PHENOLIC COMPOUNDS OF PROPOLIS FROM THE BOREAL CONIFEROUS ZONE

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S u m m a r y

The profile of phenolic compounds in 19 propolis samples from different provinces in Finland were analysed for the first time using HPLC-DAD. Nine individual flavonoids (comprising 26% of the identified phenolics), eleven cinnamic acid derivatives (36%), two caffeic acid derivatives (14%), three chlorogenic acid derivatives (14%), and three other phenolic acids (10%) were found in the propolis samples. The compounds found in the largest quantities were methyl-naringenin and caffeic acid phenethyl ester (CAPE). The phenolic profiles of Finnish propolis show marked differences when compared with *P. nigra* and *P. tremuloides* propolis of Central European and Canadian origins. The phenolic compounds found in propolis samples are commonly found in the tree species growing in Finland. Non-metric multidimensional scaling showed that samples were scattered and they did not form clear groups according to the geographical origin or age of the sample.

Keywords: propolis, phenolic compound, HPLC-DAD analysis, Finland.

INTRODUCTION

Sticky and glue-like propolis is produced by honey bees from the resins, leaf buds, mucilages, gums, and substances from the cracks in the bark of trees. These ingredients are then mixed with the beeswax and *β*-glucosidase that bees secrete during propolis collection. It is a strongly adhesive and lipophilic material with a pleasant aromatic odour (Marcucci, 1995). It is assumed that bees seek different resin sources for propolis, and collect the raw material for propolis from the vegetation near their hive. This method of collecting affects the colour, odour, and composition of propolis (Bankova et al., 2000). Despite the different sources, propolis has similarities in its chemical composition and works in the hive in the same way, preventing the growth of unwanted micro-organisms (Kujumgiev et al., 1999).

There is very little information about the sources and chemical composition of propolis collected in Northern Europe. In Lithuania, it was found that propolis collected from deciduous trees and meadows differs from the propolis collected from coniferous trees. The former carries more ferulic and coumaric acids, which are the predominant phenolic acids in Lithuanian propolis (Ramanauskienė et al., 2009). In Poland, Warakomska and Maciejewicz (1992), using the pollen analysis of propolis, found that 28% of the pollen grains in propolis came from Brassicaceae, *Salix, Pinus* and *Betula* species. The studies done by Popravko and Sokolov (1980) on propolis in Russia obtained from the Moscow area, revealed the same compounds are found in buds of *Populus tremula* L. and *Betula pendula* Roth. In North America in Canada, propolis samples from the boreal forest areas contained high concentrations of cinnamic and *p*-coumaric acid (Christov et al., 2006).

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In the warmth of the hive or as a result of honey bee activities, the compounds of propolis diffuse into honey, which means that part of the phenolic compounds in honey originate from propolis (Tomas-Barberan et al., 2001; Truchado et al., 2010). In order to be able to distinguish the plant-derived phenolics in unifloral honeys, one has also to know which phenolic compounds originate from propolis. The aim of this study was to analyse the profile of phenolic compounds of propolis collected in Finland's boreal coniferous zone. This information enables us to better characterize plant-derived phenolic compounds in Finnish unifloral honeys. We were also interested to see if there are any differences in chemical composition that are due to provenance effects. This is the first study concerning the phenolic composition and sources of propolis from the boreal coniferous zone in Scandinavia.

MATERIALS AND METHODS

We collected 19 propolis samples from 17 beekeepers of five Finnish provenances. Table 1 shows the origins and age of the samples.

The phenolics of the propolis samples were analysed using high-performance liquid chromatography (HPLC). Fifty (50) milligrams of propolis were extracted using 8.5 ml of methanol at room temperature for 30 minutes. The extract was filtered through a paper filter and using methanol, the volume was adjusted to 10 ml. 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), an instrument containing

Table 1.

The origin and age of the propolis samples.
(All samples were stored at room temperature
except sample NK-8, which was stored in a freezer)

Sample	Origin	Age	GPS-coordinates (ETRS89-GRS80)	
			N / lat	E / lon
NK-1	North Karelia	a	62.21	30.32
NK-2	North Karelia	a	63.53	29.18
NK-3	North Karelia	a	62.62	30.98
NK-4	North Karelia	a	62.57	30.15
NK-5	North Karelia	a	62.21	30.60
NK-6	North Karelia	b	62.69	30.87
NK-7	North Karelia	a	62.73	29.44
NK-8	North Karelia	a	62.87	29.77
NK-9	North Karelia	b	62.21	30.18
NK-10	North Karelia	a	63.70	29.13
NK-11	North Karelia	a	62.43	30.05
NK-12	North Karelia	a	62.33	30.86
NK-13	North Karelia	b	62.33	30.86
SK-1	South Karelia	b	61.63	29.56
SK-2	South Karelia	b	61.83	29.69
CF-1	Central Finland	b	62.02	25.48
CO-1	Central Ostrobothnia	a	63.56	23.68
CO-2	Central Ostrobothnia	a	63.56	23.68
NO-1	Northern Ostrobothnia	b	64.02	24.68

a - sample older than one year;
b - sample collected from hive in summer 2010.

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. The column used was Zorbax, SB-C18, 4.6 x 75 mm with 3.5 µm particle size. The elution solvents were aq. 1.5% tetrahydrofuran + 0.25% orthophosphoric acid (A), and 100% methanol (B). 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; 20-40 min 50% A, 50% B; 40-75 min 50% A, 50% B; 75-80 min100% B. The flow rate was 2 ml/min and the autoinjection volume was 20 µl. The temperature of the column and injector was +30°C and +20°C, respectively. The HPLC runs were monitored at 220 and 320 nm. Analysed secondary metabolites were quantified against commercial standards. The identification of the compounds was based on the HPLC-MS-identification or on comparison of retention times and spectral characteristics as described in Julkunen-Tiitto and Sorsa (2001) and Keski-Saari et al. (2005).

The quantification of the phenolic compounds is based on the commercial standards: chlorogenic acid for chlorogenic acid derivatives; ferulic acid for cinnamic acid derivatives, *p*-OH-cinnamic acid derivatives and caffeic acid derivatives, pinocembrin for pinocembrin derivatives and pinobanksin, benzoic acid for benzoic acid and benzoic acid derivatives, vanillic acid for vanillic acid, apigenin for apigenin and methyl-apigenin, naringenin-7-glucoside for methyl-naringenin and naringenin derivative, and kaempferol for di-methyl-kaempferol.

Non-metric multidimensional scaling (NMS), creating a distance matrix (Bray-Curtis) between the investigated samples, was used to visualize dissimilarities in the phenolic content of the propolis samples. Preliminary runs suggested a 3-dimensional solution. After rerunning the analysis using the suggested dimensionality, the stress value for the analysis stood at 4.4, indicating a good and reliable solution. Multivariate analysis was conducted using PC-ORD version 5.0 (McCune and Mefford, 1999).

RESULTS AND DISCUSSION

The colour and odour of the analyzed propolis samples were similar: the colour was reddish brown, and the odour fairly spicy. In total, 26 phenolic compounds were identified and quantified: nine cinnamic acid derivatives, three chlorogenic acid derivatives, caffeic acid derivative and caffeic acid phenethyl ester (CAPE), benzoic acid, one of its derivatives and vanillic acid as well as nine flavonoids (Tab. 2). There were only three propolis samples that contained all 26 compounds, and sixteen identified compounds were found in all the samples (Tab. 3).

There was great variation in the amount of the phenolic compounds in the individual propolis samples (Tab. 3). The total amount of the phenolic compounds present in propolis samples ranged from 79.8 to 156.3 µg/g, the average being 119.5 µg/g. Cinnamic acid derivatives and flavonoids comprised 64% and 26% of all phenolics, respectively. Methyl-naringenin, CAPE, *p*-OH-cinnamic acid derivative 4 and benzoic acids were found in the largest quantities (Fig. 1). High amounts of CAPE (14% of all phenolics), which is regarded as one of the biologically active components of propolis (Russo et al., 2002) were found. In some samples, flavonoids such as acacetin and methyl-apigenin, were found only in trace amounts.

The age of the propolis is expected to affect the chemical composition of propolis (Krell, 1996). Accordingly in our samples, NK-1 and NK-12 were the old samples, and their total phenolic contents were the lowest (Tab. 3). Although the NK-8 sample had been stored in a freezer, which should slow down the chemical changes, and sample NK-13 was the youngest sample, their phenolic contents were unexpectedly low. In the samples older than one year, the mean of the total amount of phenolic compounds was 117.35 µg/g,

whereas in samples of the current season (year 2010, regarded as fresh samples), the mean content was 123.1 μg/g. On the other hand, it seems obvious that there is variation within apiaries as well, since samples NK-12 and NK-13 originate from the same apiary but do not have the same phenolic profile or content. In this case, the difference in chemistry may only be partly explained by the age of the samples (NK-12 was old and NK-13 fresh). More research is needed to explain the effect of storage conditions on the phenolic content of propolis.

In addition to age, there are many other factors that may influence the composition of propolis; bee race (Silici and Kutluca, 2005), the season of the collection (Bankova et al., 1998), geographical origin (Bankova et al., 1992; Bankova et al., 2002), and above all, the plant species, or even the subspecies of plant species (Greenway et al., 1990). In temperate areas of Europe where *Populus* species are considered to be the main source of propolis, the main phenolic compounds in propolis are flavonoids, such as pinocembrin, pinobanksin, chrysin and galangin (Bankova et al., 1992; Bankova

Table 2.

Identification of propolis phenolics. (Rt = retention time (min) and MS-ions obtained by LC/single quadrupole MS). (der = derivative of mentioned compound)

Phenolic compound	Rt	Identification
Name		MS-ions
Cinnamic acid der 1*	7.5	-
Vanillic acid *	8.5	169 (M+H), 191 (M+Na)
Chlorogenic acid der 1	10.4	455
p-OH-cinnamic acid der 1 (p-coumaric acid)*	13.4	165 (M+H)
Benzoic acid*	13.5	123 (M+H), 145 (M+Na)
4-hydroxy-3-methoxy cinnamic acid (ferulic acid)*	14.4	195 (M+H), 217(M+Na)
Methyl-cinnamic acid der*	14.6	179 (M+H), 201 (M+Na)
Benzoic acid der	17.1	-
p-OH-cinnamic acid der 2	19.3	-
Cinnamic acid der 2*	22.4	-
Pinocembrin der 1*	29.2	-
Pinocembrin der 2	30.4	-
Caffeic acid der*	32.2	-
Apigenin*	34.4	271 (M+H)
p-OH-cinnamic acid der 3*	35.8	355, 179
Methyl-naringenin	38.5	287 (M+H)
Chlorogenic acid der 2*	39.9	-
Acacetin	42.9	-
Chrorogenic acid der 3*	43.4	449
p-OH-cinnamic acid der 4*	43.9	509
Caffeic acid phenethyl ester (CAPE)*	46.1	307 (M+Na)
Pinobanksin der	46.7	295 (M+Na)
Di-methyl-kaemferol	46.7	315 (M+H), 337 (M+Na)
Methyl-apigenin	47.3	285 (M+H)
Naringenin der 1	53.4	-
Cinnamic acid der 3*	70.9	-

* compounds marked with an asterisk can be found in all samples.

et al., 2002). In our propolis samples neither chrysin nor galangin was found, and the total amount of flavonoids of all phenolics was about 26% (quantification based on HPLC/DAD-analyses). In central European *P. nigra* based propolis the flavonoid content was more than 30% (quantification based on abundance of MS ions) (Bankova et al., 1992; Bankova et al., 2002). In our samples, cinnamic acid derivatives comprised about 64% (based on HPLC/DAD-analyses) of all phenolics. But Bankova et al. (1992) reported the amount of cinnamic acid derivatives for poplar propolis to be only 30% (based on abundance of MS ions) of all phenolics. Christov et al. (2006) have studied Canadian propolis collected from boreal forest. Canadian propolis, which probably originates from *P. tremuloides*, is characterized by large amounts of *ρ*-coumaric and cinnamic acids (about 30%, based on abundance of MS ions) and a low concentration of flavonoids (8.5%). Although the quantification of compounds in different studies were based on different analytical systems, these results indicate marked differences in the phenolic content between Finnish propolis and *P. nigra* and *P. tremuloides* propolis.

In temperate areas of Europe, *Populus* species are considered to be the main source of propolis (Bankova et al., 2000), but in Finland, other *Populus* species than *P. tremula* are uncommon outside gardens and parks. The most common tree species growing in Finland are coniferous species, *Pinus sylvestris* L. and *Picea abies* (L.) H.Karst and deciduous species *Betula* spp, S*alix* spp., *P. tremula* and *Alnus incana* (L.) Moench (Lampinen and Lahti, 2010). Their pollen grains are generally found

Table 3.

Content of phenolic compounds of individual propolis samples (expressed as μg/g), mean of three samples and ± standard error (der = derivative)

Comp-ound*	The origin and age of the propolis samples**									
	NK-1	NK-2	NK-3	NK-4	NK-5	NK-6	NK-7	NK-8	NK-9	NK-10
1	1.2±0.02	0.9±0.1	0.8±0.05	0.3±0.04	0.6±0.1	0.4±0.1	0.5±0.03	0.6±0.04	0.7±0.02	0.8±0.2
2	0.7±0.1	0.3±0.02	0.4±0.002	0.2±0.04	0.4±0.02	0.6±0.1	0.4±0.05	0.3±0.02	0.3±0.02	0.3±0.1
3	0.8±0.1	1.0±0.2	1.6±0.1		1.1±0.3	1.9±0.5	1.2±0.2	1.2±0.05	1.5±0.1	1.0±0.2
4	5.7±0.7	6.6±0.9	7.0±0.5	6.4±0.6	4.7±1.2	5.3±0.7	7.8±0.6	7.9±0.2	9.3±0.3	6.3±3.1
5	12.5±2.6	9.0±4.7	18.9±0.9	18.0±2.7	9.1±3.1	20.5±6.2	8.2±2.4	9.4±1.3	8.2±1.4	6.2±1.5
6	6.1±1.2	6.2±1.9	10.2±0.5	12.4±1.2	8.3±1.0	14.7±2.7	8.7±1.0	7.7±0.6	10.7±1.0	6.4±1.0
7	1.3±0.2	1.2±0.1	1.8±0.1	1.3±0.1	1.2±0.2	1.9±0.05	0.9±0.1	1.3±0.2	1.3±0.2	1.3±0.2
8	0.7±0.1	0.1±0.04	0.3±0.01		0.2±0.1	0.3±0.1				0.2±0.05
9	0.3±0.05	0.1±0.04								0.1±0.01
10	1.1±0.3	3.1±1.0	1.6±0.1	1.0±0.1	0.9±0.1	1.4±0.2	1.4±0.2	0.7±0.02	0.9±0.1	0.5±0.1
11	0.5±0.1	0.5±0.05	0.8±0.01	0.6±0.01	0.6±0.1	0.6±0.003	0.6±0.03	0.8±0.1	0.5±0.03	0.4±0.2
12		0.1±0.1	0.5±0.04		0.3±0.1		0.3±0.1		trace	
13	0.3±0.1	0.2±0.03	0.3±0.01	0.3±0.04	0.2±0.02	0.3±0.1	0.3±0.02	0.2±0.1	0.3±0.03	0.3±0.1
14	0.6±0.2	0.6±0.1	1.2±0.1	0.8±0.1	0.7±0.2	1.0±0.03	0.7±0.04	0.8±0.03	0.8±0.1	0.6±0.1
15	2.2±0.3	1.5±0.1	2.3±0.1	2.0±0.1	1.7±0.2	1.9±0.2	2.3±0.1	2.3±0.7	2.1±0.2	2.2±0.4
16		18.7±2.0	37.4±2.2		22.9±6.4		17.3±1.7	33.2±3.6	28.8±3.6	22.7±5.6
17	6.2±1.1	5.4±1.5	9.5±0.6	9.4±0.9	6.1±1.4	7.4±4.2	5.5±0.3	6.6±1.4	5.8±0.6	11.7±5.8
18	7.4±1.2	7.2±1.5	11.1±0.1	10.0±0.2	8.4±1.0	12.2±1.0	7.7±0.4	10.4±2.9	7.5±0.5	10.7±2.5
19	13.3±1.6	13.9±1.6	15.8±0.4	16.0±0.8	12.5±1,3	12.1±1.2	13.7±1.2	17.8±4.3	14.7±1.4	23.4±8.2
20	15.1±1.3	13.2±2.0	15.9±0.8	16.5±0.2	10.1±0.7	10.5±1.0	14.2±1.4	18.2±4.2	18.4±2.0	18.7±8.6
21		4.9±1.8	12.8±0.8	13.2±2.0	4.1±1.9	4.1±0.9	2.4±0.7	8.2±0.8	4.5±0.7	3.3±0.9
22		trace	trace		trace		trace	trace		
23			1.4±0.2		0.5±0.5		0.5±0.5	0.7±0.7		0.4±0.4
24	3.8±0.5	7.4±2.8	12.5±0.7	10.1±0.5	4.1±1.4	6.1±0.6	5.4±0.5	5.5±0.9	11.2±1.3	9.7±4.8
Total	79.8±8.3	102±21.8	164.1±0.5	118.6±3.2	98.4±15.9	103.2±18.1	100.2±6.7	130.4±11.0	127.4±6.8	127.2±25.2

Table 3. Continued

Compound*	The origin and age of the propolis samples**								
	NK-11	NK-12	NK-13	SK-1	SK-2	CF-1	CO-1	CO-2	NO-1
1	0.7±0.1	0.4±0.03	0.3±0.03	0.5±0.02	0.8±0.03	0.3±0.04	1.5±0.03	1.2±0.1	0.9±0.1
2	0.4±0.02	0.4±0.04	0.2±0.04	0.3±0.01	0.5±0.02	0.2±0.01	0.6±0.1	0.6±0.1	0.5±0.03
3	1.5±0.1	0.7±0.04	2.4±0.4	1.3±0.1	0.9±0.1	1.3±0.3	0.8±0.04	0.9±0.1	1.2±0.04
4	6.8±0.3	2.8±0.2	5.2±0.5	5.7±0.3	6.0±0.8	3.1±0.4	8.4±1.0	6.0±0.6	7.1±0.7
5	11.8±2.9	5.3±1.4	20.6±2.9	18.4±1.7	14.3±3.0	6.2±1.0	7.2±1.3	8.2±0.9	9.9±1.1
6	9.3±1.0	5.2±0.5	9.7±1.1	7.3±0.2	9.5±1.8	4.5±0.5	6.4±0.1	6.5±0.8	10.3±0.5
7	1.5±0.2	1.9±0.2	1.4±0.2	1.2±0.04	1.6±0.1	1.0±0.1	1.5±0.2	1.2±0.1	1.7±0.1
8	0.3±0.03	0.4±0.03	0.1±0.1		0.4±0.05	trace	0.5±0.1	0.6±0.1	0.3±0.01
9	0.3±0.1	0.1±0.01	0.2±0.02	0.2±0.01	0.1±0.02		0.3±0.04	0.3±0.02	0.1±0.01
10	0.7±0.2	0.4±0.04	1.3±0.1	1.0±0.2	1.1±0.2	0.3±0.02	1.3±0.1	1.8±0.3	1.0±0.2
11	0.8±0.1	0.1±0.03	1.0±0.2	1.3±0.1	0.6±0.1	0.7±0.1	0.7±0.05	0.4±0.1	0.2±0.1
12	0.7±0.1	0.1±0.02			0.1±0.1		0.6±0.1		
13	0.3±0.01	0.2±0.02	0.4±0.01	0.3±0.04	0.2±0.02	0.2±0.03	0.3±0.02	0.3±0.01	0.3±0.1
14	1.3±0.2	1.1±0.2	0.8±0.03	1.4±0.1	0.7±0.1	0.7±0.1	0.7±0.1	0.6±0.1	0.8±0.1
15	3.0±0.05	0.9±0.3	1.9±0.2	2.2±0.03	2.0±0.3	1.5±0.3	2.6±0.05	2.3±0.1	2.5±0.2
16	42.0±2.1	35.8±4.8			15.8±1.0	43.0±7.1	22.5±2.4	16.1±2.5	25.2±7.1
17	7.9±0.8	5.8±0.5	13.9±0.4	9.2±0.9	4.9±0.6	6.7±0.7	5.3±0.5	5.6±0.2	6.3±0.7
18	10.1±0.6	11.1±0.9	11.4±0.6	10.6±1.4	8.4±0.6	6.9±0.4	7.5±0.4	5.5±0.2	8.4±0.9
19	18.7±1.4	10.1±1.4	15.3±0.9	16.6±0.6	12.6±1.1	13.4±1.8	19.9±0.5	15.4±1.0	17.7±1.7
20	16.5±0.8	10.5±0.8	15.4±1.0	30.3±3.2	17.7±3.0	12.0±1.5	20.8±2.6	19,9	15.1±2.1
21	11.7±2.3	9.2±1.1	29.0±3.6	30.3±2.0	3.1±0.3	12.3±1.6			4.5±1.2
22	trace	trace			trace		1.5±0.8		
23	2.4±0.6	1.9±0.1			0.3±0.3		0.8±0.4		
24	7.5±1.7	5.4±1.5	5.4±1.5	6.4±0.6	7.5±2.5	4.7±0.4	6.7±0.6	6.5±0.9	8.7±2.3
Total	156.3±11.5	109.7±9.1	135.8±10.6	144.5±3.6	109.3±10.0	119.1±14.7	118.2±3.9	99.9±5.9	122.7±15.4

*Compounds: **1** Cinnamic acid der 1, **2** Vanillic acid. **3** Chlorogenic acid der , **4** ρ-OH-cinnamic acid der 1, **5** Benzoic acid, **6** Ferulic acid, 7 Methyl-cinnamic acid der, **8** Benzoic acid der, **9** ρ -OH-cinnamic acid der 2 **10** Cinnamic acid der 2, **11** Pinocembrin der 1, **12** Pinocembrin der 2, **13** Caffeic acid der **14** Apigenin, **15** ρ -OH-cinnamic acid der 3, **16** Methyl-naringenin, **17** Chlorogenic acid der 2, **18** Chrorogenic acid der 3, **19** ρ -OH-cinnamic acid der 4 **20** Caffeic acid phenethyl ester (CAPE), **21** Pinobanksin der, **22** Di-methyl-kaemferol, **23** Naringenin der 1, **24** Cinnamic acid der 3. (der = derivative of mentioned compound).
** see Table 1.

in Finnish honey samples (Salonen et al., 2009). These trees are expected to be the main plants providing raw material for Finnish propolis. The phenolic compounds that we found in our propolis samples (Tab. 2) are commonly found in the tree species growing in Finland. As an example, *p*-coumaric and ferulic acids can be found in *P. abies* and *Juniperus communis* L. (Strack et al., 1988), apigenins have been found in *B. pendula* buds (Peltonen et al., 2006), and cinnamic acid derivatives are found in the leaves of *B. pendula* and *Salix* spp. (Laitinen et al., 2002). Interestingly, we did not find any salicylates, which are the main components of *Salix* spp. and *P. tremula* leaves and bark (e.g. Julkunen-Tiitto and Sorsa, 2001; Julkunen-Tiitto, 1985). The presence of methyl naringenin is interesting; some samples contained significant amounts (from 10 mg/g to 42 mg/g), other samples did not contain even traces of this compound (Tab. 3). This flavanone has been found from *Betula* bud exudates (Lahtinen et al., 2006) and in the leaves of Norway spruce (Rummukainen et al., 2007). The compounds found in propolis from Northern Russia, and therefore also assumed to originate from *B. verrucosa*

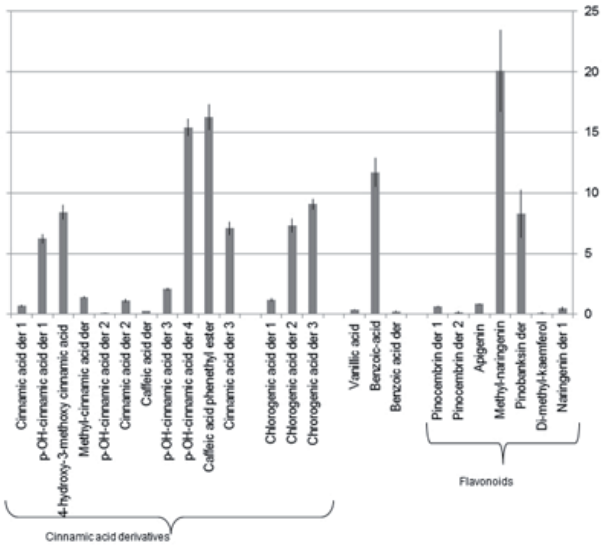

Fig. 1. Average amounts (μg/g propolis ± standard errors) of the identified phenolic compounds.

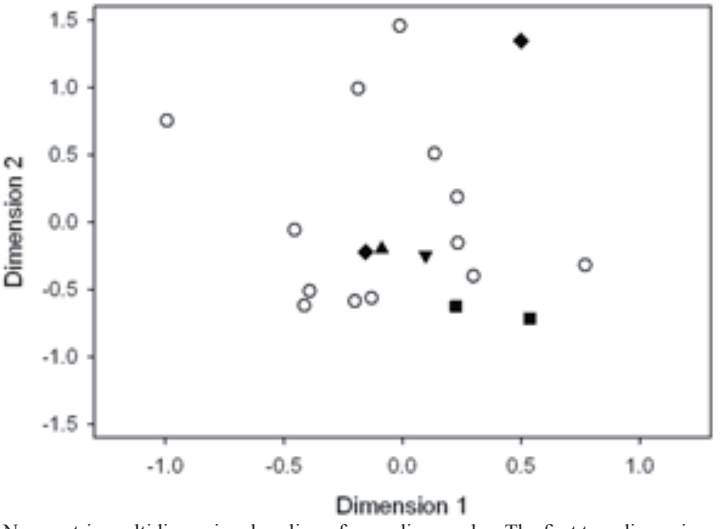

Fig. 2. Non-metric multidimensional scaling of propolis samples. The first two dimensions are shown. Symbols indicating sample origin: open circle = North Karelia, black diamond = South Karelia, upward black triangle = Central Finland, black square = Central Ostrobothnia, downward black triangle = Northern Ostrobothnia.

(*B. pendula*), are acacetin, apigenin, ermanin, rhamnocitrin and kaempferid (Popravko and Sokolov, 1980). Pinocembrin, galangin, ρ-coumaric acid, ferulic acid, benzyl ferulate and benzoic acid have been found in the buds of *P. tremula* (Bankova et al., 1992). Our results indicate that no Finnish tree species can be pointed out as the sole source for Finnish propolis.

Some similarities in phenolic acid profiles between Finnish and Lithuanian propolis was found. Ramanauskienė et al. (2009) found gallic, caffeic, coumaric, ferulic, cinnamic and rosmarinic acids in Lithuanian propolis samples and they

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noted that ferulic and coumaric acids were the predominant phenolic acids in their samples. Both of these acids were found in all of our samples; coumaric acid ranged from 2.79 to 9.30 μg/g and ferulic acid from 4.48 to 14.68 μg/g.

Non-metric multidimensional scaling (NMS) was used to visualize dissimilarities in the phenolic content of the propolis samples. This scaling showed that the samples were scattered and they did not form clear groups according to the geographical origin or age of the sample (Fig. 2). Such results indicate the great variation in the phenolic profiles of the samples and maybe the need for a larger number of samples.

Our purpose was to find out if the phenolic content of the propolis can help us to define the plant-derived phenolic compounds in Finnish unifloral honeys. Our purpose was partly realized. According to our results, it seems that 12 out of the 26 phenolic compounds found in propolis are found in Finnish unifloral honey samples (Salonen et al., 2011).

Finnish propolis, with a high cinnamic acids content, could be a potential promising source for biologically active compounds. It would also be very interesting to test the effect of Finnish propolis against microbes, cancer cells and HIV-virus. More research is needed to draw firmer conclusions on propolis and its effect on honey composition. Continued research should involve sampling from the Southern parts of Finland, which are the main honey producing areas in Finland.

CONCLUSIONS

1. Finnish propolis samples contained 26 individual phenolic compounds.

2. The phenolic profiles of Finnish propolis showed marked differences when compared with *P. nigra* and *P. tremuloides* propolis of Central European and Canadian origins.

3. Non-metric multidimensional scaling showed that samples were scattered and they did not form clear groups according to the geographical origin or age of the sample.

4. No Finnish tree species can be pointed out as the sole source for Finnish propolis.

5. Finnish propolis with high cinnamic acids content could be a potential promising source for biologically active compounds.

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ZWIĄZKI FENOLOWE PROPOLISU Z LASÓW IGLASTYCH STREFY BOREALNEJ

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Streszczenie

Propolis jest lepką substancją o właściwościach antybakteryjnych produkowaną przez pszczoły z wydzielin drzew, wosku pszczelego oraz β-glukozydazy. Celem badań była analiza związków fenolowych propolisu pochodzącego z lasów iglastych strefy borealnej w Finlandii. Zebrane informacje ułatwiają charakterystykę pochodzenia związków fenolowych w miodach odmianowych produkowanych w Finlandii. Umożliwiają dodatkowo poznanie różnic w składzie chemicznym wynikających z różnego miejsca pochodzenia.

Za pomocą HPLC-DAD zbadano 19 próbek propolisu pochodzącego z różnych rejonów Finlandii. W badanych próbkach stwierdzono dziewięć flawonoidów (co stanowi 26% zidentyfikowanych związków fenolowych), jedenaście pochodnych kwasu cynamonowego (36%), dwie pochodne kwasu kawowego (14%), trzy pochodne kwasu chlorogenowego (15%) oraz trzy inne kwasy fenolowe (10%). Metylo-naringenina oraz CAPE zostały wykryte w największych ilościach. Profile fenolowe propolisu pochodzącego z Finlandii różnią się znacznie od propolisu *P. nigra* i *P. tremuloides* pochodzącego z centralnej Europy oraz Kanady. Związki fenolowe znalezione w naszych próbkach propolisu są powszechnie spotykane wśród drzew rosnących w Finlandii, np. kwas p-kumarowy i kwasy ferulowe w *Pinus abies* oraz *Juniperus communis*. Wielowymiarowe niemetryczne skalowanie próbek wykazało, że były rozproszone i nie tworzyły wyraźnych grup w zależności od pochodzenia geograficznego jak i wieku próbki.

Słowa kluczowe: propolis, związki fenolowe, analiza HPLC-DAD, Finlandia.