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Development, bioactive and sensory analysis of the honey-filled chocolate pralines infused with ground ivy (*Glechoma hederacea* L.) extract

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Abstract

Chocolate is consumed largely worldwide and it is known as one of the most craved foods. The market of chocolate products is growing steadily and is expected to continue to grow in the coming years. The aim of the present study was to formulate chocolate pralines with enriched sensory and bioactive attributes by incorporating different types of honey (false indigo, buckwheat, rapeseed, mandarin and sage) and ground ivy (Glechoma hederacea L.) extract as ingredients of the filler. The honey samples were subjected to physico-chemical analysis, sugar analysis using the HPLC-RID methodology, as well as to evaluation of antioxidant capacity. The bioactive and sensory properties and sugar composition of the formulated pralines were determined. Bioactive characterization included the determination of phenolic profile by HPLC-PAD methodology. Buckwheat honey showed the most pronounced antioxidant capacity with the value of 3.21 and 2.06 µmol Trolox/g dmb, determined by ABTS and DPPH assays, respectively, while the lowest values were measured for mandarin honey (0.41 and 0.32 µmol Trolox/g dmb). Specific bioactive compounds of cocoa: epicatechin, theobromine and caffeine, were detected in all formulated chocolate pralines, as well as the most predominant phenolic compounds of ground ivy: chlorogenic, caffeic, rosmarinic acid and rutin. Such enriched bioactive composition contributed to the pronounced antioxidant capacity of chocolate pralines. Finally, chocolate pralines prepared with mandarin honey were sensory evaluated as the sweetest and gained the highest scores for the overall acceptability.

Keywords: chocolate pralines, ground ivy, honey, polyphenols, sensory evaluation

Introduction

Chocolate is one of the most consumed foods in the world and can be considered an affordable luxury for personal satisfaction (Prete and Samoggia, 2020), as its consumption enhances positive mood, particularly when consumed mindfully (Meier et al., 2017). In 2021, the chocolate confectionery market generated a revenue of approximately 0.99 trillion US dollars worldwide and the generated revenue is expected to increase in the coming years reaching a value of 1.33 trillion dollars in 2027 (Statista, 2022). Chocolate consumption is widespread in Western Europe, with Switzerland, Austria, Germany and Ireland accounting for ~8 kg of chocolate consumed per capita per year (Statista, 2018). Given their popularity, it would be ingenious to enrich chocolate products with ingredients that provide health benefits to offer healthy confectioneries to consumers. It is important to point out that chocolate products themselves, especially dark chocolate, have various health benefits due to the presence of cocoa polyphenols - proanthocyanidins, flavanols and anthocyanins, but can also serve as a great carrier for the delivery of incorporated bioactive compounds, as they can mask unpleasant flavours (Faccinetto-Beltrán et al., 2021). As an illustration, Belščak-Cvitanović et al. (2012) have shown that the addition of dried raspberry leaves in dark chocolate had led to an increase in total polyphenolic content. To implement such enriched confectioneries in regions such as the Balkans, where chocolate consumption is less common, specifically, in Croatia it is 2.2 kg per capita per year (GAIN, 2016), the use of local plant species with traditional applications would be an interesting approach. A potentially suitable plant for this purpose is ground ivy (Glechoma hederacea L.). Ground ivy belongs to the Lamiaceae family and has been used for centuries to treat various diseases such as the common cold, inflammation, diabetes, bronchitis, asthma, jaundice, gallstones, cholecystitis and urinary tract stones (Chou et al., 2019). In addition to traditional plants, natural sweeteners, such as honey, can also be used as a natural source of various bioactive compounds. Honey is a sweet viscous liquid produced by several species of honeybees (Genus Apis) (Farooq et al., 2020) and is considered a superfood with several pharmaceutical properties. In general, honey contains about 200 compounds such as sugars, proteins, enzymes, minerals, vitamins, amino acid and a wide range of polyphenols (Ranneh et al., 2021).

The aim of the present study was to formulate innovative chocolate pralines with incorporated bioactive compounds originated from honey and ground ivy extract. False indigo, buckwheat, rapeseed, mandarin and sage honey, as honeys produced in small quantities, were used and analysed for sugar content and antioxidant capacity. The same analyses were performed on formulated chocolate pralines, along with sensory evaluation and determination of phenolic profile.

Materials and methods

Chemicals

Rosmarinic acid (97%), caffeic acid (HPLC standard), chlorogenic acid (95%), rutin trihydrate (>97%), (S)-6-Methoxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich (USA). Ethanol and formic acid were purchased from Carlo Erba (Germany), methanol from Panreac (Spain) and acetonitrile from Fisher Scientific (USA). Potassium peroxydisulfate, standards of D-glucose, D-fructose and sucrose were purchased from Fluka (Germany) and petroleum ether from Kemika (Croatia). All the chemicals used for experimental procedures were of analytical or HPLC grade.

Materials

Ground ivy was collected in April 2020 in the area of Bilogora (Bjelovar, Croatia). Aerial parts were separated from roots, air-dried at room temperature, ground and sieved to obtain a fraction <450 μm that was used for further applications. A voucher of ground ivy was stored in Flora Croatica Database (University of Zagreb, Faculty of Science, Department of Botany, Croatia) under number 71767.

Honey samples were obtained directly from beekeepers from different locations across Croatia, and before analyses stored in glass containers at room temperature. The year of harvesting for all samples was 2022. The floral origin of honey was specified by the beekeepers.

Cocoa liquor and cocoa butter were purchased from Barry Callebaut (Switzerland), sunflower lecithin from Nutrimedica d.o.o. (Croatia), powder sugar from Franck d.d. (Croatia) and xanthan gum from FREE by Doves farm (UK).

Determination of moisture and dry matter content in honey samples

The moisture and dry matter content in honey samples was determined according to the refractometric method defined by International Honey Commission (2009).

Determination of pH and electrical conductivity of honey samples

The pH of honey samples was measured using a pH meter (Five Easy FE20, Mettler Toledo, Switzerland). Electrical conductivity was measured in 20% (w/v) honey water solutions using a conductivity meter (Lab 945, SI Analytics, Germany).

Determination of sugars by HPLC-RID methodology in honey samples

For the HPLC determination of sugars, 1 g of sample was homogenized in 100 mL of distilled water. Determination of sugars was performed on a Hi-Plex Ca column (300 × 7.7 mm) and the Agilent 1200 Series chromatographic system (Agilent Technologies, USA) coupled with a refractive detector (RID; Agilent Technologies, USA). The mobile phase was water. With respect to refractometric detection, isocratic elution of the analyte at a flow rate of 0.6 mL/min for 15 min was established. The temperature of the column was 80 °C and that of the detector 40 °C. The volume of injected samples was 10 μL. Sucrose, glucose and fructose identification was performed by comparing the retention time with commercially available standards, while quantification was enabled by establishing calibration curves. The analysis was performed in duplicate. All samples were filtered through a 0.45 μm membrane filter (Nylon Membranes, Supelco, USA) prior to the analysis.

Determination of antioxidant capacity of honey samples

Determination of the antioxidant capacity by applying DPPH radical cation decolorization assay was performed with Trolox as a standard for the calibration curve (Brand-Williams et al., 1995). The reaction mixture consisted of honey homogenate in water (100 μL) and 0.094 mM DPPH solution in methanol (3.9 mL). The absorbance was measured after 30 min at 515 nm. The analysis was performed in duplicate.

Determination of antioxidant capacity by applying ABTS•+ radical cation decolorization assay was performed with Trolox as a standard for the calibration curve (Re et al., 1999). The 7 mM ABTS solution (4.912 mL) was mixed with 140 mM potassium peroxydisulfate (88 μL) in water and left to react for 16 h in the dark. Prior to the analysis, the ABTS•+ radical solution was diluted with ethanol to an absorbance of 0.700 at 734 nm. The reaction mixture consisted of honey homogenate in water (40 μL) and of the ABTS•+ radical solution (4.0 mL). The absorbance was measured at 734 nm after 6 min. The analysis was performed in duplicate.

Preparation of ground ivy extract

Extraction was performed with 1 g of ground ivy sample and 100 mL of distilled water for 10 min at 100 °C in water bath (Inko VKZ ERN, Inkolab d.o.o., Croatia). After completion of the extraction, the extract was centrifuged (Thermo Scientific SL8/8R centrifuge, USA; 9500 rpm, 20 min, 4 °C) and the supernatant was concentrated to 10-fold volume under vacuum (IKA RV8, Germany).

Formulation of chocolate pralines

Chocolate pralines were formulated to contain 15% of filler. The filler was prepared to contain 1% of xanthan in the homogenate of the honey prepared in the ground ivy extract (honey:water = 1:1 (w/w)). A total of 5 formulations were prepared with different types of honey – false indigo (sample CP_F), buckwheat (sample CP_B), rapeseed (sample CP_R), mandarin (sample CP_M) and sage (sample CP_S). The chocolate coating contained 69.5% of cocoa liquor, 15% of cocoa butter, 15% of sucrose and 0.5% of sunflower lecithin.

Characterization of chocolates pralines

The prepared chocolate pralines (~10 g) were crushed, transferred to a 50 mL Eppendorf tubes in which 20 mL of petroleum ether was added in order to remove the fat from the samples. The samples were stirred on a magnetic stirrer for 15 min, centrifuged (9500 rpm, 15 min), after which the supernatants were discarded and the residues were defatted once again with petroleum ether. Defatted pralines were used for the determination of sugars, polyphenols and methylxanthines. The sugar extraction was performed in a water bath (Inko VKZ ERN, Inkolab d.o.o., Croatia) at 80 °C for 2 h with distilled water as a solvent. The sugar content was analysed using HPLC-RID methodology as described earlier. For the extraction of polyphenols and methylxanthines, 1 g of sample and 20 mL of methanolic solution (80%, v/v) were used. The extraction was performed in ultrasound bath (Elmasonic 2 120, Elma, Singen, Germany) with a nominal power of 200 W and a frequency of 37 kHz during 15 min at 50 °C. After the completion of the extraction, the samples were centrifuged (9500 rpm, 15 min), after which the supernatants were collected and the residues were extracted once again under the same parameters. Supernatants of the first and second extractions were merged and analysed. The prepared extracts were subjected to HPLC analysis on Agilent Series 1200 chromatographic system (Agilent Technologies, USA) coupled with a photodiode array detector (PAD) and using Zorbax Extend C18 (4.6 × 250 mm, i.d., 5 μm) chromatographic column (Agilent Technologies, USA). The elution was performed in a gradient with a two-component mobile phase consisting of 1% (v/v) formic acid solution in water and 1% (v/v) formic

acid solution in acetonitrile as described in the study by Šeremet et al. (2021). The same extracts were used for the determination of antioxidant capacity. The analysis was performed in duplicate. All samples were filtered through a 0.45 μm membrane filter (Nylon Membranes, Supelco, USA) prior to the HPLC analysis.

Sensory evaluation of chocolate pralines

The sensory evaluation of pralines was performed to evaluate the selected parameters including sweetness, sourness, honey aroma and herbal aroma using a 9-point scale where 9 represents a high intensity, while 1 represents a low intensity. A 9-point hedonic scale including 9 liking degrees (points) - from "dislike extremely" (1) to "like extremely" (9) was used in assessment of overall acceptability. The samples were presented to 10 trained panel members between the ages of 20 and 45. The chocolate pralines were presented to the members of the panel at room temperature.

Statistical analysis

One-way ANOVA and Tukey's post hoc test were performed in the Statistica (v.14, TIBCO Software Inc.) software. The differences were considered significant at $p<0.05$.

Results and discussion

Physico-chemical parameters of honey samples

The content of dry matter, values of pH and electrical conductivity of honey samples are presented in Table 1.

The content of dry matter was in the range from 82.68% (mandarin honey) to 85.97% (rapeseed honey). EU regulations require a maximum moisture content of 20% in all types of honey, except heather honey, for which up to 23% is allowed (EC, 2001). In the present study, moisture content below 20% was determined in all samples. Low water content is desirable because honey may begin to ferment and lose its fresh quality (Tafere, 2021). Generally, the pH values of honey range from 3.2 to 6.5. High acidity results from the fermentation of honey, while the low pH of honey inhibits the growth of microorganisms (Čalopek et al., 2016). In the present study, pH of honey samples was in a narrow range from 3.51 (buckwheat honey) to 4.24 (sage honey). Electrical conductivity is a property that largely depends on the concentration of mineral salts, organic acids and proteins in honey (Chua et al., 2012). Thus, higher electrical conductivity is linked with the higher total mineral content. The lowest electrical conductivity (187.7 μS/cm) was determined in false indigo honey and the highest (406.0 μS/cm) in rapeseed honey. According to the regulations for honey, according to which the highest permissible value of electrical conductivity is 0.8 mS/cm (Croatian Regulation, 2015), all samples are of correct quality.

Sugar content in honey samples

Sugars are produced by honeybees from nectar 's sucrose that is converted by the action of enzymes α- and β-glucosidase, α- and β-amylase and β-fructosidase. Monosaccharides are the most abundant carbohydrates in honey and account for 65 - 80% of total soluble solids (Kolayli et al., 2012). Sugar analysis was performed using HPLC-RID methodology and the results are presented in Table 2.

As expected, glucose and fructose were dominant sugars in the honey samples. The highest content of glucose (37.80% dmb) and fructose (40.18% dmb) was found in mandarin honey. The lowest content of glucose (30.71 and 30.67% dmb) was determined in buckwheat and rapeseed honeys and the lowest content of fructose (34.64 and 34.94% dmb) in buckwheat and sage honeys. The results are in accordance with the study of Alshammari et al. (2022), who determined glucose content to be in the range from ~20% to ~32% and fructose from ~28% to ~37%. Generally, the average ratio of fructose to glucose is 1.2:1, but this ratio depends largely on the source of the nectar from which the honey was obtained. This ratio is used to evaluate the crystallization of the honey, since glucose is less soluble in water as compared to fructose (da Silva et al., 2016). It is reported that a ratio of fructose to glucose of 1.14 or less would indicate fast granulation, while values over 1.58 are associated with no tendency to granulation (Kolayli et al., 2012). According to the presented results, only rapeseed honey showed no tendency to fast granulation.

Table 1. Dry matter content, pH and electrical conductivity of honey samples

Sample	Content of dry matter (%)	pH	Electrical conductivity (μS/cm)
False indigo	84.47±0.12^{ab}	3.56±0.04^{ab}	187.7±0.3
Mandarin	82.68±0.57	3.56±0.01^{ac}	210.1±0.9
Buckwheat	84.57±0.33^{ac}	3.51±0.00^{bc}	374.5±3.5
Rapeseed	85.97±0.58	3.78±0.02	406.0±3.0
Sage	84.50±0.07^{bc}	4.24±0.03	356.5±4.5

Means denoted in the same column with the same superscript letters are not significantly different ($p>0.05$).

Table 2. Sugar content in honey samples

Sample	Glucose (% dmb)	Fructose (% dmb)	Σ (glucose and fructose)	Fructose/glucose ratio
False indigo	33.06±0.05^{a}	36.20±0.26	71.27±0.31	1.16±0.01
Mandarin	37.80±0.08	40.18±0.19	77.98±0.27	1.06±0.00^{a}
Buckwheat	30.71±0.03^{b}	34.64±0.08^{a}	65.35±0.11	1.13±0.00
Rapeseed	30.67±0.45^{b}	37.27±0.24	67.94±0.69^{a}	1.22±0.01
Sage	32.51±0.35^{a}	34.94±0.10^{a}	67.46±0.46^{a}	1.07±0.01^{a}

dmb-dry matter basis; Means denoted in the same column with the same superscript letters are not significantly different ($p>0.05$).

Antioxidant capacity of honey samples

The antioxidant capacity of the honey samples is presented in Figure 1. Buckwheat honey showed the most pronounced antioxidant capacity – 3.21 and 2.06 μmol Trolox/g dmb, determined by ABTS and DPPH assays, respectively, while the lowest values were measured for mandarin honey – 0.41 and 0.32 μmol Trolox/g dmb. The values of antioxidant capacity of the honey samples determined by ABTS method are slightly higher than those determined by DPPH method, which can be explained by the ability of ABTS radical to react with a broader range of antioxidant compounds (Mareček et al., 2017). Generally, antioxidant capacity of honey originates from different antioxidants, such as organic acids, flavonoids, phenolic acids, carotenoid derivatives, enzymes (catalase, glucose-oxidase), vitamins (ascorbic acid) and amino acids (Nicewicz et al., 2021).

Sugar content of chocolate pralines

The content of sucrose, glucose and fructose in chocolate pralines is presented in Table 3.

The sucrose content in chocolate pralines was within the expected range (10 – 12%) as the chocolate liquor used for the formulation contained 15% sucrose and constituted ~85% of the praline. In accordance with the analyses of sugar content in the honey samples (Table 2), the highest content of glucose (13.71 mg/g) and fructose (15.29 mg/g) was determined in pralines with mandarin honey (sample CP_M).

Bioactive characterization of chocolate pralines

Bioactive characterization of the chocolate pralines included determination of antioxidant capacity and content of individual phenolic compounds and methylxanthines. The results are presented in Figure 2 and Table 4.

Means denoted with the same superscript letters are not significantly different (p>0.05).

Figure 2. Antioxidant capacity of chocolate pralines

In accordance with the determination of antioxidant capacity of the honey samples (Figure 1), the highest antioxidant capacity (60.02 and 58.86 μmol Trolox/g) was determined in pralines with buckwheat honey (sample CP_B).

Specific bioactive compounds of cocoa – epicatechin, theobromine and caffeine, were detected in all formulated chocolate pralines, as well as the most predominant phenolic compounds of ground ivy – chlorogenic, caffeic, rosmarinic acid and rutin.

Sensory evaluation of chocolate pralines

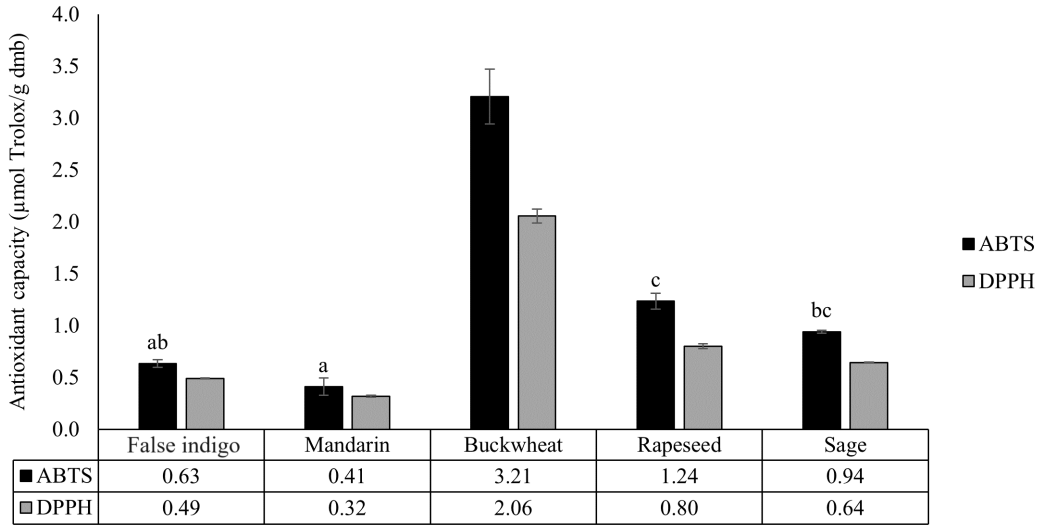
Sensory evaluation of the chocolate pralines is presented in Figure 3.

All chocolate pralines were sensory evaluated as mildly bitter, with scores in a narrow range from 4.3 (samples CP_M and CP_B) to 4.9 (sample CP_R). The sweetness of the pralines was of higher intensity and pralines with mandarin honey were evaluated as the sweetest with a score of 5.6 (sample CP_M). Honey aroma was most pronounced in the pralines with mandarin (sample CP_M) and buckwheat (sample CP_B) honey with scores of 5.2. and 5.5, respectively. Herbal aroma, originating from incorporated ground ivy extract, was not perceived by the panellist as the highest score was 3.1 (sample CP_B). Finally, all chocolate pralines were generally scored as highly acceptable. The lowest liking degrees (points) of overall acceptability (6.1) was determined for pralines with rapeseed honey (sample CP_R) and the highest (7.0) for pralines with mandarin (sample CP_M).

Table 3. Content (mg/g) of sugars in chocolate pralines

Sample	CP_F	CP_M	CP_B	CP_R	CP_S
Sucrose	127.58±1.20^{a}	121.52±1.38	116.67±0.28	111.42±0.11	109.32±0.31^{a}
Glucose	10.86±0.06	13.71±0.08	10.25±0.10^{a}	10.31±0.07^{ab}	10.48±0.06^{b}
Fructose	13.38±0.05	15.29±0.08	12.51±0.00	11.40±0.03	10.72±0.06

Means denoted in the same row with the same superscript letters are not significantly different (p>0.05).



dmb-dry matter basis; Means denoted with the same superscript letters are not significantly different (p>0.05).

Figure 1. Antioxidant capacity of honey samples

Table 4. Content of polyphenols and methylxanthines in chocolate pralines

Polyphenols and methylxanthines from chocolate liquor (mg/g)					
	CP_F	CP_M	CP_B	CP_R	CP_S
Theobromine	2.52±0.00	3.97±0.00	3.67±0.01	3.83±0.00	4.19±0.01
Caffeine	0.32±0.00	0.49±0.00[a]	0.40±0.00	0.43±0.01	0.49±0.00[a]
Epicatechin	0.51±0.01	0.80±0.01[a]	0.66±0.01	0.73±0.01	0.78±0.00[a]
Polyphenols from ground ivy extract (µg/g)					
Chlorogenic acid	3.01±0.08	4.23±0.05	3.85±0.02	4.77±0.03[a]	4.88±0.05[a]
Caffeic acid	2.69±0.00	4.35±0.01	3.30±0.02	3.75±0.01	3.84±0.02
Rosmarinic acid	1.05±0.04[a]	1.84±0.07	1.18±0.09[a]	1.35±0.05[b]	1.42±0.02[b]
Rutin	1.27±0.07	2.97±0.11	1.53±0.03	2.26±0.16[a]	2.11±0.05[a]

Means denoted in the same row with the same superscript letters are not significantly different (p>0.05).

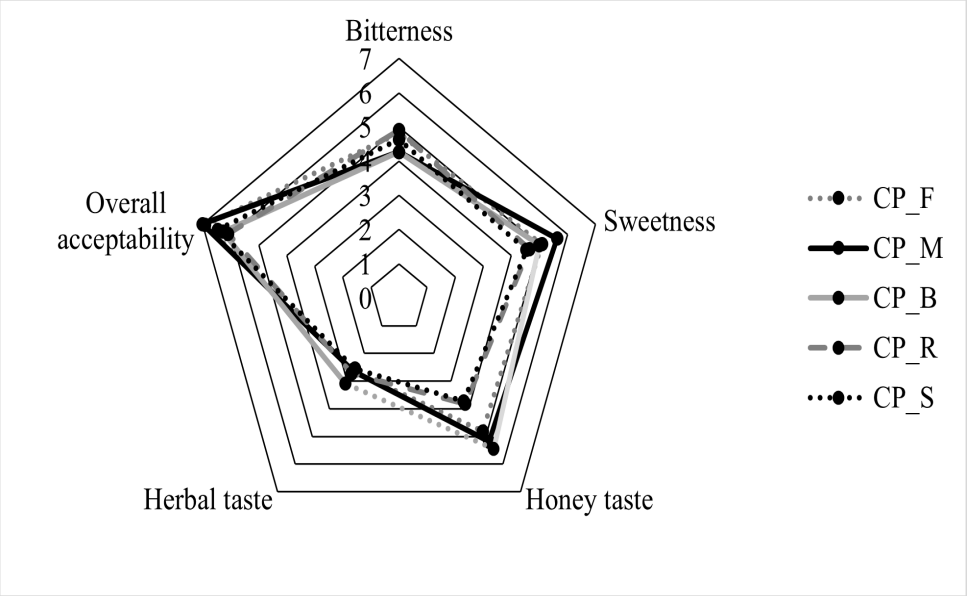


Figure 3. Sensory evaluation of chocolate pralines

Conclusions

The investigated honey samples showed satisfactory quality in terms of physico-chemical characterization, as well as sugar analysis. They also showed to possess a certain antioxidant capacity. Ground ivy extract and investigated honey samples were successfully used in combination as a filler for the chocolate pralines, as all the formulated pralines were sensory evaluated as highly acceptable. Ground ivy extract served successfully as a natural source of bioactive compounds, as its bitterness in the chocolate pralines was masked by honey and chocolate liquor.

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