

Theses of doctoral (PhD) dissertation

**ANALYSIS OF MONOFLORAL HONEYS
ENRICHED WITH DRIED HERBS, SPICES AND
LYOPHILIZED FRUITS**

Written by: Emőke Anita Topa

Supervisors: Dr. Béla Róbert Kovács
academic

Dr. Nikolett Czipa
associate professor



UNIVERSITY OF DEBRECEN
Kerpely Kálmán Doctoral School

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1. Background and objectives of the doctoral dissertation

Honey is a natural substance produced by *Apis mellifera* bees, which can originate from plant nectar, honeydew, or a mixture of bee-collected nectar with bee secretions. Numerous historical records have been found indicating that honey has been known and used since the ancient times. Pre-conquest records from Hungary regarding to honey production are also available. After our ancestors had occupied the Carpathian Basin, they learned and continued the craft of beekeeping. The unique climate and technological options are remarkably beneficial for honey production (SZALAY, 1992). We produce important monofloral honeys, such as linden, canola, acacia, sunflower, phacelia, silkweed and chestnut honey, but forest, lavender and sage honeys are also becoming more popular. The climate of Hungary is beneficial for the demands of many plants, such as acacia, therefore our beekeepers produce significant quantity of acacia honey. Due to its reputation and significance regarding to its purity, the Hungaricum Committee has declared acacia honey a Hungaricum in 2014.

It is commonly known that honey has various properties which might be remarkably beneficial for the human organism (CIUCIRE – GEANA, 2019). It contains sugars, moisture, amino acids, proteins, pigments, minerals, volatile compounds and enzymes in variable proportions. Furthermore, it contains phenolic compounds and flavonoids, which have antimicrobial, anti-inflammatory, anticancer and antioxidant effects. Phenolic compounds found in honeys contribute to the total biological activity, quality, colour and other organoleptic characteristics of honey (CAN et al., 2015). However, these compounds are present in honey in low concentrations. Only a few research was published on similar studies, but these investigations prove that these values might increase by the addition of specific plants. The aim of my study was the analysis of well known monofloral honeys, and the improvement of the quality parameters of these honeys by the addition of different fruits, herbs and spices.

I analysed acacia, linden, canola and sunflower honey. I have enriched canola and sunflower honeys with 5 fruits (strawberry, raspberry, sour cherry, blackberry, blueberry), while acacia and linden honeys were enriched with 10 herbs and spices (basil, marjoram, lovage, sage, rosemary, garlic, anise, peppermint, lemon balm, ginger).

My research included the determination of polyphenol-, flavonoid and element content of the applied fruits, spices and honeys. Besides, I have also analysed moisture and sugar content, electrical conductivity, pH, HMF, proline content and diastase activity of the honeys enriched with the above-mentioned materials.

The analytical measurements were followed by the statistical analyses of the data collected. I have calculated mean and standard deviation. To determine statistically verified differences between the results, I have applied one-way ANOVA, during which I have used Tukey and Dunnett's T3 tests considering the homogeneity of the samples. In case of the monofloral honeys, I have carried out a Linear Discriminant Analysis (LDA), and I have also carried out the Pearson correlation analysis in case of the analysed parameters.

2. Material and methods

The measurements were carried out in the Institute of Food Science, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen. Honeys and fruits were collected from domestic producers and commercial units. Canola and sunflower honeys were enriched by lyophilized, crumbled fruits (strawberry, raspberry, sour cherry, blackberry and blueberry) in the following concentrations: 2.5%, 5.0%, 7.5%, 10%. The analysis was carried out 1 week after the enrichment, during which I have not removed the fruits from the samples.

Acacia and linden honeys were enriched by spices and herbs (basil, marjoram, lovage, sage, rosemary, garlic, anise, peppermint, lemon balm, ginger) in the following concentrations: 1.25%, 2.5%, 3.75%, 5.0%. In this case, I have carried out the analyses for 6 months in every 2nd month. I have removed the spices from the samples in every case before the measurements. Spices were collected in a dried form from commercial units.

Prior to the enrichments, I have collected 10 of each samples from different apicultures. I have chosen the most applicable honeys for the enrichment based on the analyses carried out.

2.1. Lyophilization and determination of dry matter content of the fruits

I have collected fresh fruits from primary producers. Following the purification, I have placed the samples on trays and froze them in a freezer. After that, I have carried out the drying (-45 °C, 24-28 hrs) by a freeze-drying apparatus (Heto Powerdry PL 9000). After the drying, I have powdered the fruits with a pestle or electric grinding device.

Dry matter content of the fruits was determined by an oven-drying method (Memmert UF 75 Universal Oven, Memmert GmbH & Co. KG, Schwabach, Germany). I have ground the samples with a mixer, then placed them in a petri dish. The drying was carried out at 55 °C for a few hours, followed by cooling the samples in a desiccator. After reaching room temperature, the weight of the samples were measured by an analytical scale (LPC-313, VWR International). I have calculated the dry matter content, expressed in %.

2.2. Determination of moisture and sugar content

Moisture and sugar content of the honeys were measured jointly by a handheld refractometer (ATAGO 2514-E03, MASTER-HONEY/BX, Japan). This device was developed specifically for the analysis of honey samples. A few drops of honey is dripped on the prism, then it was closed airtight. Turning the device towards light, moisture and sugar content of the sample can be read from the scales of the device, expressed in %.

2.3. Determination of total polyphenol content

Principle of the determination of polyphenols, or phenolic compounds is based on Folin-Ciocalteu reagent, which was used by Singleton et al. (1999). The reagent contains phosphotungstic and phosphomolybdic acid, which oxidizes these compounds, resulting in a blue colour change. The darker the solution is, the more phenolic compounds can be found in the sample. Colour intensity and phenolic content is proportionate, therefore the absorbance of the samples can be measured by spectrophotometer (Evolution 300 LC, Thermo Electron Corporation, England) at a wavelength of 760 nm, against the mixture of methanol and distilled water (MeOH:DW; 80:20). To prepare the calibration solutions, a gallic acid stock solution was used. Results were expressed in mg GAE (gallic acid equivalent)/100 g.

Applied chemicals: gallic acid or 3,4,5-trihydroxybenzoic acid(Alfa Aesar GmbH & Co. KG, Karlsruhe, Germany), Folin-Ciocalteu reagent (VWR International S.A.S., France), methanol (Scharlab S.L., Spain), sodium carbonate (Sigma-Aldrich Chemie GmbH, Germany).

2.4. Determination of flavonoid content

The determination of flavonoid content was also carried out by a spectrophotometer, based on the method of Kim et al. (2003). Absorbance of the samples was measured at 510 nm. To prepare the calibration solutions, catechin stock solution was used. Results were expressed in mg CE (catechin equivalent)/100 g.

Applied chemicals: catechin (Cayman Chemical Company, USA), methanol (Scharlab S.L., Spain), sodium nitrite (Scharlau Chemie S.A., Spain), sodium hydroxide (Sigma-Aldrich Chemie GmbH, Germany), aluminium chloride (Scharlab S.L., Spain).

2.5. Determination of diastase activity

The determination of diastase activity was carried out by SCHADE-WHITE-HADORN method, based on MSZ 6943-6:1981 standard. The method includes the application of an indicator and a spectrophotometric (Evolution 300 LC, Thermo Electron Corporation, England) measurement. Calculation of the results happened by graphic representation. Results are expressed in Goethe's number, which indicates the volume (ml) of 1% starch solution degraded at 40 °C in an hour by the α and β amylase found in 1 g honey sample.

Applied chemicals: iodine (Sigma-Aldrich Chemie GmbH, Germany), potassium iodide (Sigma-Aldrich Chemie GmbH, Germany), sodium acetate (Scharlab S.L., Spain), sodium chloride (VWR International S.A.S., France), starch (VWR International S.A.S., France), acetic acid (VWR International S.A.S., France).

2.6. Determination of proline content

Proline content of honey samples were measured based on the method of OUGH (1969). Its principle is that ninhydrin reacts with the proline content of the sample, forming a coloured compound. Colour intensity is measured by spectrophotometer (Evolution 300 LC, Thermo Electron Corporation, England) at 520 nm.

Applied chemicals: L-proline (Sigma-Aldrich Chemie GmbH, Germany), formic acid (VWR International S.A.S., France), ninhydrin (VWR International S.A.S., France), ethylene glycol monomethyl ether (VWR International S.A.S., France), isopropanol (VWR International S.A.S., France).

2.7. Determination of hydroxymethylfurfural (HMF) content

HMF content of honeys was determined based on MSZ 6943-5:1989 (White method) standard, which is identical to AOAC 980.23 method. Its principle is to measure the absorbance of the honey solution by spectrophotometer (Evolution 300 LC, Thermo Electron Corporation, England) against a blank solution, in which the chromophore (which has the absorption maximum at 284 nm) is degraded by hydrogen sulphite. HMF content is calculated based on the adjusted absorbance, results are expressed in mg/100g.

Applied chemicals: crystalline potassium hexacyanoferrate(II) trihydrate (Scharlab S.L., Spain), crystalline zinc acetate dehydrate (Scharlab S.L., Spain), sodium disulfite (Scharlab S.L., Spain).

2.8. Determination of pH

pH of honey samples was carried out based on MSZ 6943/3-80 standard. Mettler Toledo FiveEasy F20 (Mettler-Toledo GmbH, Switzerland) and Mettler Toledo LE438 (Mettler-Toledo GmbH, Switzerland) electrodes were applied.

2.9. Determination of electrical conductivity

Electrical conductivity of the honey samples was carried out based on the method of Bogdanov (1997). Electrical conductivity of the honey solutions (containing 20% dry matter of honey) was measured by an applicable apparatus (Mettler Toledo FiveEasy FE30; Mettler-Toledo GmbH, Switzerland). For the determination, Mettler-Toledo LE703 (Mettler-Toledo GmbH, Switzerland) was applied. Results were expressed in $\mu\text{S}/\text{cm}$.

2.10. Determination of element content

Sample preparation for the measurement was carried out based on the method of Kovács et al. (1996). Digestion was carried out at 60 and 120 °C by the addition of concentrated nitric acid (69%, VWR International LTD., Radnor, USA) and hydrogen peroxide (30%, VWR International LTD., Radnor, USA). The digestion was followed by the dilution of the samples with ultrapure distilled water (Milli-Q water purification system, Millipore SAS, Molsheim, France) and a filtration through qualitative filter paper (grade: 388, Sartorius Stedim Biotech S.A., Gottingen, Germany). Element content of the samples was measured by Inductively Coupled Plasma Optical Emission Spectrometer (Thermo Scientific iCAP 6300, Cambridge, UK). Results were expressed in mg/kg. In some cases, the element contents did not exceed the limit of detection, which are the followings: barium content of fruity honeys (0.100 mg/kg), copper content of acacia honeys enriched with spices (0.100 mg/kg).

2.11. Statistical analysis

The analytical measurements were carried out in triplicate. To evaluate the results, I have used SPSS software (version 23; SPSS Inc. Chicago, Illinois, USA). I have calculated mean and standard deviation. To find statistically verified differences between the results, I have applied One-Way ANOVA, specifically Tukey and Dunnett's T3 tests considering the homogeneity of the results. In case of the monofloral honeys, I carried out a linear discriminant analysis (LDA). Besides those, I have applied Pearson correlation analysis for the analysed parameters.

3. Results

3.1. Monofloral honeys

Honeys contain compounds such as antioxidants and minerals which have an important role in the maintenance of human health. On the other hand, these compounds are present in honeys in low concentrations. In this study, I aimed to increase the concentration of these compounds by the addition of different plants. Honey samples (canola, sunflower, acacia, linden) were chosen based on the analysis of honeys from numerous beekeepers. Since „colourful fruits” are considered outstandingly healthy, this was one of the factors influencing the choice, and the other important aspect was their cultivability in Hungary. Therefore, strawberry, raspberry, sour cherry, blackberry and blueberry was used for the enrichment. The selection of herbs and spices was carried out based on literature data. Basil, marjoram, lovage, sage, rosemary, garlic, anise, peppermint, lemon balm and ginger was applied.

Results of the canola, sunflower, acacia and linden honeys are indicated in table 1.

Table 1.: Results of the monofloral honeys

Parameter	Canola honey (n=10)	Sunflower honey (n=10)	Acacia honey (n=10)	Linden honey (n=10)
TPC (mg GAE/100 g)	26.5±2.3 (23.3-29.3)	44.8±7.0 (37.6-59.3)	17.5±1.1 (15.9-19.0)	39.2±5.1 (31.7-45.6)
Moisture content (%)	19.3±0.3 (18.8-19.6)	19.5±0.3 (18.9-19.9)	18.4±0.2 (18.2-18.9)	18.3±0.4 (17.6-18.9)
Conductivity (μS/cm)	147±10 (135-162)	520±68 (452-668)	116±8 (105-125)	830±49 (752-912)
Proline content (mg/kg)	301±47 (198-361)	991±161 (815-1273)	235±18 (206-267)	612±95 (488-744)
Diastase activity (DN)	22.1±1.0 (20.1-23.4)	19.0±1.2 (17.5-20.4)	14.8±0.6 (13.9-15.6)	23.3±2.5 (19.6-26.4)
Ca content (mg/kg)	41.1±5.2 (34.2-49.1)	108±14 (82.9-124)	11.3±2.7 (8.39-16.2)	82.4±9.0 (70.3-98.3)
K content (mg/kg)	174±14 (162-198)	554±50 (519-689)	123±9 (111-137)	1061±94 (934-1230)
Mg content (mg/kg)	12.4±0.8 (11.2-13.6)	28.8±4.9 (23.1-36.9)	4.38±0.47 (3.53-5.16)	20.0±2.8 (16.3-22.3)
Na content (mg/kg)	7.03±0.64 (6.13-7.81)	6.49±1.70 (4.32-8.89)	3.50±0.27 (3.19-3.97)	11.9±2.4 (10.1-17.1)
P content (mg/kg)	41.1±3.3 (35.5-45.5)	71.2±6.2 (62.4-79.9)	36.1±3.62 (32.0-43.8)	50.5±5.2 (42.5-58.2)
S content (mg/kg)	23.9±2.0 (20.6-27.8)	29.3±3.0 (25.2-33.9)	13.5±1.9 (12.1-18.1)	26.9±5.4 (20.1-34.5)

Source: Own editing

The highest polyphenol and proline contents were measured in the sunflower honeys, while the highest diastase activity was measured in case of linden honeys. In case of total polyphenol content, there was no statistically verified difference between linden and sunflower honeys ($p=0.271$), either in case of linden and canola honeys' diastase activity ($p=0.571$), but regarding to the proline content and electrical conductivity, every honey type showed significant difference from each other.

Sunflower honeys showed the highest calcium, magnesium, phosphorus and sulphur content, and the highest potassium and sodium contents were found in linden honeys. Statistically verified difference could not be observed only in case of the sodium content of sunflower and linden honeys ($p=0.895$), and the sulphur contents of linden and sunflower, and linden and canola honeys ($p=0.843$; $p=0.509$). In every other case, the analysed honey types showed significant differences from each other.

As differences were verified in case of several parameters of honeys, I have carried out the linear discriminant analysis (LDA) to determine whether these parameters could be suitable for the classification of the honey types. The result of the analysis is illustrated by Figure 1. Honey type was the grouping variable, while the independent variables were the analysed parameter, except for moisture content. Out of the independent variables, potassium content, calcium content and electrical conductivity had the highest effect on the discriminant function (Wilks' Lambda = 0.018; 0.020; 0.046), while sulphur and sodium content had the lowest effect (Wilks' Lambda = 0.227; 0.186). Three discriminant functions were determined, the first one explained 99.2% of total variance, while this ratio in case of the other functions were 96.0 and 87.6%. The first function contained potassium content and conductivity, the second one contained calcium proline content, phosphorus content, magnesium content and TPC, while the third one contained diastase activity, sodium and sulphur content. Based on the eigenvalues, acacia (-9.954) and canola (-9.135) honeys showed very low values in the first dimension, and these values are close to each other, but the difference between these two types are much higher in the second dimension (acacia: -3.618; canola: -1.007). Linden honeys showed the highest eigenvalue in the first dimension (17.666), while sunflower honeys showed the highest one in the second dimension (7.814). In the third dimension, canola honeys had the highest value (3.823), while the lowest was shown by acacia honeys (-3.191). The cross-validation verified my hypothesis, that monofloral honeys could be classified into groups based on the parameters, since the result of the cross-validation was 100%.

Therefore, we can declare that the analysed parameters are suitable for the classification of these monofloral honeys.

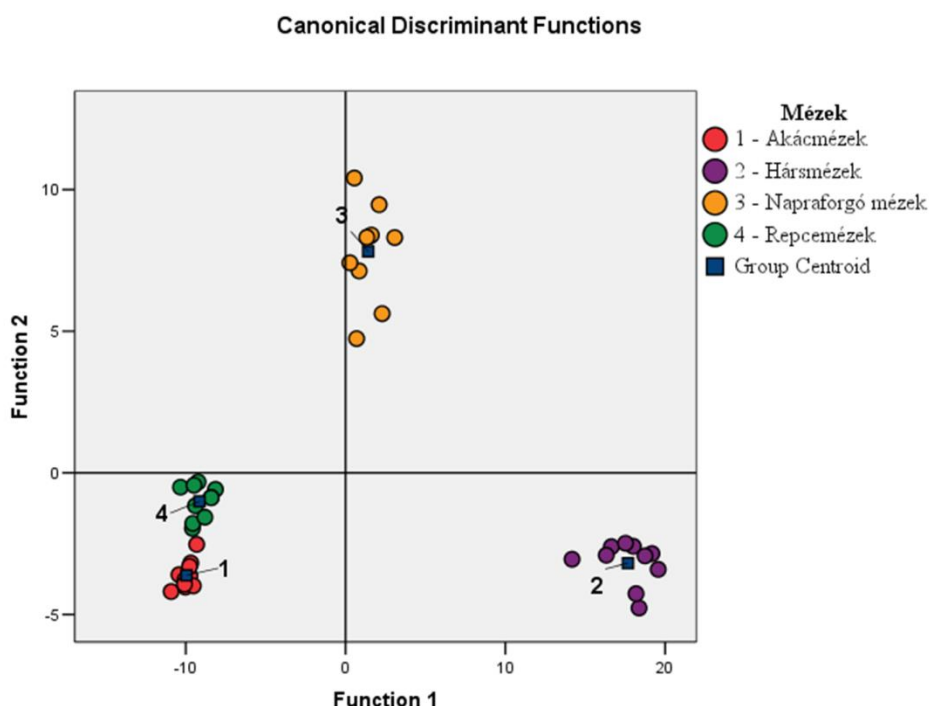


Figure 1: Linear Discriminant Analysis

Source: Own editing

3.2. Results of the honeys enriched with fruits

The addition of lyophilized fruits caused an increase in the electrical conductivity (Figure 2.). The increase was much stronger in case of canola honeys than in case of sunflower, because sunflower honeys showed three times higher electrical conductivity than canola honeys. The addition of strawberry, raspberry and sour cherry caused similar increase in case of canola honeys. In case of 2.5% fruit content, it caused 2-2.5 times higher (285-318 $\mu\text{S}/\text{cm}$), in case of 5.0% it caused 3-3.5 times (410-482 $\mu\text{S}/\text{cm}$), in case of 7.5% it caused 4-5 times (541-639 $\mu\text{S}/\text{cm}$), while in case of 10% it caused 5-6 times higher results. Due to the addition of blueberry, the increase of the electrical conductivity was lower, twice as high in vase of 2.5% fruit content (274 $\mu\text{S}/\text{cm}$), and 3.5 times (454 $\mu\text{S}/\text{cm}$) higher in case of 10% enrichment. Similar increase could be observed in case of sunflower honeys, which was 1.5 times higher (459-499 $\mu\text{S}/\text{cm}$) in case of 2.5% fruit content, 1.7 times higher (564-616 $\mu\text{S}/\text{cm}$) in case of 5%, twice as high (644-685 $\mu\text{S}/\text{cm}$) in case of 7.5%, and 2.2 times (719-792 $\mu\text{S}/\text{cm}$) higher in case of 10% fruit content in case of every

fruit. Maximal electrical conductivity of honey is 800 $\mu\text{S}/\text{cm}$ based on Directive no. 1-3-2001/110 Codex Alimentarius Hungaricus, which criterion was met by every sample.

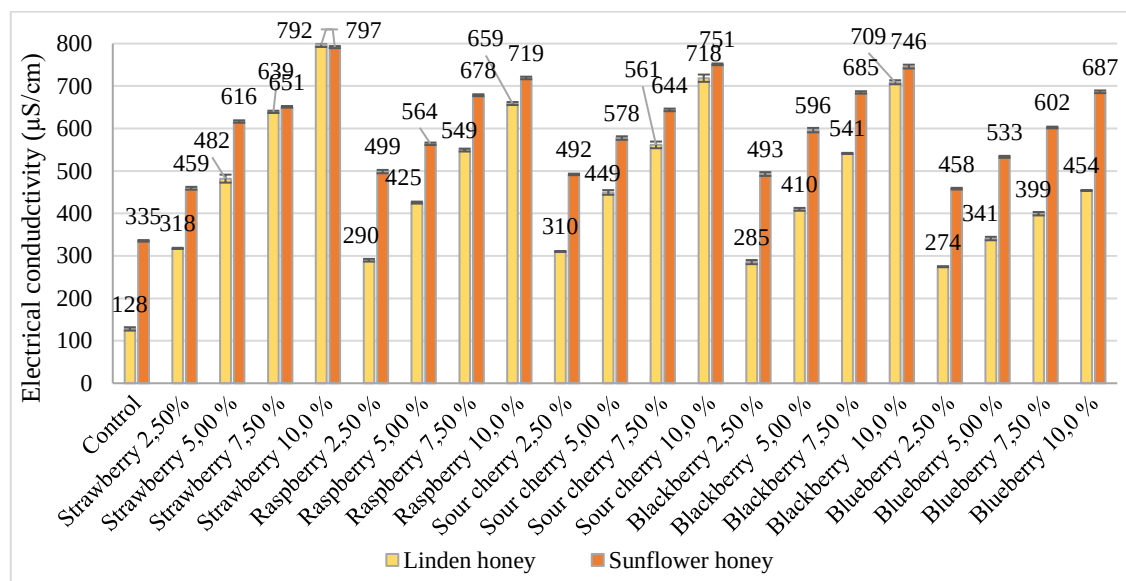


Figure 2.: Electrical conductivity of honeys enriched with fruits

Source: Own editing

Based on Directive no. 1-3-2001/110 Codex Alimentarius Hungaricus, the minimal diastase activity of honeys is 8 DN. Based on the results, honey samples used for the enrichment showed high diastase activity, but the addition of lyophilized fruits caused a decrease in this parameter. The more fruit was added, the lower diastase activity was measured. The decrease was similar in case of the two honey types, which could be explained by the similar diastase activity of the control samples. The enrichment in 2.5% already decreased the enzymatic activity to 50% or lower in case of the honeys flavoured by strawberry and blackberry, and sunflower honey flavoured by blueberry. In case of honeys enriched with raspberry in 2.5%, the diastase activity was 13 DN, which decreased significantly in case of 5% fruit content – it was around 6 DN. It is important to mention, that in case of a 5% fruit content, diastase activity decreased to 50% except for two samples, in which I have measured a lower decrease, namely canola and sunflower honeys enriched with sour cherry. These samples showed 6 and 28% decrease in case of 2.5% fruit content, while 36 and 37% decrease in case of 5.0% fruit content. Based on the results, 5% fruit content decreased the enzymatic activity in a volume that they no longer met the criteria of Codex Alimentarius Hungaricus, except for canola honeys enriched with sour cherry and blueberry, and sunflower honeys enriched with sour cherry and blackberry. However, in case of canola honey enriched with sour cherry and blueberry in

7.5%, sunflower honey enriched with sour cherry in 10%, and sunflower honey enriched with blackberry in 7.5% also had a diastase activity lower than 8 DN.

The concentration of antioxidant compounds increased due to the enrichment; both polyphenol and flavonoid content was increased by the enrichment with blackberry, strawberry and raspberry the most efficiently. Similar trends could be observed in case of both monofloral honey types, which is illustrated by Figure 3. The addition of blackberry increased polyphenol content proportionate to the quantity of fruit added, causing 4, 6, 8 and 10 times higher concentrations (134-352 mg GAE/100 g). This level of increase was 3, 5, 6 and 7 times higher (107-270 mg GAE/100 g) in case of blueberry, while 2, 5, 6 and 7 times higher (126-270 mg GAE/100 g) in case of the addition of strawberry. There are some differences in case of flavonoid contents, because the enrichment with blackberry and blueberry caused 7, 9, 15 and 19 times (33,6-94,2 mg CE/100 g), and 5, 7, 10 and 13 times higher (26,0-67,2 mg CE/100 g) flavonoid contents in case of sunflower honeys, while it caused 4, 9, 12 and 15 times (19,4-69,2 mg CE/100 g), and 4, 6, 9 and 11 times higher (17,4-50,4 mg CE/100 g) concentrations in case of canola honeys, therefore the enrichment caused higher flavonoid contents in case of sunflower honeys. On the other hand, the enrichment with strawberry increased flavonoid content of canola honeys mostly, which means a 7, 9, 12 and 15 times higher (34,2-68,7 mg CE/100 g) concentration, while it was 4, 6, 11 and 14 times higher (20,0-68,3 mg CE/100 g) in case of sunflower honeys.

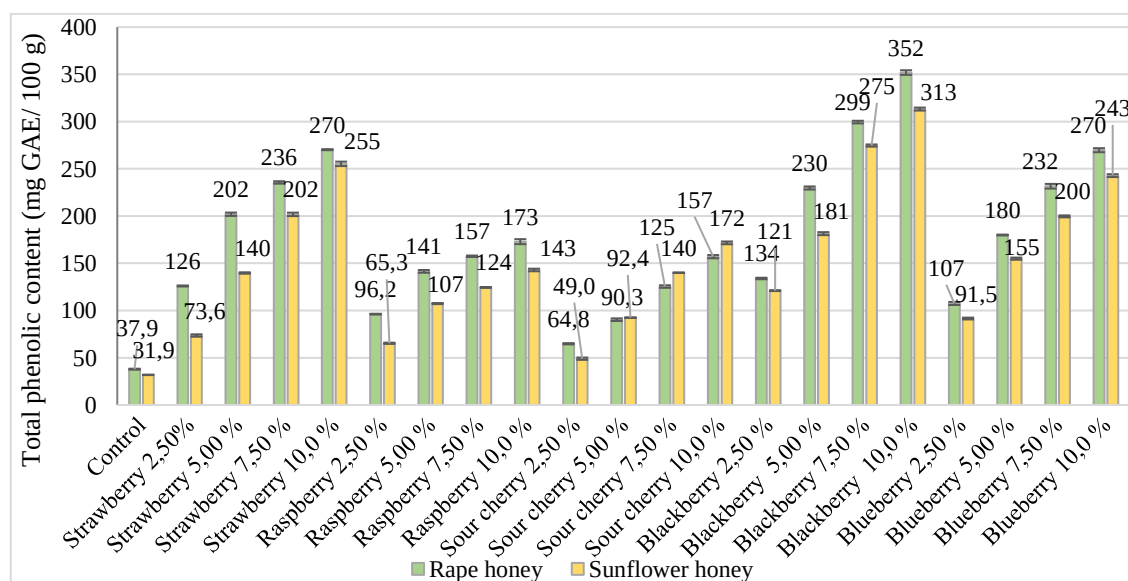


Figure 3.: Polyphenol content of honeys enriched with fruits

Source: Own editing

Despite blackberry and blueberry showed the lowest polyphenol contents, these fruits increased the concentrations of the honey samples the most efficiently, while in case of flavonoid contents, fruits with high flavonoid contents caused the highest concentrations found in the products.

Proline content was increased due to the enrichment with fruits in every case compared to the control sample, proportionate to the quantity of fruit added. In case of canola honeys, strawberry, sour cherry and blueberry had the highest effect on the proline content, while in case of sunflower honeys, the addition of sour cherry, blackberry and blueberry was the most efficient.

Regarding the pH of the samples, I have observed that the pH of the honeys enriched with fruits was lower than the control sample's. The addition of strawberry decreased pH the least, only by 3% in case of the 10% enrichment, which is negligible. The highest effect on pH was shown by raspberry in case of both honey types. In the order of increasing addition of the fruit, the decrease was 12%, 15% and 17% in case of canola honeys, while it was 5% in case of sunflower honeys enriched in 2.5%, and in case of the other quantities, I have observed a 7% decrease in the pH.

In case of the macro elements, the addition of lyophilized fruits to the honeys increased their quantity, their concentration increased proportionate to the quantity of fruit added in case of both honey types. Strawberry increased calcium (97,0-241 mg/kg), potassium (500-1660 mg/kg) and magnesium (43,4-138 mg/kg) contents the most effectively, while sodium content was elevated the most by the addition of blackberry in case of canola honeys. The addition of blackberry also caused the highest increase in the calcium, magnesium, phosphorus and sulphur content in case of sunflower honeys, although it contained lower amounts of calcium (196-364 mg/kg), magnesium (66,1-180 mg/kg) and phosphorus (112-275 mg/kg) than strawberry, however the difference between their concentration was not great. It is obvious in case of sulphur that blackberry – which had the highest sulphur content – increased the concentration of this element the most efficiently, while the highest potassium content was shown by the honey enriched with strawberry. Sodium contents were increased mostly by strawberry and blackberry. Just like in case of canola honeys, blueberry – which was the least rich in macro elements – caused the slightest increase in the element content of honeys. Overall, fruits had a solid effect on the element content of honeys, which resulted in a multiple increase in the concentration of the analysed parameters.

In case of micro elements, the same conclusions can be drawn regardless the honey types. Strawberry caused the greatest increase of iron, manganese and strontium content, but similarly high iron concentrations could be measured in the honeys enriched with blackberry, and similarly high contents of manganese could be observed in the samples containing raspberry. The addition of sour cherry resulted in the highest boron content, while the enrichment with blackberry resulted in the highest copper and zinc content. In case of zinc, significant increase could be reached by the addition of raspberry too. Aluminium and barium are not essential for the human organism, what is more, numerous adverse effects of these elements are known. The increase in the barium content of the enriched honeys was not that great due to the low barium content of fruits, except for samples containing strawberry, as this fruit contained the above-mentioned elements in many times higher concentrations than the other fruits. The increase of aluminium content was the highest in case of honeys enriched with blueberry, which contained this element in the highest quantity. Based on the results, the concentrations of micro elements measured is corresponding to the element content of the applied fruits.

1.3. Results of the honeys enriched with spices

The addition of spices to the honey samples caused increase in the electrical conductivity in this case too. It should be highlighted that the increase in acacia honeys (control: 122-127 $\mu\text{S}/\text{cm}$) was much higher than in case of linden honeys (control: 542-548 $\mu\text{S}/\text{cm}$), since the electrical conductivity of linden honey was 4 times higher than acacia honey's, which had an effect on the rate of increase. The addition of lovage caused the highest increase in the conductivity. In case of acacia honeys, the 1.25% enrichment resulted in 2.5-3 times higher (315-381 $\mu\text{S}/\text{cm}$), while the 5.0% enrichment caused 5.8-6 times higher (709-787 $\mu\text{S}/\text{cm}$) electrical conductivity than measured in the control sample. In case of linden honey, the 1.25% enrichment showed only 1.1-1.2 times higher (574-673 $\mu\text{S}/\text{cm}$), while 5.0% enrichment caused only 1.3-1.5 times higher (732-826 $\mu\text{S}/\text{cm}$) electrical conductivity than the control sample's.

Directive no. 1-3-2001/110 Codex Alimentarius Hungaricus prescribes a minimal diastase activity of 8 DN in case of acacia and linden honeys too. Based on the results, the honeys used for the analysis had high diastase activity, however – as opposed to the other parameters – the enrichment decreased the enzymatic activity proportionate to the quantity and the soaking time too. Although linden honey (26,2-24,5 DN) had a

significantly higher diastase activity than acacia honey (12,9-12,4 DN), the enzymatic activity decreased in both cases similarly. More significant decrease could be observed due to the addition of sage and ginger in case of acacia honeys. Both enrichments caused similar decreases in the enzymatic activity. The 1.25% enrichment caused 12-23% (11,3-9,57 DN), while the 5.0% enrichment caused 21-33.5% (10,2-8,25 DN) of decrease in the diastase activity. In case of linden honeys, peppermint and ginger honey samples showed the most significant decrease in diastase activity. In case of peppermint, the 1.25% enrichment resulted in 1-4.5% (26,3-23,4 DN), while the 5.0% enrichment caused 22-25% (20,5-18,3 DN) decrease in the enzymatic activity, while these values varied between 11 and 25%. Considering the results, every sample met the criteria of Codex Alimentarius Hungaricus. However, the addition of spices in higher quantities causes lower enzymatic activity, which decreases by time too, so it could become lower than 8 DN.

HMF content also gives information on the freshness of honey. There is no HMF present in fresh honeys, but it can appear due to long storage or heating. The maximal HMF content is 40 mg/kg in case of monofloral honeys based on Codex Alimentarius Hungaricus. Based on the results, fresh acacia and linden honey was used for the research, on which neither the enrichment, nor the 6 months soaking time had no effect, since HMF content of each sample was under the limit of detection.

The proline content was increased the addition of herbs and spices in every case compared to the control sample's, however the concentration decreased by the lengthened soaking time. Based on the results, proline content of linden honey was pretty high without any kind of enrichment, therefore it did not decrease under the prescribed value with the longest soaking time either. Proline content of the acacia honey was much lower, which decreased under the prescribed minimal value due to the long soaking time in spite of the enrichment of spices. This decrease was the most observable in case of the sixth month in the control sample too. After the 1.25% enrichment, only the proline content of basil, marjoram and lovage honey exceeded the minimum value, while after the 2.5% enrichment, sage, anise, peppermint and lemon balm honeys also exceeded 180 mg/kg of proline content. In case of the addition of higher amounts of spices, every result exceeded the minimum value.

During the analysis of pH, I have observed higher values in case of the samples enriched with spices than the control sample's, which was also influenced by the soaking time, since the pH of the control sample also increased during storage. The addition of ginger decreased the least the pH of both honey types, which was only 4% in case of acacia, and 9% in case of linden honey after 6 months of soaking with a concentration of 5.0%. Marjoram and lemon balm had the highest effect on this parameter in case of acacia honeys, while lovage and garlic had the highest effect in case of linden honeys, however the increase was not significant in any case. The rate of increase was similar in case of samples enriched with marjoram and lemon balm. In case of 1.25% enrichment, the increase was 4-11% (4,06-4,39 and 4,10-4,33), while in case of the 5.0% enrichment, the increase was 15-21% (4,62-4,99 and 4,55-4,98). Lovage and garlic had similar effect on the pH of linden honey too. 1.25% enrichment caused 3-10% (4,11-4,27 and 4,12-4,43), while 5.0% enrichment resulted in 12-14% (4,52-4,71 and 4,51-4,70) increase in the pH.

In case of macro elements, the addition of spices increased the concentrations measured in honeys, and the higher quantity of spices and longer soaking time also increased the concentrations of these elements in case of both honey types. Calcium content was elevated the most by lovage, calcium content was increased by basil, magnesium content was increased by lemon balm, while phosphorus and sulphur content was increased by garlic in acacia honey the most efficiently. The highest calcium content was measured in lovage, the highest potassium and magnesium content could be found in basil, the highest phosphorus content was shown by garlic, and the highest sulphur content was contained by sage, therefore the results of the honeys enriched with those were in partial compliance with my expectations. The lowest calcium and potassium content was measured in garlic honey, the lowest magnesium and sulphur content was shown by rosemary honey, while the lowest phosphorus content was measured in anise honey samples. In case of the spices, the lowest calcium and magnesium content was measured in garlic, the lowest potassium and sulphur content was shown by anise, and the lowest phosphorus content was shown by rosemary. Considering the lowest element contents, the results did not meet my expectation. In case of linden honeys, the highest calcium and magnesium content was measured in the samples containing lovage, the highest potassium content was measured in basil honey sample, while the highest phosphorus and sulphur content was shown by samples containing garlic. The lowest calcium content was measured in the sample enriched with anise, the lowest potassium content was found in ginger honey

sample, and the lowest magnesium, phosphorus and sulphur content was shown by honey enriched with rosemary, therefore these results also were in partial compliance with my expectations.

The amount of micro elements was increased by the enrichment with herbs and spices similarly. The highest boron and copper content was reached by the addition of lemon balm, the highest barium and manganese content was measured in samples containing lovage, while the highest iron and aluminium contents were measured in the samples containing marjoram in case of both honey types. Strontium content of acacia honeys was the highest due to the addition of marjoram, while the content of the same element was the highest due to the addition of lovage in the linden honeys. Zinc content was elevated the most by the addition of sage in case of acacia honey, while it was increased the most by the enrichment with garlic in case of linden honeys. The highest boron content could be observed in rosemary, the highest barium and aluminium content was measured in lemon balm, the highest iron concentration was measured in marjoram, the highest manganese content was shown by peppermint, the highest copper content could be found in basil, the highest amount of strontium could be found in rosemary, while the highest zinc content was found in sage. The results did not verify my expectations except for the iron content. Overall, the addition of herbs and spices and lengthening the soaking time resulted in an increase of the micro element contents in case of every sample.

4. New scientific results of the dissertation

1. I have verified that acacia, linden, sunflower and canola honeys can be classified by linear discriminant analysis based on their origin, if polyphenol content, electrical conductivity, proline content, diastase activity, potassium, sodium, calcium, magnesium, phosphorus and sulphur content of the samples are applied as independent variables.
2. I have stated that the addition of strawberry, raspberry, blackberry and blueberry increased polyphenol and flavonoid content of canola and sunflower honeys in every case. The most significant increase could be observed in case of samples enriched with blackberry, mostly in case of 10.0% enrichment. Flavonoid content of canola honey increased from 4.59 mg CE/100 g to 69.2 mg CE/100 g, while it changed from 5.07 mg CE/100 g to 94.2 mg CE/100 g in case of sunflower honey. Polyphenol content of canola honey increased from 37.9 mg GAE/100 g to 352 mg GAE/100 g, and this value changed from 31.9 mg GAE/100 g to 313 mg GAE/100 g in case of sunflower honey.
3. I have stated that calcium, potassium and magnesium content was mostly increased by strawberry, phosphorus and sulphur content was mostly increased by raspberry, while sodium content was mostly increased by blackberry, in case of canola honey. Calcium, magnesium, phosphorus and sulphur content of sunflower honey was increased by strawberry to the greatest extent. Similar declarations could be made observing the micro element contents. Strawberry increased iron and manganese content the most significantly. Highest boron contents were shown by samples containing sour cherry, while the highest copper and zinc content was caused by the addition of blackberry.
4. I have made the following statements regarding to the directives of Codex Alimentarius Hungaricus on the quality parameters of honey: Electrical conductivity and proline content of both canola and sunflower honeys were still under the limiting value in case of 10.0% enrichment with lyophilized strawberry, raspberry, sour cherry, blackberry and blueberry.

5. Diastase activity of canola and sunflower honeys containing 2.5% of lyophilized strawberry, raspberry, sour cherry, blackberry and blueberry; canola and sunflower honeys containing 5.0% of lyophilized sour cherry; canola honey containing 5.0% of lyophilized blueberry; and sunflower honeys containing 5.0% of lyophilized blackberry or 7.5% of lyophilized sour cherry did not exceed Diastase Number (DN) 8 specified by Codex Alimentarius Hungaricus.
6. I have stated that the addition of spices (basil, marjoram, lovage, sage, rosemary, garlic, aniseed, peppermint, balm, ginger) increased polyphenol and flavonoid content of acacia and linden honeys, and the most significant increase was caused by marjoram. After 6 months of soaking, polyphenol content of acacia honey was increased from 20.6 mg GAE/100 g to 368 mg GAE/100 g by the enrichment rate of 5.0%, while flavonoid content was increased from 2.89 mg CE/100 g to 70.3 mg CE/100 g. By the same enrichment, polyphenol content of linden honey was increased from 24.6 mg GAE/100 g to 222 mg GAE/100 g, and its flavonoid content changed from 1.75 mg CE/100 g to 26.2 mg CE/100 g.
7. I have determined that the enrichment with spices increased potassium, calcium, magnesium and sulphur content. Calcium content was increased by lovage, potassium content was increased by basil, magnesium concentration was increased by balm, while phosphorus and sulphur content was increased by garlic to the greatest extent in case of acacia honey. By observing linden honey, samples containing lovage showed the highest calcium and magnesium concentrations, samples containing basil had the highest potassium content, while samples containing garlic showed the highest phosphorus and sulphur content. In case of both mono-floral honeys, the highest boron and copper content was caused by balm, the highest manganese content was measured in the samples containing lovage, and the highest iron content was measured in the samples containing marjoram. By observing strontium content, marjoram caused the highest increase in case of acacia honey, while lovage caused the highest increase in case of linden honey. The highest zinc content was measured in case of the addition of sage to acacia honey, and the addition of garlic to linden honey.

5. Practical results

1. I have classified the analysed acacia, linden, sunflower and canola honeys by linear discriminant analysis based on their origin. Polyphenol content, electrical conductivity, proline content, diastase activity, potassium, sodium, calcium, magnesium, phosphorus and sulphur content of the samples were applied as independent variables. Linear discriminant analysis is suitable for the classification of these honey types based on the above-mentioned parameters.
2. The concentration of antioxidant compounds of both canola and sunflower honeys could be increased by the addition of strawberry, sour cherry, raspberry, blackberry and blueberry. However, to avoid spoilage along with too high moisture content, lyophilisation of the fruits before addition is recommended. Lyophilisation is a gentle procedure which does not modify polyphenol and flavonoid content of fruits significantly. It is also recommended to apply crystallized honeys for this aim, mostly honeys containing smaller sugar crystals, since more homogenous products can be prepared from cream honeys.
3. Polyphenol and flavonoid content of acacia and linden honeys could also be increased by the addition of dried spices (basil, marjoram, lovage, sage, rosemary, garlic, aniseed, peppermint, balm, ginger), out of which marjoram and basil caused the most significant changes. In case of 2.5-5.0% spice content, spices should be soaked for at least 2 months, and then removed by filtration. Increasing soaking time can result in the increase in the antioxidant concentrations in greater extent.

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7. Publications



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List of publications related to the dissertation

Hungarian scientific articles in Hungarian journals (5)

1. Kántor, A., Alexa, L., **Topa, E.**, Kovács, B., Czipa, N.: Különböző fűszerekkel dúsított kenyerek makroelem tartalmának meghatározása és hozzájárulásuk a táplálkozási referencia értékhez. *Élelmiszervizsgalati Kozlemen.* 68 (3), 4036-4046, 2022. ISSN: 2676-8704.
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2. **Topa, E.**, Alexa, L., Kántor, A., Kovács, B., Czipa, N.: Piros gyümölcsök minőségi jellemzői friss és liofilizálás utáni állapotban. *Élelmiszervizsgalati Kozlemen.* 67 (4), 3649-3656, 2021. ISSN: 2676-8704.
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3. Kántor, A., Alexa, L., **Topa, E.**, Kovács, B., Czipa, N.: Száritott bazsalikkal dúsított kenyerek vizsgálata és eredményeinek értékelése. *Élelmiszervizsgalati Kozlemen.* 67 (4), 3665-3671, 2021. ISSN: 2676-8704.
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