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JAPANESE KNOTWEED (*FALLOPIA JAPONICA*): LANDSCAPE INVASIVE PLANT VERSUS HIGH QUALITY HONEY SOURCE

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Abstract

Scientific studies show that the darker the honey, the higher its bio-active properties are. Fallopia japonica (Japanese knotweed) is an invasive plant, growing shrub reaching heights of 3 m. Flowering occurs in late summer, when small, greenish-white flowers develop in long panicles in the axils of the leaves. Plants are dioeciously with flowers intensely visited by the bees, the honey obtained from nectar of this plant being a mild-flavored version of buckwheat honey; dark in color, appearing dark red when held to light. The present study aim to determine the chemical composition of Japanese knotweed honey and also their bioactive properties derived from the chemical composition. Different physico-chemical, gravimetric, spectrophotometric and chromatographic determinations were used in the study. Analyzed honey samples are very good sources of minerals, especially K and Na (1187-6196 mg/kg K and 58.8-68.8 mg/kg Na). Also high amounts of Ca were determined. High amounts of total polyphenols and flavonoids were determined in Japanese knotweed honey samples, from several western places of Romania. This could reduce the impact that Fallopia japonica invasive plant have on the habitat, and if this plant is kept under observation and far from the inhabited areas, it could be highly valuable for beekeepers and honey production.

Key words*: Fallopia japonica, plant, honey, chemical composition, antioxidant effect.*

INTRODUCTION

Japanese knotweed (*Fallopia japonica* Houtt., *Reynoutria* spp.), is one of the most troublesome exotic species in Europe and elsewhere (Saintenoy-Simon, 2003; Weber, 2003; Muller, 2004). It has been included in the list of the "100 of the World's Worst Invasive Alien Species" (ISSG). Originating from East Asia, it was introduced in Europe at the end of the 19th century and is now found in many regions and countries. *F. japonica* is a shrub-like rhizomatous geophyte and thus belongs to a functional type not represented in the native vegetation. In Europe, the reproduction of *F. japonica* is only vegetative. Fragments of rhizomes and stems easily re-sprout and can be carried by streams or animals (Weber 2003). The main dispersion agent is human activity through the movement of topsoil containing plant fragments (Dassonville et al., 2007, Fennel et al., 2018).

Knotweeds are perennial herbs with long branched rhizomes, multiple high erect stems and large leaves with ovate or broadly elliptic blade. Inflorescences are axillary or terminal with small white-yellowish flowers (Figure 1). Flowering occurs in late summer, when the flowers develop in long panicles in the axils of the leaves. Plants are dioeciously with flowers intensely visited by the bees. Flowers are functionally monosexual, male with long stamens and short pistils, female with short stamens and distinct pistils (Patocka et al., 2017). The management of this species is very difficult and most of the time not successful. *F. japonica* has been extensively studied. Published studies concern its past and present distribution (Pysek et al., 2001; Mandák et al., 2004), possible impact of climate change on its future distribution (Beerling et al., 1995), genetic diversity (Hollingsworth & Bailey, 2000), impacts on native plant and animal communities (Maertz et al., 2005) and management (Child et al., 2001).

The invasive, strong root system may damage concrete foundations, buildings, roads, paving and architectural sites.

In Romania, this plant grows mainly on the riverbanks of western part of the country, but

also in other places in Transilvania (Dumitraşcu et al., 2012, 2014).
Scientific studies show that the darker the honey, the higher its bio-active properties are. Honey obtained from nectar of this plant being a mild-flavoured version of buckwheat honey, is dark in colour (Figure 1), appearing dark red when held to light. The present study aim to determine the chemical composition of Japanese knotweed honey and their bioactive properties derived from the chemical composition.

Figure 1. *Fallopia japonica* invasive plant and honey

MATERIALS AND METHODS

Honey samples. Fallopia japonica declared honeys (three samples) from the western part of Romania were collected from beekeepers. The botanical origin of honey samples was determined by pollen and physico-chemical analysis. Each honey sample was kept in closed containers in the dark at 4^{o}C until analysis and testing.
Honey Analysis. Honey analysis were carried out in the Laboratory for Quality Control of Bee Products and Bee Diseases in the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. The physicochemical parameters were determined according to Romanian and International Legislation for honey or food ingredients, following standard methods or original methods developed in the lab. Palinological analysis is used for botanical origin determination (Louvreaux et al. 1978). Sugar profile was determined by high performance liquid chromatography (HPLC) with refractive index detection, following the method described in International Honey Commision Methods (Bonta et al., 2008). For the quantification of main sugars, a calibration curve in the range 4–0.5 g/100 g, with regression coefficient of R^2=0.9982 for a mixture of 9 standards (glucose, fructose, saccharose, trehalose, maltose, turanose, isomaltose, erlose, melezitose) was used. Results were expressed in g/100g honey. Water and HMF content, electrical conductivity and diastazic index, were determined following the methods from International Honey Commission, methods also validated in APHIS Laboratory, USAMV Cluj-Napoca (Bobiş et al., 2010; Cimpoiu, et al., 2013). The content of total lipids was determined using Soxhlet method (Soxtherm, Gerhardt, Germany), with an adapted method from literature (Almeida Muradian et al., 2005). Protein content was determined by Kjeldahl digestion , distillation and titration, following an adapted method of Lujerdean and Varga (2002). Total Phenolic Content (TPC) in extracts was determined according to the Folin-Ciocâlteu procedure described by Singleton et al. 1999 with some modifications. Briefly, an aliquot of 25 µL honey solution was mixed with 125 µL Folin Ciocâlteu 0.2N and incubated at room temperature for 5 min. Next, 100 µL of sodium carbonate solution (75g/L) was added and allowed to stand for 2 h at room temperature in the dark. The absorbance of the reaction mixture was read at 760 nm using multichannel spectrophotometer (model Sinergy 2 Biotek). A methanolic gallic acid solution (0.001- 0.15 mg/mL) was used for calibration curve. The $AlCl_3$ method was used for estimation of flavone/flavonol content of the samples (Meda et al., 2005). 150 µL of honey solution was mixed with the same amount of 2% $AlCl_3$ methanolic solution. The mixture was shaken and after 10 min of incubation, absorbance was read at 415 nm in the Sinergy 2 Biotek spectrophotometer. Flavonoid content was calculated from the calibration curve of quercetin standard and expressed as mg quercetin equivalents/g sample. Different essential and trace elements such as Ni, Cr, Fe, Mg, Ca, Mn, Pb, Na, Cd and K in honey samples were analysed using atomic absorption spectrophotometer (Aanalyst 800, CromatecPlus, U.S.A) equipped with graphite furnace. The inert argon gas flow and the temperature parameters were followed as recommended by the manufacturer. The absorption wavelength for determination of each element together with its linear working

range and correlation coefficient of calibration graphs are given in Table 1.

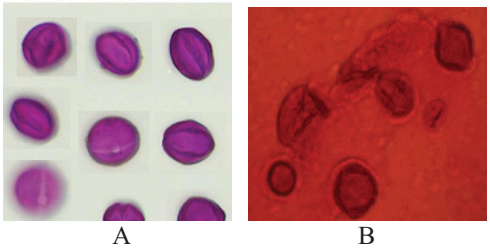
Tab.1. Operating parameters for working elements

Elements flow	Wavelength (nm)	Slit coefficient (nm)	Width correlation (mA)
Fe	248.3	0.2	0.9999
Mg	285.2	0.7	0.9988
Ca	422.7	0.7	0.9984
Na	589.0	0.2	0.9990
K	766.5	0.7	0.9967
Pb	283.3	0.7	0.9923

Statistical analysis. All data are expressed as mean from three replicates of every sample. Results were analyzed using Statistical Package for Windows®. Differences between samples are tested by one-way ANOVA. P values of <0.05 were considered significant.

RESULTS AND DISCUSSIONS

The results of the melissopalynological analyses of honeys declared as Japanese knotweed honey show that the specific pollen was present but not overrepresented (Figure 2).



A B

Fig. 2. Fallopia pollen: A- http://pollen.tstebler.ch; B-knotweed honey pollen

Fallopia japonica plant belongs to Polygonaceae family and honey obtained from the nectar of this plant has similar characteristics as buckwheat honey (Panseri et al., 2013). It is known that buckwheat honey is dark in colour; possess a strong taste due to the volatile profile and very good biological characteristics (antioxidant, antibacterial).

Sensory determination of analysed samples show a sweet aromatic honey, dark brown in colour (Figure 1), crystallized with fine uniform crystals. The aroma is different from any other flower honey and different of buckwheat honey (even the plants belong to the same family).

Main chemical composition parameters and bioactive molecules for the analysed samples are presented in Table 2. They are in accordance with the values stated by existing standards or literature studies.

Tab.2. Analyzed chemical parameters of honey samples.

Parameter	Sample 1	Sample 2	Sample 3
Water content (%)	18.9	20.0	15.6
Electrical conductivity(mS/cm)	0.697	0.647	0.541
Diastazic index (DN)	13.34	11.52	18.74
HMF content (mg/kg)	9.93	0.29	2.54
Fructose (%)	39.83	38.91	38.90
Glucose (%)	31.48	29.30	35.24
Sucrose (%)	0.29	3.18	0.67
Turanose (%)	1.29	1.08	0.74
Maltose (%)	1.96	1.36	2.18
Trehalose (%)	0.44	0.27	0.34
Erlose (%)	0.13	0.22	0.11
Na (mg/kg)	64.62	58.81	68.88
Mg (mg/kg)	15.74	16.27	4.86
Ca (mg/kg)	28.77	46.09	41.84
Fe (mg/kg)	3.68	2.07	3.09
K (mg/kg)	1187.36	1414.56	6196.83
Total nitrogen (%)	1.05	0.47	0.64
Total lipids (%)	0.12	0.52	0.41
Total polyohenols (mgGAE/100g)	195.0	100.0	145.0
Total flavonoids (mgQe/100g)	55.0	20.0	35.0

Water content ranged between 15.6 and 20%, being in the limits of standard. Although Japanese knotweed honey is dark in colour, its electrical conductivity is at the lower limit of the standard for honeydew honey (0.600 mS/cm) (Bogdanov et al., 1997). High diastazic index and low HMF content show an authentic, fresh honey. The main sugars present in the samples were fructose and glucose, with the fructose content higher that glucose (F/G ratio in the analysed samples is higher than 1.2, denoting that honeys remain fluid for a long period of time). The sucrose content is below the upper limit of the standard (5%). In the samples other di and trisaccharides were present, namely turanose (0.74 – 1.29%), maltose (1.36 – 2.18%), trehalose (0.27 – 0.44%), erlose (0.11 – 0.22%).

Lipid content of honey came from the residual pollen present in the sediment. Lipid content of the analysed samples ranged between 0.12 – 0.52%, which is high for honey. The same situation was observed for nitrogen content expressed as total proteins: 0.47 – 1.05%. These parameters make Japanese knotweed honey an important source of protein and amino acids and give this type of honey a high nutritional value.

Mineral content in honey is related to the geographical origin, with the presence of

specific minerals in the soil where the nectar plants are growing. These substances are of nutritional and health importance. Some of the minerals found in honey include calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc. Their amounts are different, with geographical and botanical origin of honeys. Five minerals were determined in the honey samples, and the highest mineral present is by far potassium (118.7 – 619.7 mg/100 g). This amount is very high compared to literature (Tuzen et al., 2007; Alvarez Suarez et al., 2012; Ajibola et al., 2012; Solayman et al., 2015). Also high amounts of calcium and sodium were found (2.87 – 4.61 mg/100 g and 5.88 – 6.88 mg/100 g respectively).

High amounts of total phenolics were determined by means of Folin Ciocâlteu method: 100 – 195 mg GAE/100 g honey as well as flavones/flavonols: 20 – 55 mg QE/100 g. These amounts are higher than those found in honeydew honeys and other dark colour honeys (Gheldorf et al., 2002; Yao et al., 2003; Meda et al., 2005; Lachman et al., 2010).

Even for buckwheat honey, Kaškoniene et al. (2009) found lower amounts of polyphenols, which give our honey important qualities that need to be better explored.

CONCLUSIONS

Honey consumption, as a nutraceutical product, and this type of honey specifically, is associated with various nutritional benefits and therapeutic potential (Cianciosi et al., 2018). The biological activity of honey is determined always by its complex and important components. The composition of honey is strongly influenced by a multitude of factors, which vary with botanical and geographical origins. Minerals are minor constituents of honey, but they play important roles in determining honey quality. The bioactive compounds from the class of polyphenols play also important roles in the honey nutraceutical properties. Further studies are needed on a higher number of samples, but for start, this type of honey, specific to the Western part of Romania need all our attention to elucidate all its properties. This could reduce the impact that *Fallopia japonica* invasive plant have on the habitat, and if this plant is kept under observation and far from the inhabited areas, it could be highly valuable for beekeepers and honey production.

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