Characterisation of two unique unifloral honeys from the boreal coniferous zone: lingonberry and mire honeys

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Studies of unifloral honeys are rare in Finland, and thus there are no common traits accepted for their characterization. In our study lingonberry and mire honeys were characterized. The physico-chemical, organoleptic and melissopalynological properties and phenolic composition of the honeys were analysed and the floral origin of the mire honey was considered. Lingonberry honey was reddish and had a flavour of toffee, whereas mire honey had very strong aroma and reddish colour. Both honeys had high electrical conductivity and pH values when compared to Finnish polyfloral honeys. They were both rich in fructose, while the number of disaccharides in lingonberry honey was four and in mire honey six. Lingonberry honey had three unifloral specific phenolic compounds whereas mire honey had no unifloral-specific phenolic compounds, and its floral origin was not clearly defined.

Key words: unifloral honey, physico-chemical properties, phenolic compounds, melissopalynology

Introduction

Honey can be classified in several ways, e.g. by its source. The European Council Directive 2001/110/ EC concerning honey allows specific denominations of honeys where the simple product name "honey" can be supplemented by information on the floral, vegetable, regional, territorial or topographical origin (European Commission 2002). Thus, if the nectar from which the bees have derived the honey is gathered mainly from the flowers of one specific plant species in the foraging area of the beehive, the honey is called unifloral honey and it can be named after the plant from which the nectar originates (White 2005). On the other hand, the source of the 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 nature due to amount and combination of various components specific to this honey. In these cases EU directive concerning honey allows the specific denomination, where the product name "honey" is supplemented by the information on the topographical origin. Well known examples of these kinds of honeys are jungle or pasture honeys (Fukuda et al. 2011). Unifloral and biotope honeys may have a special taste or consist of substances that are beneficial to human health, and this may often make their commercial value greater than that of multifloral honeys (Bogdanov et al. 2008).

In Finland, predominant forest types range from mesic nutrient rich to oligotrophic dry heath coniferous forests (Ahti et al. 1968) and about 30% of the land area is classified as mire ecotype (Vasander et al. 2003). In eastern and northern Finland the main honey yield comes from wild forest plants typical of boreal coniferous zone flora (Salonen et al. 2009). Under favourable weather conditions lingonberry (*Vaccinium vitis-idaea*), which is one of the most common berry plants in Finnish forests (Lampinen and Lahti 2011) can produce an adequate amount of nectar to build up a honey yield with a special aroma. Moreover,

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plant species growing in a mire biotope, such as several species of *Vaccinium* family, *Rubus chamaemorus*, *Menyanthes trifoliata* and *Geranium sylvaticum* can also produce a nectar yield that produces honey with a characteristic aroma. This honey is called mire honey.

In recent years, some Finnish beekeepers have started produce lingonberry honey and mire honey for commercial purposes in central, eastern and northern Finland (Lapland), and they have also been able to export both honeys at a good price. Therefore there is a commercial need to characterize these two unique honeys. The previous basic data on Finnish honey by Varis et al. (1983) gives very little information on the composition of unifloral honeys from the boreal coniferous forests. More detailed data on the properties of boreal unifloral honeys from raspberry and fireweed has been reported by Salonen et al. (2011). In this study, we investigated the physico-chemical values for Finnish lingonberry and mire honey and described here their sensorial properties and melissopalynology as well as the phenolic acids and flavonoids as a basis for distinguishing these two unifloral honeys from other Finnish unifloral and multifloral honeys. In addition, the botanical origin of the mire honey was considered.

Material and methods

Honey samples

Seven lingonberry and eight mire honey samples were collected in summer 2007 and 2008 with the help of the Finnish Beekeepers Association and Finnish beekeepers. All samples were from commercial honey batches. Honey samples were selected by those organoleptic characteristics that are typical of these two unifloral honeys (colour, taste and odour) in order to find truly representative samples. The honey samples were stored at +5 °C in the dark until analysis.

Analyses of physical properties and invertase activity

All physical properties and invertase activity were analysed following the analytical methods harmonized by the International Honey Commission (Bogdanov 2009). A digital refractometer was used for the determination of water content directly from the honey (Atago 4422 PAL-22S Digital Pocket Honey Refractometer). Conductivity was measured with a Multiline P4 Universal Meter from a 10 g dry matter in a dose of honey dissolved in 50 ml of MilliQ water. The 10% honey-water solution was used in pH-measurements using a PHM 210 standard pH Meter (Meter Lab Radiometer, Copenhagen). Invertase activity was determined by the methods published by Bogdanov (2009) and 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.

Organoleptic properties

The method presented by Piana et al. (2004) was used in the analyses of organoleptic properties. Visual, olfactory and taste characteristics were noted on an evaluation form.

Sugar analyses

The method for sugar has been described in Salonen et al. (2011). The 0.5% honey water-acetonitrile solution were eluted with isocratic 75% acetonitrile-water elution solvent 1.4 ml/min using the High-Performance Liquid Chromatography-instrument (HPLC) (Agilent, Series 1100, Germany) containing a binary pump (G1316A), a thermostated autosampler (G1329A), a thermostated column oven (G1316A) and a refractive index detector (RID) (G1362A) combined with HP Chem Station Software. The column

was Zorbax, carbohydrate, 4.6×150 mm, particle size 5 μ m. For the qualification and quantification of the saccharides the HPLC-chromatograms of the samples were compared to those of commercial standards. The standards were fructose, glucose, maltose (Merck), D-turanose, panose, erlose, melezitose, isomaltose (Sigma-Aldrich), gentiobiose, raffinose (Sigma), saccharose (VWR) and trehalose (ICN Biomedicalsinc)

Melissopalynological analyses

The qualitative melissopalynological characteristics of the honey samples were analysed according to Louveaux et al. (1987). At least 400 pollen grains were counted from a 10 g washed and centrifuged honey sample.

Analyses of phenolic compounds

Phenolic compounds were extracted and reversed phase HPLC analysed as published by Salonen et al. (2011). Phenolics in 25 g 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 with 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 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) containing a binary pump (G1316A), a thermostated autosampler (G1329A), a thermostated column oven (G1316A) and a Diode Array Detector (DAD) (G1315B) combined with HP Chem Station Software, as previously published by Salonen et al. (2011). The column was Zorbax, SB-C18, 4.6×75 mm, particle size3.5 μ m. 1.5% tetrahydrofuran + 0.25% ortho-phosphoric acid water (=A) and 100% methanol (=B) were the elution solvents. The samples were eluted according to the following gradient: 0-5 min 100% A; 5–10 min 85% A, 15% B; 10–20 min 70% A, 30% B; 20–50 min 50% A, 50% B; 50% B; 50–55 min 100% B. The flow rate was 2 ml min⁻¹ and the auto-injection volume was 20 μ l. The temperatures of the column and injector were +30 and +20 °C, respectively. The HPLC runs were monitored at 220 and 320 nm.

The identification of phenolic compounds was based on a comparison of retention times and spectral characteristics of the HPLC/MS-identification of the MS-ions as described in Julkunen-Tiitto and Sorsa (2001) and Keski-Saari et al. (2005).

The quantification of phenolic compounds was based on the commercial standards: protocatechuic acid (Sigma-Aldrich) for protocatechuic acid; ferulic acid (Aldrich) for ferulic acid, cinnamic acid derivatives, p-OH-cinnamic acid derivatives and caffeic acid derivative; chlorogenic acid (Roth) for chlorogenic acid derivatives; vanillic acid (Fluka AG) for vanillic acid; benzoic acid (Sigma) for benzoic acid and benzoic acid derivatives; kaempferol 3-O-rhamnoside (Apin Chemicals Ltd) for kaempferol 3-O-rhamnoside; quercetin (Aldrich) for flavonoid derivative; isorhamnetin (Roth) for rhamnetin derivatives; galangin (Aldrich) for galangin derivatives; apigenin (Roth) for apigenin; naringenin7-glucoside (Roth) for methyl-naringenin; and acacetin for acacetin (Roth).

Results

Lingonberry honey

The electrical conductivity of lingonberry honey was 0.5 milli Siemens cm⁻¹ (mS cm⁻¹) and pH 4.5. These results are markedly higher than those of the average Finnish polyfloral honeys. The water content was low and the invertase activity value high (Table 1).

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|------------------------|---|---------------|---|------------------|-----------|--------------------------|-----------|--|
| Lingonberry honey | | | | Mire honey | Finnish p | Finnish polyfloral honey | | |
| Parameter | n | $Mean \pm SE$ | n | Mean \pm SE | n | Mean | Range | |
| Moisture (%) | 7 | 16.6 ± 0.5 | 8 | 16.6 ± 0.3 | 1151 | 16.9** | 13.2-21.6 | |
| Conductivity (mS cm-1) | 7 | 0.5 ± 0.76 | 8 | 1.08 ± 0.33 | 1151 | 0.27** | 0.1-1.88 | |
| pН | 7 | 4.5 ± 0.1 | 8 | 4.9 ± 0.1 | 158 | 4.0* | 3.6-4.6 | |
| Invertase activity | 6 | 150 ± 14.2 | 5 | 145.4 ± 16.4 | 1151 | 92.5** | 9-267 | |

Table 1. The physical parameters of lingonberry, mire and Finnish polyfloral honey samples.

SE = standard error of the mean.

* Reference: Varis et al. 1983, ** Reference: Sample material described in Salonen et al. 2009.

In sensorial analysis the colour of lingonberry honey had a dark intensity and reddish tone. The intensity of odour was medium and the descriptions of the odour were "resinous, dry hay, toffee, fresh, orange, cedar, pungent and apricot". The sweetness assessments varied from weak to strong. Acidity of the honey was medium and bitterness weak. The intensity of the aroma was medium and the descriptions were "toffee, citrus fruit, candied fruits, fruits and exotic fruit". The persistence or after-taste value was between short and medium.

Lingonberry honey was rich in fructose and its fructose/glucose ratio was over 1.4 and glucose/water ratio 1.7 (Table 2), suggesting that this honey granulates slowly. All the samples contained the disaccharides sucrose, turanose, maltose or trehalose (peaks overlapping) and isomaltose. In addition, lingonberry honey contained oligosaccharides (erlose or melezitose, peaks overlapping). However, the disaccharides maltose and trehalose and the two oligosaccharides erlose and melezitose could not be separated with the novel carbohydrate column used because the peaks overlapped.

| Compound | Lingonberry honey | | Mir | Mire honey | | Finnish polyfloral honey | | |
|----------------------------------|-------------------|-----------------|-----|-----------------|-----|--------------------------|--|--|
| | n | Mean \pm SE | n | Mean \pm SE | n | Mean Range | | |
| Fructose (g 100 g-1) | 7 | 40.3 ± 0.8 | 8 | 39.2 ± 0.41 | 158 | 47.3*** 38.5-58.4 | | |
| Glucose (g 100 g-1) | 7 | 27.7 ± 1.5 | 8 | 28.2 ± 0.34 | 158 | 43.9*** 34.3-54.5 | | |
| Saccharose (g 100 g-1) | 6 | 0.26 ± 0.11 | 6 | 0.11 ± 0.03 | | | | |
| Turanose (g 100 g-1) | 6 | 2.22 ± 0.10 | 6 | 2.79 ± 0.11 | | | | |
| Unknown 1 | 6 | 0 | 6 | peak | | | | |
| Unknown 2 | 6 | 0 | 6 | peak | | | | |
| Maltose/Trehalose (g 100 g-1)* | 6 | 1.11 ± 0.09 | 6 | 1.35 ± 0.09 | | | | |
| Isomaltose (g 100 g-1) | 6 | 2.17 ± 0.16 | 6 | 2.56 ± 0.15 | | | | |
| Gentiobiose (g 100 g-1) | 6 | 0 | 6 | 0 | | | | |
| Melezitose/ Erlose (g 100 g-1)** | 6 | 0.53 ± 0.10 | 6 | 0.69 ± 0.04 | | | | |
| Raffinose (g 100 g-1) | 6 | 0 | 6 | peak | | | | |
| Panose (g 100 g-1) | 6 | 0 | 6 | 0 | | | | |
| Fructose/Glucose ratio | 7 | 1.48 ± 0.07 | 8 | 1.39 ± 0.02 | 158 | 1.1 | | |
| Glucose/Water ratio | 7 | 1.68 ± 0.11 | 8 | 1.70 ± 0.03 | | | | |
| Fructose+Glucose (g 100 g-1) | 7 | 68.0 ± 2.24 | 8 | 67.4 ± 0.49 | | | | |

Table 2. Composition of mono-, di- and oligosaccharides, fructose/glucose and glucose/water ratios and total amount of mono-saccharides in lingonberry and mire honey samples.

SE= standard error of the mean

* Peaks overlapping, calculated as trehalose, ** Peaks overlapping, calculated as melezitose, *** Reference: (Varis et al. 1983). Method: thin layer chromatography.

We identified eighteen different phenolic compounds in the samples of lingonberry honey (Fig. 1): seven cinnamic acid derivatives and flavonoids, benzoic, vanillic and protocatechuic acids and tetragalloylglucose (Table 3). The most abundant were vanillic and benzoic acids. The amount of flavonoids was very low, only 0.57 μ g g⁻¹, and the ratio of phenolic acids to flavonoids was 19.5. Phenolic compounds typical for lingonberry honey samples were cinnamic acid derivative 1 (retention time, t_R 3.1), p-OH-cinnamic acid derivative 2 (t_R 23.9) and acacetin (t_R 44.1) (Fig. 1).



Fig. 1. HPLC chromatograms (DA-detection, monitored at 220 nm) of the mire and lingonberry honey samples. 1 Cinnamic acid der 1, 2 Protocatechuic acid, 3 Cinnamic acid der 2, 4 Vanillic acid, 5 Chlorogenic acid der , 6 p-OH-cinnamic acid der 1, 7 Benzoic acid, 8 Ferulic acid, 9 Tetragalloylglucose, 10 Benzoic acid der, 11 Cinnamic acid der 3, 12 p-OHcinnamic acid der 2, 13 Kaempferol 3-O-rhamnoside, 14 flavonoid der , 15 Rhamnetin der, 16 p-OH-cinnamic acid der 3, 17 Galangin der 1, 18 Galangin der 2, 19 Apigenin, 20 Methyl-naringenin, 21 Acacetin. der = derivative of mentioned compound

About 42% of the pollen grains in the lingonberry honey samples originated from *Vaccinium* species, mainly *V. vitis-idaea* (Fig. 2) and *V. myrtillus* origin (Fig. 3) (Table 4). Some samples also contained pollen grains of *V. oxycoccus* (Fig. 4). Other pollen grains typical of the samples of this honey came from Rosaceae, *Salix*, *Trifolium* and Apiaceae species.



Fig. 2. Pollen grain of Vaccinium vitis-idaea.

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| | 1 | Lingonberry | <u> </u> | | |
|---------------------------------------------------|----------------|-----------------------|-----------------|--------------------|-----------------------|
| Phenolic compound | | honey | Mire honey | Identificat Uv- | ion |
| μg g ⁻¹ | t _R | $Mean \pm SE$ | Mean \pm SE | spectrum | MS-ions |
| Cinnamic acid der 1* | 3.1 | 0.22 ± 0.05 | | х | |
| Protocatechuic acid* | 4.0 | trace | 0.46 ± 0.10 | х | 155 (M+H) |
| Cinnamic acid der 2* | 7.5 | 1.46 ± 0.22 | 0.56 ± 0.10 | х | |
| Vanillic acid* | 8.5 | 3.08 ± 0.59 | 1.11 ± 0.12 | х | 169 (M+H), 191 (M+Na) |
| Chlorogenic acid der** | 10.4 | - | trace | х | 455 |
| p-OH-cinnamic acid der 1** | 13.3 | 1.54 ± 0.43 | 2.52 ± 0.22 | х | 165 (M+H) |
| Benzoic acid* | 13.5 | 2.28 ± 0.20 | 2.70 ± 0.52 | х | 123 (M+H), 145 (M+Na) |
| Ferulic acid** | 14.4 | 1.17 ± 0.28 | 1.20 ± 0.12 | х | 195 (M+H), 217(M+Na) |
| Tetragalloylglucose** | 16.2 | trace | trace | х | |
| Benzoic acid der** | 17.1 | - | 0.48 ± 0.07 | х | 303 |
| Cinnamic acid der 3* | 22.4 | 0.49 ± 0.13 | 0.59 ± 0.05 | Х | |
| p-OH-cinnamicacid der 2** | 23.9 | 0.85 ± 0.15 | - | х | |
| Kaempferol 3-O-rhamnoside** | 27.4 | trace | trace | х | 455 (M+H) |
| flavonoid der** | 28.1 | trace | - | Х | |
| Rhamnetin der** | 29.1 | - | trace | х | |
| p-OH-cinnamicacid der 3** | 31.3 | trace | - | х | |
| Galangin der 1* | 33.2 | 0.21 ± 0.04 | 0.23 ± 0.06 | х | 271 (M+H) |
| Galangin der 2* | 33.8 | trace | - | Х | 271 (M+H) |
| Apigenin** | 34.4 | trace | - | х | 271 (M+H) |
| Methyl-naringenin** | 38.3 | 0.36 ± 0.01 | 0.40 ± 0.10 | х | 287 (M+H) |
| Acacetin** | 44.1 | trace | - | х | |
| Total amount of phenolic compou | ınds µg g | g ⁻¹ 11.22 | 10.06 | | |
| Amount of phenolic acids $\mu g \; g^{\text{-1}}$ | | 11.08 | 9.61 | | |
| Amount of flavonoids $\mu g g^{-1}$ | | 0.57 | 0.63 | | |
| Ratio: phenolic acids/ flavonoids | | 19.49 | 15.25 | | |

Table 3. Identified phenolic compounds of lingonberry and mire honeys.

trace = very low amount in one sample, tR = retention time, SE = standard error of the mean, der = derivative of mentioned compound, * monitored at 220 nm, ** monitored at 320 nm.



Fig. 3. Pollen grain of Vaccinium myrtillus.



Fig. 4. Pollen grain of Vaccinium oxycoccos.

| Pollen group | Lingonberry honey Mean ± SE | Mire honey Mean ± SE | Finnish polyfloral honey %* |
|-----------------------|--------------------------------|-------------------------|-----------------------------|
| n | 7 | 8 | 734 |
| Apiaceae | 7 ± 3 | 7 ± 2 | 3 |
| Brassicaceae | 0 | 0 | 32.0 |
| Epilobium | present | present | present |
| Geranium | present | present | present |
| Menyanthes trifoliata | present | 2 ± 2 | present |
| Rosaceae | 18 ± 3 | 10 ± 2 | 30 |
| Rubus chamaemorus | present | 2 ± 2 | present |
| Salix | 13 ± 4 | 25 ± 6 | 8 |
| Trifolium | 7 ± 2 | 3 ± 3 | 15 |
| Vaccinium | 43 ± 7 | 45 ± 7 | 3 |
| Others | 12 ± 6 | 7 ± 2 | 9 |

Table 4. Melissopalynological analysis of lingonberry and mire honeys (expressed as the percentage of a pollen group out of all counted pollen grains).

n=number of samples, SE = standard error of the mean, * Reference: Salonen et al. (2011).

Mire honey

The electrical conductivity of the mire honey was over 1.0 mS cm⁻¹, the water content was low, the invertase activity value high and the pH is around 4.9 (Table 1).

In sensory analyses mire honey showed medium colour intensity and a reddish colour tone. The intensity of the odour was medium and it was described as "straw, dry hay, mint, solvent, fresh, orange blossom". Tasting assessments varied greatly: sweetness, acidity, bitterness, aroma and persistence or after-taste were evaluated with all the scores (0–3). Aroma was described as "refreshing, apricot, solvent, aniseed, eucalyptus, dates prunes and fruit".

All the mire honey samples contained six disaccharides: sucrose, turanose, maltose or trehalose (peaks overlapping), isomaltose and two unknown disaccharides (Table 2). Of the oligosaccharides, mire honey samples contained raffinose in trace amounts, as well as erlose, melezitose or both (peaks overlapping).

Only fourteen phenolic compounds could be identified in mire honey samples (Fig. 1): four cinnamic acid derivatives and flavonoids, benzoic, vanillic and protocatechuic acids, chlorogenic acid derivative, benzoic acid derivative and tetragalloylglucose. The total amount of flavonoids was very low (0.63 μ g g⁻¹), and the

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ratio of phenolic acids to flavonoids was 15.25 (Table 3). Benzoic acid and p-OH-cinnamic acid derivative 1 were found in the highest amounts. Mire honey had no unifloral specific phenolic compounds.

Melissopalynological analyses indicated that 44% of the pollen grains in the mire honey came from the *Vaccinium* family, mostly from species such as *V. myrtillus* (Fig. 3) and *V. vitis-idaea* (Fig. 2) and only few from *V. uliginosum* (Fig. 5) and *V. oxycoccos* (Fig. 4) (Table 3), which grow on mires. The amount of pollen grains from the *Salix* species was more abundant than that in Finnish polyfloral honey. Other pollen grains typical of this unifloral honey came from Rosaceae, Apiaceae and *Trifolium* species, which are also common in Finnish polyfloral honey samples and from other plant species growing on mire biotopes, *R. chamaemorus* (Fig. 6) and *M. trifoliata* (Fig. 7) (Table 3).





Fig. 6. Pollen grain of Rubus chamaemorus.

Fig. 5. Pollen grain of Vaccinium uliginosum.



Fig. 7. Pollen grain of Menyanthes trifoliata.

Discussion Lingonberry honey

The average electrical conductivity of Finnish polyfloral honeys is under 0.3 mS cm⁻¹ (Table 1). The high electrical conductivity of the lingonberry honey (0.5 mS cm⁻¹) and the special organoleptical properties can be used as indicators when separating lingonberry honey from other Finnish honey varieties. Lingonberry honey was rich in fructose which means that the ratio of fructose/glucose was 1.48 and the ratio of glucose/water was 1.68. These ratios indicate that lingonberry honey granulates slowly.

In lingonberry honey pollen grains of Vaccinium species were found in highest amount (Tabel 3). Pollen

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grains of *Salix* and Apiaceae species were also found in higher amounts than in Finnish unifloral honeys (Salonen et al. 2009), since these species flower at the same time as lingonberries. In addition, pollen grains of *Geranium. sylvaticum*, which is a common plant in Finnish nature (Lampinen and Lahti 2011) and flowers at the same time as lingonberries, were expected to be found in lingonberry honey in greater amounts than in Finnish unifloral or polyfloral honeys. However, the total amount of pollen grains of *G. sylvaticum* was very low, although it has been observed (personal communication with beekeepers) that bees visit *G. sylvaticum* very diligently. The size of *G. sylvaticum* pollen grains (Fig. 8) is huge, and it is known that bees are able to filter large pollen grains out of the nectar easily as they fly from the forage flowers to the hive (Bryant and Jones 2001). This means that pollen of *G. sylvaticum* may be highly under-represented in honey samples. We know very little of the importance of *G. sylvaticum* as a potential nectar plant. This issue needs to be further researched in the near future.



Fig. 8. Pollen grain of Geranium sylvaticum.

From the phenolic acids in lingonberry honey, vanillic acid was found in highest amount. Its amount was notably higher than in mire or in other Finnish unifloral honey (Salonen et al. 2011). Vanillic acid has also been found in Bulgarian (Dimitrova et al. 2007) unifloral honeys. Phenolic compounds typical for lingonberry honey samples were cinnamic acid derivative 1, p-OH-cinnamic acid derivative 2 and acacetin. These compounds were not found either in Finnish unifloral raspberry or in fireweed honey samples (Salonen et al. 2011), while acacetin can be found in acacia honey (Marghitas et al. 2010).

Lingonberry honey is collected from mid-June till the end of June. Lingonberries bloom every year, but it seems that nectar production varies greatly from year to year and that the yield of the lingonberry honey depends very much on weather conditions in June. Thanks to its special toffee odour and taste and its reddish colour, lingonberry honey can easily be recognised even from the honeycombs in the hive.

Mire honey

The electrical conductivity of the mire honey (Table 1) was the highest (over 1.0 mS cm⁻¹) when compared with polyfloral or unifloral honeys collected in Finland (Salonen et al. 2011). According to EU's honey directive, the electrical conductivity of a honey should not exceed the limit of 0.8 mS cm⁻¹, unless the honey is derived from honeydew. In the melissopalynological analysis we found very few honeydew marks, proving that mire honey was nectar honey. For this reason mire honey should be added to the list of exceptions in honey directive, where unifloral nectar honeys having electrical conductivity over 0.8 are

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mentioned (European Commission 2002). Interestingly, the pH of mire honey was very high, although in a mire biotope the substrate is acidic (Kaakinen et al. 2008). Like lingonberry honey, mire honey was rich in fructose and it granulates slowly, as well.

Generally, when compared with other Finnish poly- and unifloral honeys, mire honey has a very peculiar and strong odour and taste, resembling, for example, chestnut, lime or heather honeys (Persano Oddo and Piro 2004), and it is highly valued in Finland. Based on its organoleptic characteristics, mire honey samples win the category of unifloral honeys in the Finnish honey competition almost every year.

Mire honey had no unifloral specific phenolic compounds. In addition, the low amount of phenolic compounds was rather surprising. It was expected that mire honey could contain abundant amounts of phenolics that might contribute to some extent to the organoleptic characteristics of the honey. Vanillic acid is a phenolic of this kind; however, its content was low, as much as three times lower when compared with lingonberry honey, but nevertheless six times higher than in Finnish raspberry and fireweed honeys (Salonen et al. 2011).

To collect mire honey, beekeepers transfer their hives from Central Finland to mire areas, mainly to Lapland and Northern Ostrobothnia at the end of May. Melissopalynological analyses (Table 4) indicated that 44% of the pollen grains in the mire honey came from *V. myrtillus*, *V. vitis-idaea*, *V. uliginosum* and *V. oxycoccos*, which grow on mires. The amount of pollen grains from the *Salix* species was more abundant than that in Finnish polyfloral or lingonberry honey samples because mire honey is collected early in the summer, when *Salix* species are the most important food plants for bees, and some *Salix* species flower on mires in June, as well. The amounts of pollen grains from plant species typical for mire biotopes, *R. chamaemorus* and *M. trifoliate* were low.

The denomination of unifloral honey may be difficult to establish. In the EU Council Directive relating to honey it is stated that "In trade the simple product name "honey" is used and it can be supplemented by the information of the floral, vegetable, regional, territorial or topographical origin" (European Commission 2002). Mire honey is commercially named "Cloudberry honey" or "Honey from cloudberry mire", because cloudberry (*R. chamaemorus*) is widespread in mire biotypes and a very highly valued berry in Finland. However, there is no research on the amount of nectar secretion of cloudberry flowers in Finland. Cloudberry is a dioecious plant species and there seems to be no agreement of the amount of nectar in female or male plant individuals (Brown and McNeil 2009). According to our melissopalynological analyses, the amount of *R. chamaemorus* pollen grains was very moderate in cloudberry honey, so it is unlikely that *R. chamaemorus* would be at least the main source of nectar for mire honey.

Pollen grains of *M. trifoliata* are generally found in mire honey samples in low amounts. This plant commonly grows on Finnish mires (Lampinen and Lahti 2011), but there is no information about its nectar production. Secondary metabolites, such as phenolic acids and flavonoids, could be used as markers of the floral origin of the honey (Tomas-Barberan et al. 2001). *M. trifoliata* leaves contain many phenolic compounds, such as iridoids and secoiridoids, secologanate, dihydrofoliamenthin, quercetin-glycoside and kaempferol-glycoside, in a fairly high concentration (Martz et al. 2009). In our study these phenolic compounds were not found in the mire honey samples. Nevertheless, pollen grains of *M. trifoliate* in mire honey proved that bees visit the plant.

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Conclusions

This study discribes the physico-chemical composition and phenolic content of two different types of Finnish unifloral honey. These unique honeys differ in terms of their odour, flavour, taste, electrical conductivity and also their sugar and phenolic content. According to our results, the best tools in order to discriminate these honeys from other Finnish poly- and unifloral honeys are the value of electrical conductivity, special taste and odour and the phenolic content of lingonberry honey. Although these two unifloral honeys are not widely produced, they are of high interest. Their collection demands more work, which affects the production cost and therefore their price is higher compared to other Finnish unifloral honeys. They are a very welcome addition to the fairly modest Finnish unifloral honey selection. In the near future more research will be conducted on such issues as their floral origin, volatile compounds and antibacterial properties, as well as their influence on human health.

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References

Ahti, T., Hämet-Ahti, L. & Jalas, J. 1968. Vegetation zones and their sections in northwestern Europe. *Annales Botanici Fennici* 5: 169–211.

Bogdanov, S., Jurendic, T., Sieber, R. & Gallmann, P. 2008. Honey for Nutrition and Health: a Review. *American Journal of the College of Nutrition* 27: 677–689.

Bogdanov, S. 2009. *Harmonised methods of the International Honey Commission*. Cited 16 January 2012. Available on the Internet: http://www.bee-hexagon.net/files/file/fileE/ IHCPapers/IHC-methods_2009.pdf.

Brown, A.O. & Mcneil, J.N. 2009. Pollination ecology of the high latitude, dioecious cloudberry (Rubus chamaemorus; Rosaceae). *American Journal of Botany* 96: 1096–1107.

Bryant, V.M. & Jones, G.D. 2001. The R-value of honey: Pollen coefficients. Palynology 25: 11–28.

Dimitrova, B., Gevrenova, R. & Anklam, E. 2007. Analysis of phenolic acids in honeys of different floral origin by solid-phase extraction and high-performance liquid chromatography. *Phytochemical Analysis* 18: 24–32.

European Commission 2002. Council Directive 2001/110/EC concerning honey. *Official Journal of European Communi*ties Jan 12th 2002. L10/47–52.

Fukuda, M., Kobayashi, K., Hirono, Y., Miyagawa, M., Ishida, T., Ejiogu, E.C., Sawai, M., Pinkerton, K.E. & Takeuchi, M. 2011. Jungle Honey Enhances Immune Function and Antitumor Acivity. *Evidence-Based Complementary and Alternative Medicine* Article ID 908743, 8 p.

Julkunen-Tiitto, R. & Sorsa, S. 2001. Testing the drying methods for willow flavonoids, tannins and salicylates. *Journal of Chemical Ecology* 27: 779–789.

Kaakinen, E., Aapala, K. & Kokko, A. 2008. The Diversity and Current Condition of Finnish Mires. In: Korhonen, R., Korpela, L. & Sarkkola, S. (eds.). *Finland - Fenland: Research and sustainable utilisation of mires and peat.* The Finnish Peatland Society. Helsinki, Maahenki Ltd. p. 34–52.

Keski-Saari, S., Pusenius, J. & Julkunen-Tiitto, R. 2005. Phenolic compounds in seedlings of *Betula pubescens* and *B. pendula* are affected by enhanced UV-B radiation and different nitrogen regimes during early ontogeny. *Global Change Biology* 11: 1180–1194.

Lampinen, R. & Lahti, T. 2011. Kasviatlas 2010. Helsingin Yliopisto, Luonnontieteellinen keskusmuseo, Kasvimuseo,

A. Salonen & R. Julkunen-Tiitto (2012) 21: 159-170

Helsinki. Cited 16 January 2012. Available on the Internet : http://www.luomus.fi/kasviatlas.

Louveaux, J., Maurizio, A. & Vorwohl, G. 1978. Methods of melissopalynology. Bee World 59: 139–157.

Marghitas, L.A., Dezmirean, D.S., Pocol, C.B., Ilea, M., Bobis, O. & Gergen, I. 2010. The development of a biochemical profile of acacia honey by identifying biochemical determinants of its quality. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 38: 84–90.

Martz, F., Turunen, M., Julkunen-Tiitto, R., Lakkala, K. & Sutinen, M-L. 2009. Effect of the temperature and the exclusion of UVB radiation on the phenolics and iridoids in Menyanthes trifoliata L. leaves in the subarctic. *Environmental Pollution* 157: 3471–3478.

Persano Oddo, L. & Piro, R. 2004. Main European unifloral honeys: descriptive sheets. Apidologie 35: S38-S81.

Piana, M L., Persano Oddo, L., Bentabol, A., Bruneau, E., Bogdanov, S. & GuyotDeclerck, C. 2004. Sensory analysis applied to honey: state of the art. *Apidologie* 35: S26–S37.

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

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

Tomas-Barberan, F.A., Martos, I., Ferreres, F., Radovic, B.S. & Anklam, E. 2001. HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *Journal of the Science of Food and Agriculture* 81: 485–496.

Vasander, H., Tuittila, E-S., Lode, E., Lundin, L., Ilomets, M., Sallantaus, T., Heikkilä, R., Pitkänen, M-L. & Laine, J. 2003. Status and restoration of peatlands in northern Europe. *Wetlands Ecology* 11: 51–63.

Varis, A-L., Helenius, J. & Koivulehto, K. 1983. Composition and properties of Finnish honey and their dependence of the season, region, bee race and botanical origin. *Journal of theSscientific Agricultural Society of Finland* 55: 451–463.

White, J.W. 2005. Honey. In: Graham, J.M. (ed.). The hive and the honey bee. Dadant & sons, Hamilton, Illinois, p. 869–927.