the most abundant in lingonberry honey (Apendix 5).

3.1.5 Raspberry honey

Most of the honey collected in Central Finland is raspberry honey (I). The colour of raspberry honey was determined using the PerkinElmer Lambda 1050 spectrophotometer (III). It was observed that raspberry is more chromatic (i.e. a* and b* values are higher) than fireweed honey. The colour of the raspberry honey was close to that of Finnish polyfloral honey (III). In sensory analyses the weak odour of raspberry honey was described as "fruity, floral, pear apple, beeswax, candied fruits, hyacinth and subtle". Tasting assessments indicated that this honey with medium sweetness, weak acidity and bitterness and weak taste was described as "vanilla, floral, citrus, pear apple, candied fruits and even leafy wood" (III). Raspberry honey has low electrical conductivity values (Appendix 1), lower than that of rape honey (Ruoff, 2003) or Finnish polyfloral honey (III). The diastase number is also low. Amounts of fructose and glucose were highest in raspberry honey samples (Appendix 2). Its F/G ratio is high and the G/W ratio is over 2.1 (Appendix 2), indicating that raspberry honey crystallises rather quickly. The quantity of pollen grains may also affect the crystallisation of raspberry honey (III). All samples contained disaccharides sucrose, turanose, maltose/trehalose, isomaltose and oligosaccharide melezitose/erlose (III). These results are in line with the findings of Mauritzio (1964), who found low quantities of disaccharides and hardly any oligosaccharides in raspberry honey.

Melissopalynological analyses revealed that in raspberry honey samples more than 70% of the counted pollen grains belong to the *Rubus* species (*R. ideaus*, wild or cultivated, *R. arcticus* L. and *R. saxatilis* L.), while all other pollen groups had percentages of less than 5% (III). Pollen grains of *Rubus* are found in almost every Finnish pollen sample (I).

Twenty-five different phenolic compounds were identified in raspberry honey: six cinnamic acid derivatives, two chlorogenic acid derivatives, caffeic acid phenethyl ester (CAPE), benzoic, vanillic, ellagic and protocatechuic acids, tetragalloylglucose, cis-stillbene and twelve flavonoids or their derivatives (Appendix 4). *p*-OH-cinnamic acid derivative 1 and ferulic acid showed the greatest amounts (III). The total amount of phenolic compounds, phenolic acids and flavonoids were 10.48, 5.93 and 2.62 μg/g of honey, respectively, and the ratio of phenolic acids to flavonoids was 2.26 (Table 5). Ferreres *et al*. (1996) suggested that ellagic acid could be one biochemical marker for heather honey; however, it was also found in the raspberry and fireweed honey samples in this study (III). According to the results it seems that there is no phenolic compound that could be confirmed as a botanical marker of raspberry honeys (Appendix 4).

The most abundant free amino acid in raspberry honey was glutamine. The amounts and quantities of other amino acids in raspberry honey resemble those of fireweed honey (Appendix 5), but PCA did not form a clear group for raspberry honey samples (Fig 1).

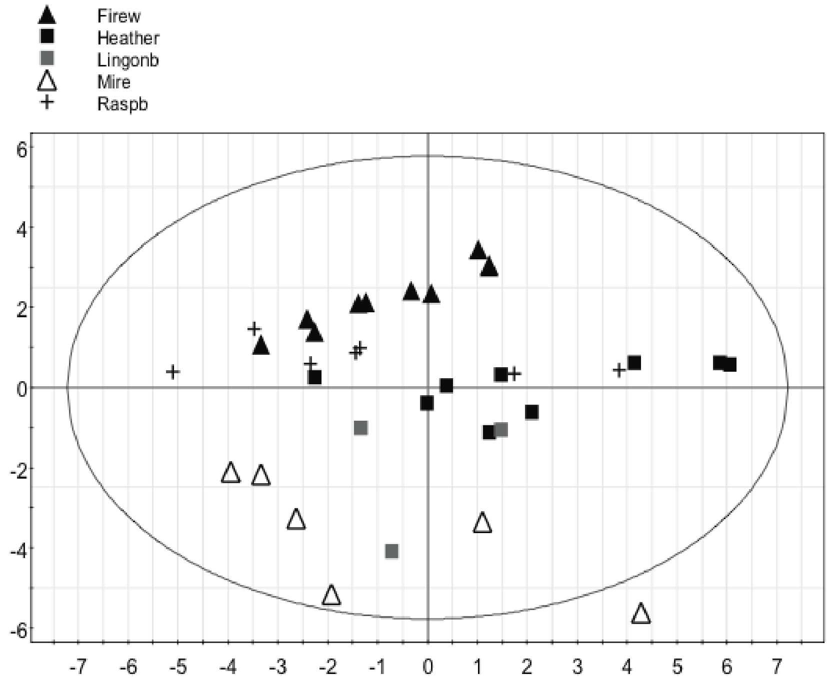

Figure 1. SIMCA-P +12.0.1 (Umetrics AB, Sweden) was used in the principal component analysis (PCA) of the results of amino acids. PCA1 (X) explains the largest variation (41%) of the space and PCA2 (Y) explains 26% of the variation of the space.

3.1.6 Fireweed honey

3.1.6.1 Nectar as a raw material of fireweed honey

Nectar is the ultimate raw material of honey. Twelve nectar samples from fireweed (*E. angustifolium*) were collected in summer 2009. In parallel it was observed from a pollen trap in a hive near the growing place of the plants that bees actually did visit *Epilobium* flowers.

The total brix value from nectar samples varied from 12 to 15%. The most abundant carbohydrates were fructose (43%), glucose (30%) and sucrose (22%). Nectar di- and oligosaccharides were turanose, isomaltose, melezitose/erlose and raffinose (Table 6). Bees add invertase enzyme to nectar while transporting it from flowers to the hive and storing it in honey combs in the hive, while invertase inverts sucrose to fructose and glucose during the ripening of the honey (Elton, 2005). This explains the lower amount of sucrose in honey than in nectar.

Table 6. Carbohydrate content of nectar and honey (% of all saccharides)

Carbohydrate	Nectar	Honey
Fructose	43.2	52.3
Glucose	30.4	38.6
Sucrose	21.8	1.7
Turanose	1.4	2.1
Maltose /Trehalose	0	1.06
Isomaltose	2.7	3.6
Gentiobiose	0	0
Melezitose / Erlose	0.2	0.6
Raffinose	0.2	tr
Panose	tr	tr

Twenty-one phenolic compounds were identified from the nectar samples (Table 7; Appendix 4): seven flavonoid derivatives, gallic, protocatechuic, chlorogenic and ellagic acid and ten ellagitannin derivatives. Only five of these can be found in fireweed unifloral honey: chlorogenic acid, ellagic acid, quercetin 3-*O*-rhamnosid and kaempferol 3-*O*-rhamnoside (Fig 2; Appendix 4). Apart from phenolic compounds, L-tryptophan (rt 4.2) and one unidentified compound (a peak with rt 23.4) that exists in all unifloral honey samples were found. L-tryptophan is generally found in pollen (Zhang *et al*., 2009), but it was not found in fireweed or any other unifloral honeys in amino acid analyses. The honey samples were filtered through cotton fabric before separation in amberlite resin, which may have removed most of the pollen grains and thus the content of L-tryptophan may be under detection limits.

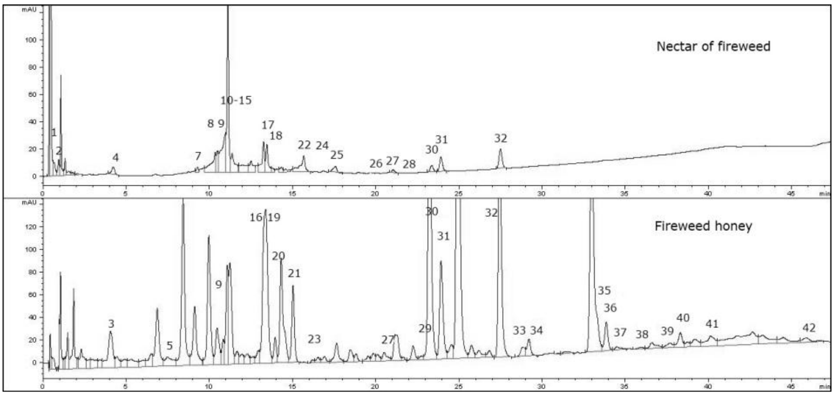

*Fig 2. HPLC-chromatograms (wavelength 220 nm) of nectar of fireweed and fireweed honey). Peak identifications: **1.** Gallic acid, **2.** Protocatechuic acid, **3.** Protocatechuic acid der, **4.** L-tryptophan, **5.** Cinnamic acid der 2, **6.** Vanillic acid, **7.** Ellagitannin der 1, **8.** Ellagitannin der 2, **9**. Chlorogenic acid der 1, **11-15.** Ellagitannin derivatives 3-8, **16.** p-OH-cinnamic acid der 1, **17.** Ellagitannin der 9, **18.** Flavonoid der 1, **19.** Benzoic acid, **20.** 4-hydroxy-3-methoxy cinnamic acid, **21.** Methyl-cinnamic acid der, **22.** Ellagitannin der 10, **23.** Tetragalloylglucose, **24.** Kaempferol glycoside 1, **25.** Kaempferol glycoside 2, **26.** Hyperin, **27.** Ellagic acid, **28.** Isoquercitrin der, **29.** Cinnamic acid der 3, **30.** Unknown, **31.** Quercetin 3-O-rhamnoside,**32.** Kaempferol 3-O-rhamnoside, **33.** Flavonoid der 2, **34.** Rhamnetin der 1, **35.** Galangin der 1, **36.** Galangin der 2, **37.** Apigenin, **38.** ρ-OH-cinnamic acid der 5, **39.** Kaempferol der, **40.** Methyl-naringenin, **41.** Chlorogenic acid der 3, **42.** CAPE. (der = derivative)*

Table 7. Phenolic content of 12 nectar samples (μg/ml)

Rt	Phenolic compound	mean ± s.e.
1.7	Gallic acid	tr
2.7	Protocatechuic acid	tr
9.3	Ellagitannin der 1	1.15 ± 0.14
10.0	Chlorogenic acid der 1	1.46 ± 0.36
10.3	Ellagitannin der 2	1.33 ± 0.22
10.5	Ellagitannin der 3	1.17 ± 0.16
10.8	Ellagitannin der 4	1.68 ± 0.39
11.1	Ellagitannin der 5	2.45 ± 0.63
11.4	Ellagitannin der 6	0.90 ± 0.15
12.5	Ellagitannin der 7	tr
13.2	Ellagitannin der 8	tr
13.4	Ellagitannin der 9	tr

13.5	flavonoid der	0.99 ± 0.08
15.6	Ellagitannin der 10	tr
16.8	Kaempferol glycoside 1	0.23 ± 0.03
17.5	Kaempferol glycoside 2	0.58 ± 0.09
20.6	Hyperin	tr
20.8	Ellagic acid	tr
21.0	Isoquercitrin der	1.36 ± 0.19
23.9	Quercetin 3-O-rhamnoside	4.51 ± 0.49
27.4	Kaempferol 3-O-rhamnoside	4.89 ± 0.54

3.1.6.2 Properties of fireweed honey

Because of its tendency to remain in a non-crystallised state, fireweed honey is highly valued by Finnish honey-buying customers. Sensory assessments and colour analysis confirmed that fireweed honey has very light colour intensity and a water-like colour tone (III). Its weak odour was described as "dry hay, weak pale malt, exotic fruit and fruit (apple)". Its sweetness was between medium and strong, and acidity as well as bitterness were weak, although the pH of fireweed honey is the lowest of the honeys studied (Appendix 1). Descriptions of the taste were "floral, exotic fruit, floral, brown sugar, a trace of grapefruit and almond paste". Like raspberry honey, fireweed honey also presents low electrical conductivity values and low diastase number. High fructose content and low F/G and G/W ratios (Appendix 2) show that pure fireweed honey crystallises very slowly and remains in a liquid, non-crystallised state for a long time. The highest sucrose and isomaltose contents were found in fireweed honey (Appendix 3). The oligosaccharide content consists of small amounts of melezitose/erlose and traces of raffinose and panose.

Pollen grains from *Epilobium* are known to be highly underrepresented in honey samples (Bryant and Jones (2001). In the fireweed honey samples of this study the average amount of *Epilobium* pollen grains was only 3.3%, while 44.5% of the pollen grains originated from Rosaceae species and 18.4% from *Trifolium* species (III). This indicates that pollen content is not the discriminating factor for fireweed honey.

The phenolics content of fireweed honey is presented in Appendix 4. The amount of kaempferol 3-O-rhamnoside was clearly greater than that in other unifloral honeys (III). The total amount of phenolic compounds (phenolic acids 3.56 μg/g and flavonoids 4.0 μg/g) in fireweed honey is 7.58 μg/g of honey, and the ratio of phenolic acids to flavonoids was much lower (0.89) than that found in other honey varieties (Table 5). Gheldof *et al*. (2002) have reported a different phenolic content in fireweed honey samples from North-America. Their samples contained *p*-hydroxybenzoic acid, *p*-coumaric acid, cinnamic acid, pinobanksin, pinocembrin and chrysin. Kaempferol 3-*O*-rhamnoside or quercetin 3-*O*-rhamnoside were not reported in their fireweed honey, although these compounds obviously are plant derived compounds (Table 7). However, Gheldof *et al*. (2002) found antioxidant properties in fireweed honey.

Amino acid analyses indicated that the amount of leucine is highest in fireweed honey, while the amount of histadine is two times higher than in other unifloral honey samples. On the other hand, the amount of asparagine is much lower than in other honey varieties. The amounts of amino acid in fireweed honey resemble those of raspberry honey (Appendix 5). In PCA amino acid contents of fireweed honey samples formed a clear group (Fig 1).

3.1.7 Dandelion honey

The production of dandelion honey is subject to the same problems as willow honey: it blooms early in the summer, and if the weather conditions are not favourable, bees need honey collected from dandelions to support their colony. Nevertheless, dandelion honey is produced in many European countries. Its colour is bright yellow and the taste intense and pungent (Persano Oddo *et al.*, 1995). The water content, electrical conductivity and pH of the dandelion honey samples in this study were 16.4%, 0.24 mS/cm and 4.2 respectively (Table 2; Appendix 1). Corresponding values in European dandelion honeys were 16–16.9%, 0.48–0.52 mS/cm and 4.5, respectively (Persano Oddo *et al.*, 1995; Persano Oddo & Piro, 2004; Ruoff, 2006). Dandelion honey is mentioned in the group that has a naturally low diastase number (Persano Oddo & Piro, 2004). The diastase number obtained for dandelion honey in this study varied from 6 to 14 (Table 2).

Although the glucose content of Finnish dandelion honey samples was the highest (Appendix 2), they contain more fructose than glucose, whereas in other studies their amounts were either equal (Ruoff, 2006) or the glucose content was higher (Persano Oddo *et al.,* 1995; Persano Oddo & Piro, 2004). Di- and oligosaccharides were analysed only from one dandelion honey sample, indicating the absence of sucrose and high amounts of turanose, isomaltose and maltose/trehalose and very low amounts of oligosaccharides (Appendix 3). The F/G (1.0) and G/W (2.3) ratios are equal in all European studies (Persano Oddo *et al.,* 1995; Persano Oddo & Piro, 2004; Ruoff, 2006), but in Finnish samples the F/G ratio was higher (1.2) and the G/W ratio (2.0) lower (Appendix 2). In any case it is generally known that dandelion honey crystallises quickly and becomes very hard (Crane *et al.,* 1984), and this is known to happen with Finnish dandelion honey, too. Similarly, Finnish unifloral rape honey crystallises as fast as dandelion honey, while its F/G ratio is between 0.9 and 1.2 and G/W ratio between 1.8 and 2.2 (Ruoff, 2003).

Melissopalynological analysis of the dandelion honey samples of this study indicated that the most abundant pollen groups in dandelion honey are Rosaceae and *Salix*. The percentage of *Taraxacum* pollen was only one per cent (Table 3). Nevertheless, all dandelion honey samples had the typical sensory characteristics of dandelion honey. Future studies on dandelion honey could be rewarding, while the use of dandelion honey has been shown to have some health effects, e.g. reduced gastric juice acidity (Bogdanov *et al.,* 2008).

3.1.8 Mire honey

The source of honey may be the mixed flora of a specific biotope in the foraging area of the beehive, and in some cases this kind of honey may have a unique organoleptic character due to the amount and combination of various components typical of the honey. This type of honey can be called regional or biotope honey (European Commission, 2002). The honey that bees collect from the plants growing on the mire biotope is called mire honey. When compared with other Finnish poly- and unifloral honeys, it has a very peculiar and strong odour and taste resembling, for example, chestnut, lime or heather honeys (e.g. Tomás-Barberán *et al.*, 2001), and it is highly valued in Finland. In sensory analyses, mire honey showed a reddish colour tone. The intensity of the medium odour of mire honey was described as "straw, dry hay, mint, solvent, fresh, orange blossom". Tasting assessments varied greatly: sweetness, acidity, bitterness and aroma were evaluated with all the scores (0–3) and the taste was described as "refreshing, apricot, solvent, aniseed, eucalyptus, dates prunes and fruit" (IV).

The electrical conductivity of mire honey is the highest compared with polyfloral (VI) or unifloral honeys (Appendix 1) collected in Finland, which is in disagreement with the EU's honey directive (European Commission, 2002). All the mire honey samples had values over 0.9 mS/cm, although mire honey is assumed to be nectar honey. This strongly indicates that mire honey could be honeydew honey. However, mire honey is collected at the beginning of summer, and generally it is assumed that in Finnish weather conditions honeydew honey is not collected before August. The water content of mire honey is low and its invertase activity value high (IV). Interestingly, the pH is very high (4.7-4.9) (Appendix 1), although in a mire biotope the substrate is acidic (e.g. Kaakinen *et al.,* 2008). All mire honey samples contained the disaccharides sucrose, turanose, very large amounts of maltose/trehalose and isomaltose as well as oligosaccharides raffinose in trace amounts and melezitose/erlose (Appendix 3).

Amiot *et al.* (1989) observed that dark-coloured honeys contain more phenolic compounds than light-coloured honeys. It was expected that mire honey could contain abundant amounts of phenolics, which might also contribute to some extent to the special organoleptic characteristics of the honey, but the findings of this study did not support these expectations. The total amount of phenolic compounds is not high (Table 5), and only fourteen phenolic compounds could be identified in the mire honey samples: four cinnamic acid derivatives and flavonoids, benzoic, vanillic and protocatechuic acids, chlorogenic acid derivative, benzoic acid derivative and tetragalloylglucose (Appendix 4). Benzoic acid and *p*-OH-cinnamic acid derivative 1 were found in the highest amounts (IV). The total amount of flavonoids is very low (0.63 μg/g), which means that the ratio of phenolic acids to flavonoids is 15.25, much higher than in other unifloral honeys (table 5). Mire honey has no unifloral specific phenolic compounds (IV).

Melissopalynological analyses showed that 44% of the pollen grains in the mire honey originated from the *Vaccinium* family, mostly from *V. myrtillus* L. and *V. vitis-idaea* and only a few from *Vaccinium uliginosum* L. and *V. oxycoccos* L. (IV), which commonly grow on mires (e.g. Lampinen & Lahti, 2010). Other pollen groups were *Salix*, Rosaceae, Apiaceae and *Trifolium,* plant species from mire biotopes, *Rubus chamaemorus* L. and *Menyanthes trifoliata* L. Mire honey's commercial name is "Cloudberry honey" or "Honey from cloudberry mire", but according to the results of melissopalynological analyses, the amount of *R. chamaemorus* pollen grains is very moderate in cloudberry honey, so it is unlikely that *R. chamaemorus* is the main source of nectar for mire honey (IV).

In free amino acids analyses mire honey indicated high amounts of proline, glutamine and aspargine, and the total amount of the amino acids was also the highest compared with other unifloral honeys (Appendix 5). In PCA amino acid contents of mire honey samples formed a clear group (Fig 1).

3.1.9 Honeydew honey

Honeydew honey is a honey that bees produce from the excretions of plant sucking insects (*Hemiptera*) or the secretions of living part of plants. In Europe, honeydew honey is collected from various plant sources (both Coniferae and Latifoliae), and this unifloral honey variety is highly valued by customers (Persano Oddo *et al.*, 2004). In Finland there is very little information on the honeydew of plants or honeydew-inducing insects. Honeydew honey is sold occasionally, but the only criteria distinguishing honeydew honeys from other honey varieties have been their special taste and the harvesting time, which usually is late in August. The taste of honeydew honey is probably derived from its different oligosaccharide contents.

Seven honeydew honey samples, which were selected by beekeepers as honeydew honey, were analysed. Their colour varied from golden brown, orange and dark brown and most of them crystallised quite slowly (Appendix 2). The EU value for the electrical conductivity of a honeydew honey is at minimum 0.8 mS/cm. The electrical conductivity value was over 0.8 mS/cm in only one honeydew sample. The average was 0.51 mS/cm and the range from 0.32 to 0.8 mS/cm. In this study the amount of fructose was lowest in honeydew honey (Appendix 2). The EU-value for the total content of fructose and glucose for honeydew honey is 45 g/100 g. In the honeydew honey samples in this study the range was 56–74 g/100g (Appendix 2). The analysed honeydew honey sample did not contain any sucrose or maltose/trehalose, but contained large amounts of turanose and isomaltose (Appendix 3). According to the literature, honeydew honey should contain raffinose and melezitose (Bogdanov *et al.*, 2004). In this study the amount of melezitose/erlose was highest in the honeydew honey samples, but raffinose was not found and panose was found only in trace amounts (Appendix 3).

In honeydew honey the ratio of the number of honeydew elements to the number of pollen grains should be over 3 (von der Ohe *et al.*, 2004). This limit was overstepped in only one of the honeydew honey samples in this study (Table 8). In four samples the number of honeydew elements was very low. The main pollen group for honeydew honeys samples was Rosaceae (Table 3).

Table 8. The ratio of the number of honeydew elements to the number of pollen grains in honeydew honey samples

Sample	Number of honeydew elements	Number of pollen grains	Ratio
700	3	446	0.01
701	9	481	0.02
702	78	418	0.2
703	291	147	2.0
704	446	120	3.7
706	133	357	0.4

3.2 PHENOLIC COMPOUNDS OF PROPOLIS IN UNIFLORAL HONEYS

Analyses of the phenolic content of propolis were needed because plant-derived phenolic compounds in honey originate from nectar or propolis. Finnish propolis proved to be different from *Populus* originating propolis from Middle Europe (II). The 26 identified phenolic compounds were nine cinnamic acid derivatives, three chlorogenic acid derivatives, caffeic acid derivative and caffeic acid phenethyl ester (CAPE), benzoic acid, one benzoic acid derivative and vanillic acid, as well as nine flavonoids (Appendix 4). The variation in the total amount of phenolic compounds in propolis samples was high, ranging from 79.8 to 156.3 μg/g, the average being 119.5 μg/g. Cinnamic acid derivatives and flavonoids comprised 36% and 26% of all phenolics, respectively. The biologically active component of propolis and the one promising health effects, CAPE (Russo, Longo & Vanella, 2002) was also found in large amounts (14% of all phenolics). Also methyl-naringenin, *p*-OH-cinnamic acid derivative 6 and benzoic acid were found in large quantities. Flavonoids acacetin and methyl-apigenin, were detected only in trace amounts in some samples (II).

Fourteen of the phenolics found in propolis were also found in unifloral honey samples (Appendix 4). *p*-OH-cinnamic acid derivative 1 (tentatively identified as *p*-coumaric acid) and benzoic acid were found in all the honey and propolis samples. Benzoic acid in honey samples may originate partly from propolis, but also partly from the amberlite resin used in the process of purification of honey samples. It is likely that compounds, such as cinnamic acid derivative 2, vanillic acid, feculic acid, cinnamic acid derivative 3, apigenin, methyl-naringenin and CAPE, which are found in honey samples, originate from propolis (II; Appendix 4). On the other hand, cinnamic acid derivative 1, protocatechuic acid, tetragalloyl-glucose, flavonoid derivatives 2, rhamnetin derivatives 1 and 2 and galangin derivatives 1 and 2 are found only in honey samples (Appenxid 4). In many Finnish unifloral honey varieties there are very few propolis-derived phenolics or flavonoids; lingonberry and mire honey, which are harvested in June, had only minor amounts of compounds originating from propolis, whereas raspberry and fireweed honeys contained more of them (Appendix 4). The reason for this is unknown.

3.3 TOOLS FOR DISCRIMINATION OF FINNISH UNIFLORAL HONEYS

One of the aims of this study was to find tools for the process of discriminating Finnish unifloral honeys. Suggestions for these tools are presented in Table 9.

The easiest way to start the discrimination process is sensorial observation. Generally, this is the most useful tool with all the unifloral honeys. The person who is conducting the observations must have enough experience in recognising unifloral honey varieties. However, the results of sensorial analyses should always be confirmed by melissopalynological and physico-chemical analysis. In this study, sensorial analyses worked very well with all honey varieties. Thus, it is not possible to discriminate raspberry honey only with the help of sensorial analyses.

In this study melissopalynological analysis was a reliable discrimination tool only for willow honey. When combined with sensorial analysis, logical results were also obtained for raspberry and lingonberry honeys. The pollen of fireweed is highly under-represented in fireweed honey and the pollen of raspberry is normally-represented (III). The amounts of *Vaccinium* pollen in lingonberry and mire honeys were almost the same (IV). The results of melissopalynological analyses of the other unifloral honey varieties are rather confusing (Table 3). For instance, the pollen grains of buckwheat and dandelion honeys seem to be under-represented in honey samples, although in many studies they have been normally-represented (Sawyer, 1988; Persano Oddo & Piro, 2004). More analytical samples are needed before drawing any future conclusions on the pollen content of Finnish buckwheat, heather, lingonberry, dandelion, mire or honeydew honeys. The short and intense growing season in Finland may cause differences in the pollen content of Finnish unifloral honeys when compared to the pollen content of honey samples from Europe.

Electrical conductivity is a usable discrimination tool with honeys having high mS/cm values, such as mire or honeydew (Appendix 1). Fireweed honey has low electrical conductivity, which is useful information when used with the results of organoleptical and pH analyses. High pH value is one recognition tool for mire honey (Fig 1). Invertase and diastase values differ greatly on the basis of the storing conditions and processing of the honey and they should not be used as discriminating tools.

Analysis of carbohydrates turned out not to be a very useful tool for discriminating Finnish unifloral honeys, because the differences in the content of individual carbohydrates are very low. However, F/G and G/W ratios may be useful in predicting the crystallisation tendency of honey (Appendix 2).

Analyses of phenolic compounds gave some tools for dis-

Table 9. Suggestions for tools that can be used in the process of discriminating between Finnish unifloral honeys

	Buckwheat	**Willow**	**Heather**	**Lingonberry**	**Raspberry**	**Fireweed**	**Mire**	**Dandelion**	**Honeydew**
water%			**x**						
electrical conductivity			**x**	**x**		**x**	**x**		**x**
pH						**x**	**x**		
fructose		**x**					**x**		
glucose				**x**	**x**			**x**	
disaccharides						**x**			
oligo-saccharides	**x**		**x**			**x**		**x**	**x**
melisso-palynological	**x**	**x**			**x**			**x**	**x**
phenolic compounds				**x**			**x**		
colour	**x**	**x**	**x**	**x**		**x**	**x**	**x**	**x**
taste	**x**		**x**	**x**		**x**	**x**	**x**	**x**
odour		**x**		**x**		**x**		**x**	
consistency			**x**					**x**	

criminating unifloral honeys. Heather, lingonberry and mire honeys had very low amounts of flavonoids. The total amount of all phenolic compounds was highest in buckwheat honey and lowest in fireweed honey (Table 5). A botanical phenolic marker was found only for lingonberry honey (VI). High vanillic acid content distinguishes lingonberry honey (IV), high tetragalloylglucose content is related to buckwheat honey (Table 4) and a large amount of methyl-naringenin can be found in willow honey (Table 4). The content of phenolic compounds was very similar in raspberry and fireweed honeys (III). The best discrimination tool is the ratio of phenolic acids to flavonoids (Table 5).

The results of PCA conducted for amino acids are presented in Fig 1. This grouping method could be a useful tool in discriminating some unifloral honeys. In this study PCA distinguished fireweed and heather from other honeys, obviously due to the high content of histidine in fireweed honey and serine and b-alanine in heather honey. For more reliable and extensive use of grouping analyses a larger set of individual samples would be needed. PCA could also be applied for other honey variables, such as phenolics.

4 Concluding remarks

In this thesis nine unifloral honeys collected in Finland were studied and their properties and composition analysed by traditional and newer methods. All the honeys have interesting sensorial properties. The unique borealis honeys mire and lingonberry honeys with their special odour and taste are appreciated by consumers and they are exported to European markets. Raspberry honey is the main honey variety produced in Central Finland, and as its taste is mild, it is good general honey. Fireweed honey is excellent honey for easy-to-use liquid honey packing, since it tends to stay in non-crystallised form for a long time. Buckwheat, heather, dandelion and honeydew honeys, which are collected all over Europe, are interesting honey varieties, each having special sensorial characters. In Finland they are produced by only a few beekeepers and thus are quite rare. Willow honey has a nice bright yellow colour and an interesting taste, but its production in Finland is quite impossible without special arrangements.

Some tools for the discrimination of Finnish unifloral honeys were found in this study. Concerning the results on physicochemical properties, the greatest variation was found in electrical conductivity. The carbohydrate content of the studied unifloral honeys was surprisingly similar, except for some variation in the di- and oligosaccharide contents. Melissopalynological analyses revealed some differences in pollen amounts compared to European unifloral honeys and some special features in the pollen content of fireweed honey, but they did not provide an answer to the question of the botanical origin of mire honey. The spectra of phenolic compounds are different in unifloral honey varieties, but a better discriminating tool is the ratio of phenolic acids to flavonoids. PCA of amino acids was also useable in distinguishing unifloral varieties.

On the basis of this study some concluding remarks can be made:

- An addition should be suggested to the Council Directive 74/409/EEC relating to honey. Mire honey should be added to the list of exceptions where those unifloral nectar honeys whose electrical conductivity is higher than 0.8 mS/cm are mentioned.
- The properties of honeydew honey collected in Finland differ greatly from those of European honeydew honeys. Its electrical conductivity is much lower than suggested in the EU directive, the average number of honeydew elements is lower than recommended and its origin is unknown. Finnish honeydew honey needs more research.
- It seems that raspberry and fireweed honeys might have naturally low diastase values. This issue needs more research since the EU's honey directive includes a list of exceptions for unifloral honeys having naturally low diastase values, and these honey varieties should be added to the list.
- The composition of Finnish propolis differs to a great extent from that of propolis from Central and Southern Europe. More research is needed, because Finnish propolis has high cinnamic acids content and it could be a potential promising source for biologically active compounds. It would also be very interesting to test the effect of Finnish propolis against e.g. microbes, cancer cells and HIV-virus
- Some honey samples in this study were obtained directly from beekeepers, who had evaluated the floral origin of the honey by means of hive location, season and available floral source. There are no strict characterisations for Finnish unifloral honeys, and beekeepers may easily mistake their honey with a distinctive taste for a unifloral honey, as they have not seen representative samples of this unifloral honey type. The consequence of this was that some honey samples in this study were not necessarily the best representative samples of their unifloral group. Anyhow, Finnish beekeepers are interested in unifloral honeys and training in the production of these honeys is needed.
- To confirm limits for the analytical parameters of different unifloral honeys, the limits should be defined in several honey la-

boratories from the same reference samples. In addition, a trained and experienced group of honey assessors is needed for official sensorial analyses.

- Collecting pure unifloral honeys is difficult, requires extra work and demands a great deal of knowledge about bees and their food plants. Considering these problems, higher prices are arguably justified. However, the higher price always offers an opportunity for malpractices. Because of this opportunity, characterisations and perhaps some kind of supervision for Finnish unifloral honeys are needed.
- The analytical data obtained in this research may be useful in future studies concerning the characterisation and properties of Finnish unifloral honeys and propolis, their antimicrobial properties, their use as sources for biologically active compounds or their impact on human health.

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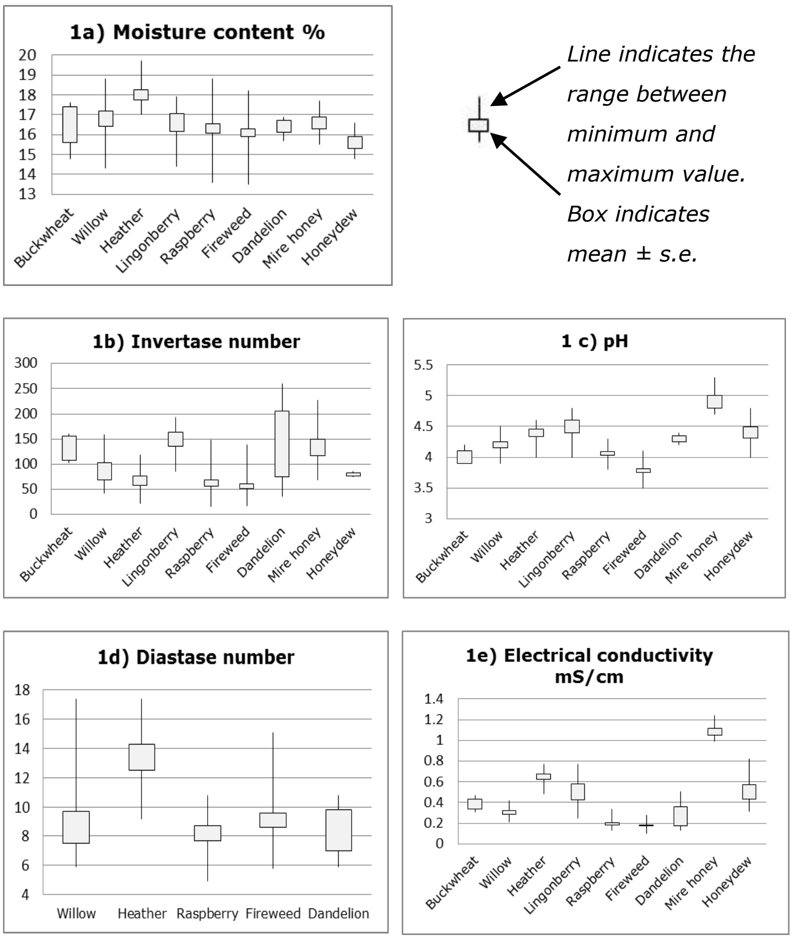
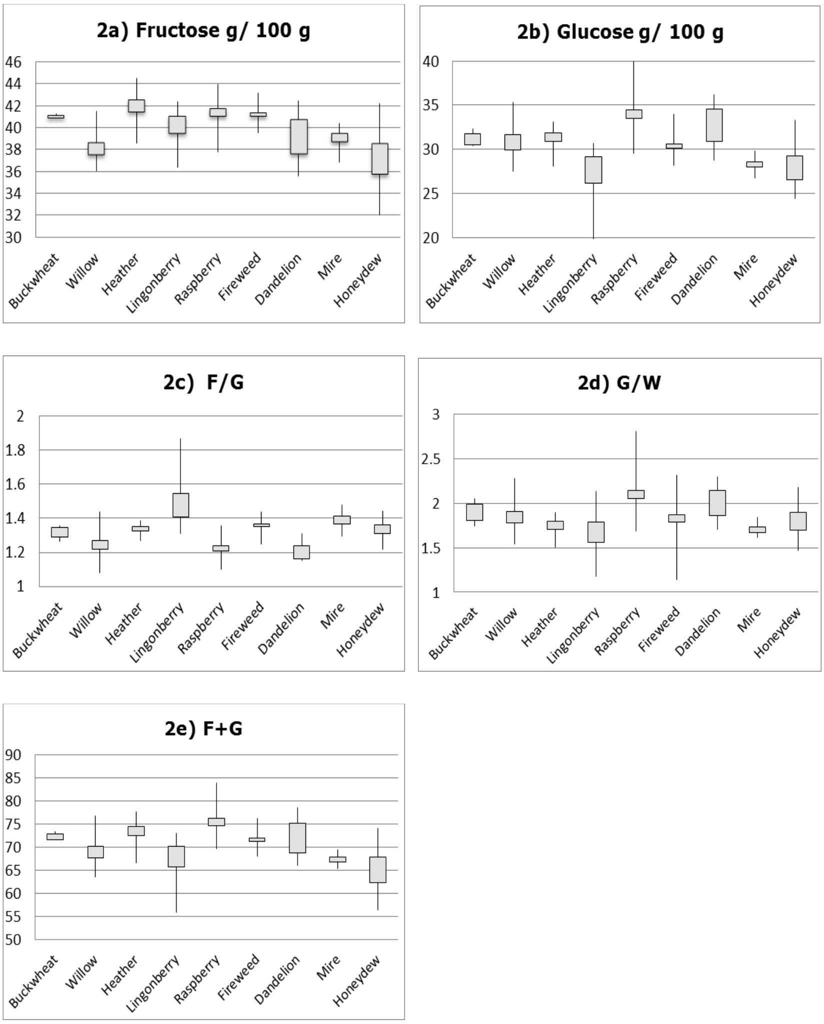
Appendix

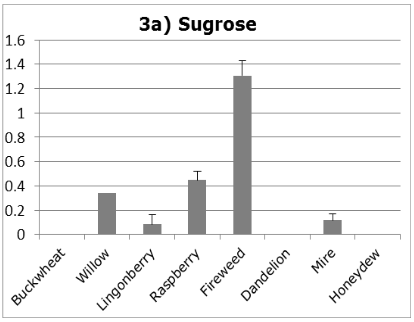
Appendix 1. Variability of the physico-chemical parameters in the uni-floral honey types studied.

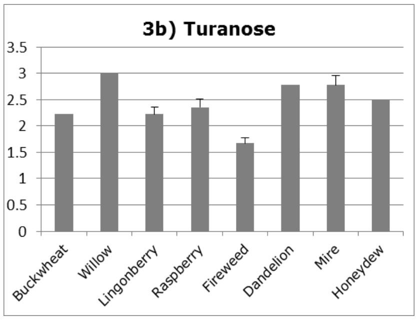


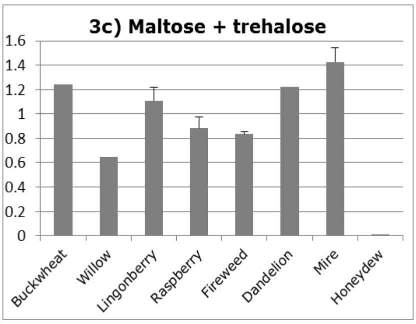
Appendix 2. Variability of the fructose, glucose and their parameters in the unifloral honey types studied.

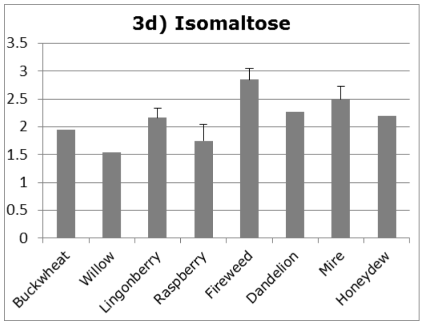


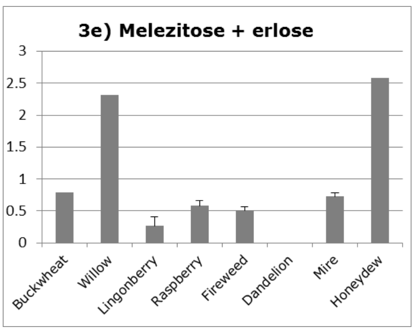
Appendix 3. Variability of the di- and oligosaccharides in 8 unifloral honey types studied (g/100 g, mean ± s.e.). In buckwheat, willow, dandelion and honeydew honeys analyses were conducted from only one sample.







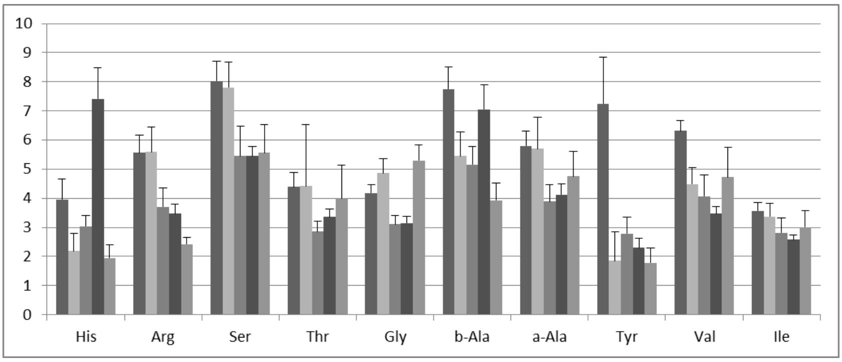


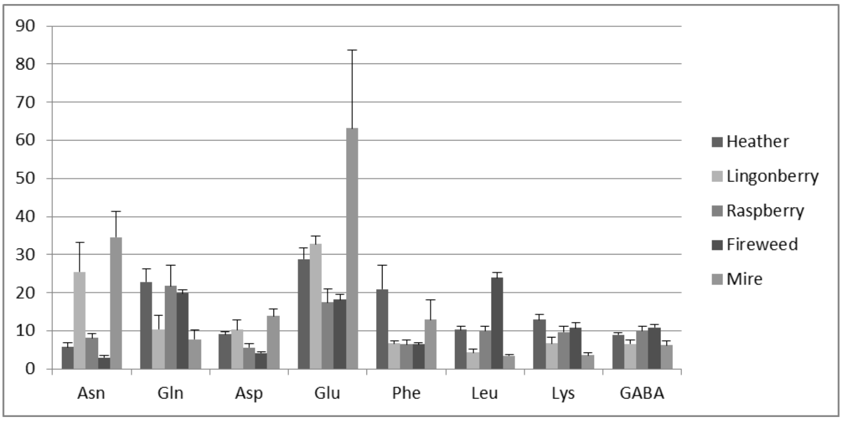


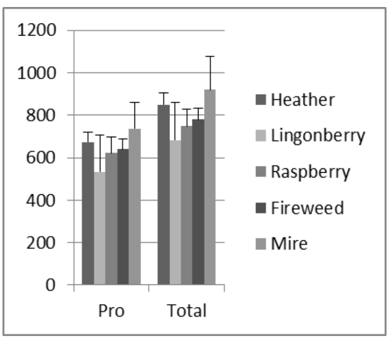
Appendix 4. Identification of 67 phenolics in honey, propolis and nectar samples.

		Phenolic compound										Identification	
	rt	**Name**	Buck wheat	Willow	Heather	Lingon berry	Rasp berry	Fire weed	Mire	Propolis	Nectar	**uv-spect rum**	**MS-ions**
1	1.7	Gallic acid									x	x	
2	2.7	Protocatechuic acid									x	x	
3	3.1	Cinnamic acid der 1		x		x						x	
4	4.0	Protocatechuic acid der	x	x		x	x	x	x			x	
5	7.5	Cinnamic acid der 2	x			x	x	x	x	x		x	
6	8.5	Vanillic acid	x	x		x	x	x	x	x		x	169 (M+H). 191 (M+Na)
7	9.3	Ellagitannin der 1									x	x	
8	10.3	Ellagitannin der 2									x	x	
9	10.4	Chlorogenic acid der 1	x	x			x	x	x	x	x	x	455
10	10.5	Ellagitannin der 3									x	x	
11	10.8	Ellagitannin der 4									x	x	
12	11.1	Ellagitannin der 5									x	x	
13	11.4	Ellagitannin der 6									x	x	
14	12.5	Ellagitannin der 7									x	x	
15	13.2	Ellagitannin der 8									x	x	
16	13.3	ρ-OH-cinnamic acid der 1 (ρ-coumaric acid)	x	x	x	x	x	x	x	x		x	165 (M+H)
17	13.4	Ellagitannin der 9									x	x	
18	13.5	Flavonoid der 1									x	x	
19	13.5	Benzoic acid	x	x	x	x	x	x	x	x		x	12 (M+H). 145(M+Na)
20	14.4	4-hydroxy-3-methoxy cinnamic acid (ferulic acid)		x		x	x	x	x	x		x	195(M+H). 217(M+Na)
21	14.6	Methyl-cinnamic acid der					x	x		x		x	179(M+H). 201(M+Na)
22	15.6	Ellagitannin der 10									x	x	
23	16.2	Tetragalloylglucose	x	x	x	x	x	x	x			x	
24	16.8	Kaempferol glycoside 1									x	x	
25	17.1	Benzoic acid der 1								x		x	
26	17.5	Kaempferol glycoside 2									x	x	
27	17.9	Benzoic acid der 2							x			x	
28	19.3	ρ-OH-cinnamic acid der 2								x		x	
29	20.6	Hyperin									x	x	
30	20.8	Ellagic acid					x	x			x	x	
31	21.0	Isoquercitrin der									x	x	
32	22.4	Cinnamic acid der 3		x	x	x	x	x	x	x		x	
33	23.4	Unknown	x	x	x	x	x	x	x		x	x	
34	23.9	Quercetin 3-O-rhamnoside					x	x			x	x	471 (M+H)
35	23.9	ρ-OH-cinnamic acid der 3				x						x	
36	25.4	Cinnamic acid der 4		x								x	
37	27.4	Kaempferol 3-O-rhamnoside	x	x		x	x	x	x		x	x	455 (M+H)
38	28.1	Flavonoid der 2				x	x	x				x	
39	29.1	Rhamnetin der 1	x	x			x	x	x			x	
40	29.2	Pinocembrin der 1								x		x	
41	30.4	Pinocembrin der 2								x		x	
42	30.5	Luteolin	x				x					x	
43	31.3	ρ-OH-cinnamic acid der 4	x			x						x	
44	32.2	Caffeic acid der								x		x	
45	33.2	Galangin der 1	x	x		x	x	x	x			x	
46	33.8	Galangin der 2	x	x		x	x	x				x	
47	34.4	Apigenin	x	x		x	x	x		x		x	271 (M+H)
48	34.5	Naringenin der	x									x	
49	34.7	Rhamnetin der 2		x			x					x	
50	35.5	Cinnamic acid der 5	x									x	
51	35.8	ρ-OH-cinnamic acid der 5					x	x		x		x	355. 179
52	37.4	Kaempferol der						x				x	
53	38.5	Methyl-naringenin	x	x	x	x	x	x	x	x		x	287 (M+H)
54	39.9	Chlorogenic acid der 2								x		x	
55	40.1	Chlorogenic acid der 3		x			x	x				x	
56	42.9	Acacetin								x		x	
57	43.4	Chrorogenic acid der 4	x							x		x	449
58	43.6	Acacetin der	x			x						x	
59	43.9	ρ-OH-cinnamic acid der 6		x						x		x	509
60	43.9	cis-stilbene					x					x	
61	45.6	Chlorogenic acid der 5	x	x								x	
62	46.1	Caffeic acid phenethyl ester					x	x		x		x	307 (M+Na)
63	46.7	Pinobanksin								x		x	295 (M+Na)
64	46.7	Di-methyl-kaempferol								x		x	315(M+H). 337(M+Na)
65	47.3	Methyl-apigenin								x		x	285 (M+H)
66	53.4	Naringenin der 2								x		x	
67	70.9	Cinnamic acid der 6								x		x	

Appendix 5. Average amounts of individual amino acids in heather, lingonberry, raspberry, fireweed and mire honeys (mean ± s.e.; *g/kg*).







Anneli Salonen

Boreal unifloral honeys: Screening of composition and properties

The aim of this thesis was to investigate the physico-chemical, organoleptic and melissopalynological characterisations of Finnish unifloral honeys. The main interest laid in those unifloral honeys which are typical for the boreal coniferous zone in Scandinavia: lingonberry, raspberry, fireweed and mire honeys. The analytical data obtained in this research may be useful in future studies concerning properties of Finnish unifloral honeys, their antimicrobial properties, their use as sources for biologically active compounds or their impact on human health.

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