



**National University of Science and Technology
POLITEHNICA Bucharest
Doctoral School of Chemical Engineering and
Biotechnologies
Domain: Chemical Engineering**



THESIS SUMMARY

**CHEMOMETRIC METHODS USED TO EVALUATE
THE QUALITY OF FOOD PRODUCTS AND THE
EFFICIENCY OF EXTRACTION OF BIOACTIVE
COMPOUNDS FROM PLANTS**

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CONTENT

APPRECIATION	ERROR! BOOKMARK NOT DEFINED.
ABSTRACT.....	ERROR! BOOKMARK NOT DEFINED.
ABSTRACT.....	3
LIST OF TABLES.....	ERROR! BOOKMARK NOT DEFINED.
LIST OF FIGURES	ERROR! BOOKMARK NOT DEFINED.
PURPOSE AND STRUCTURE OF THE THESIS...	ERROR! BOOKMARK NOT DEFINED.
1. CRITICAL STUDY OF LITERATURE DATA	ERROR! BOOKMARK NOT DEFINED.
1.1. CHEMOMETRIC METHODS.....	ERROR! BOOKMARK NOT DEFINED.
1.1.1. Unsupervised methods.....	Error! Bookmark not defined.
1.1.1.1. PCA.....	Error! Bookmark not defined.
1.1.1.2. CA.....	Error! Bookmark not defined.
1.1.2. Supervised methods.....	Error! Bookmark not defined.
1.1.2.1. LDA	Error! Bookmark not defined.
1.1.2.2. PLS.....	Error! Bookmark not defined.
1.1.2.3. MLR.....	Error! Bookmark not defined.
1.2. HONEY QUALITY ASSESSMENT.....	ERROR! BOOKMARK NOT DEFINED.
1.2.1. Physico-chemical properties of honey.....	Error! Bookmark not defined.
1.2.2. The benefits of honey	Error! Bookmark not defined.
1.3. EXTRACTION OF BIOACTIVE COMPOUNDS FROM EDIBLE PLANTS .	ERROR!
BOOKMARK NOT DEFINED.	
1.3.2. Bioactive compounds in edible plants	Error! Bookmark not defined.
1.3.3. Factors and response variables in the extraction process	Error! Bookmark not defined.
1.3.4. Edible plants used as raw material in the extraction processes carried out in Chapter 2 (ORIGINAL CONTRIBUTIONS)	Error! Bookmark not defined.
1.3.4.1. Cornflower(<i>Centaurea cyanus</i> L.)	Error! Bookmark not defined.
1.3.4.2. Basil (<i>Ocimum basilicum</i> L.)	Error! Bookmark not defined.
1.3.4.3. Chicory (<i>Cichorium intybus</i> L.).....	Error! Bookmark not defined.
1.3.4.4. Marigold (<i>Calendula officinalis</i> L.).....	Error! Bookmark not defined.
1.3.4.5. Hibiscus (<i>Hibiscus sabdariffa</i> L.).....	Error! Bookmark not defined.
1.3.4.6. Chamomile (<i>Matricaria chamomilla</i> L.)	Error! Bookmark not defined.
1.3.4.7. Sage (<i>Salvia officinalis</i> L.).....	Error! Bookmark not defined.
2. ORIGINAL CONTRIBUTIONS	ERROR! BOOKMARK NOT DEFINED.
2.1. CHARACTERIZATION AND CLASSIFICATION OF ROMANIAN ACACIA HONEY BASED ON ITS PHYSICOCHEMICAL PARAMETERS AND CHEMOMETRICS....	ERROR! BOOKMARK NOT DEFINED.
2.1.1. Materials and methods.....	6

2.1.1.1. Honey samples	6
2.1.1.2. Physicochemical analysis.....	Error! Bookmark not defined.
2.1.1.3. Statistical analysis.....	8
2.1.2. Results and discussions	Error! Bookmark not defined.
2.1.2.1. Experimental results.....	Error! Bookmark not defined.
2.1.2.2. Statistical analysis.....	Error! Bookmark not defined.
2.1.3. Conclusions	17
2.2. EFFECT OF EXTRACTION SOLVENT ON THE COMPOSITION AND ANTIOXIDANT ACTIVITY OF EDIBLE FLOWER EXTRACTS....	ERROR! BOOKMARK NOT DEFINED.
2.2.1. The effect of extraction solvent on the composition and antioxidant activity of extracts of basil (<i>O. basilicum</i>), chicory (<i>C. intybus</i>) and sage (<i>S. officinalis</i>).....	19
2.2.1.1. Materials and methods	19
2.2.1.1.1. Plant material.....	Error! Bookmark not defined.
2.2.1.1.2. Chemicals	Error! Bookmark not defined.
2.2.1.1.3. Extraction procedure	Error! Bookmark not defined.
2.2.1.1.4. Determination of total phenolic content (TPC) of plant extracts	Error! Bookmark not defined.
2.2.1.1.5. Determination of total flavonoid content (TFC) of plant extracts	Error! Bookmark not defined.
2.2.1.1.6. Evaluation of antioxidant activity of plant extracts	Error! Bookmark not defined.
2.2.1.1.7. Data processing	Error! Bookmark not defined.
2.2.1.2. Results and discussion	Error! Bookmark not defined.
2.2.1.3. Conclusions.....	Error! Bookmark not defined.
2.2.2. The effect of the extraction solvent on the composition of the extracts cornflower (<i>C. cyanus</i>), marigold (<i>C. officinalis</i>), hibiscus (<i>H. sabdariffa</i>) and chamomile (<i>M. chamomilla</i>)	Error! Bookmark not defined.
2.2.2.1. Materials and methods	Error! Bookmark not defined.
2.2.2.1.1. Plant material.....	Error! Bookmark not defined.
2.2.2.1.2. Chemicals	Error! Bookmark not defined.
2.2.2.1.3. Extraction procedure	Error! Bookmark not defined.
2.2.2.1.4. Determination of total phenolic content (TPC) of plant extracts	Error! Bookmark not defined.
2.2.2.1.5. Determination of total flavonoid content (TFC) of plant extracts	Error! Bookmark not defined.
2.2.2.1.6. Data processing	Error! Bookmark not defined.
2.2.2.2. Results and discussion	Error! Bookmark not defined.
2.2.2.3. Conclusions.....	Error! Bookmark not defined.
3. GENERAL CONCLUSIONS AND RESEARCH PERSPECTIVES.....	26
PAPER LIST	ERROR! BOOKMARK NOT DEFINED.
BIBLIOGRAPHY	30

ABSTRACT

In accordance with the title, the doctoral thesis aimed at assessing the quality of food products and the efficiency of extraction of bioactive compounds from plants using chemometric methods.

The thesis is structured in 2 main parts, *i.e.*, **1. CRITICAL STUDY OF LITERATURE DATA**, containing relevant information from the literature regarding chemometric methods, assessment of honey quality and extraction of bioactive compounds from edible plants, and **2. ORIGINAL CONTRIBUTIONS**, in which the results obtained by the author are presented.

In chapter **2.1. Characterization and classification of Romanian acacia honey based on physicochemical parameters and chemometrics**, the results of the physicochemical analysis for samples of pure acacia honey (P), indirectly adulterated honey (I), obtained by feeding bees with 3 different industrial syrups, and directly adulterated honey (D), prepared by mixing P honey with the same syrups, are presented. The study aimed at measuring the relevant physicochemical parameters of the honey samples and to discriminate pure from adulterated honey based on these parameters, using PCA (Principal Component Analysis) and LDA (Linear Discriminant Analysis) as chemometric tools. The contents of moisture, ash, 5-hydroxymethylfurfural (HMF), reducing sugars and sucrose, free acidity, diastase activity, and $\Delta\delta^{13}C = \delta^{13}C_H - \delta^{13}C_P$ were the physicochemical parameters considered in the multivariate analysis.

Chapter **2.2. The effect of the extraction solvent on the composition and antioxidant activity of edible flower extracts** is structured in 2 subchapters, one referring to the extracts obtained from the aerial parts of chicory (*C. intybus*), basil (*O. basilicum*) and sage (*S. officinalis*) plants, the other to the extracts obtained from the flowers of cornflower (*C. cyanus*), pot marigold (*C. officinalis*), hibiscus (*H. sabdariffa*) and chamomile (*M. chamomilla*). The extracts were obtained by maceration with agitation, using ethanol, methanol and two mixtures of ethanol and methanol (containing 40% and 60% ethanol) as extraction solvents. The total polyphenol content (TPC), total flavonoid content (TFC) and percentage inhibition (PI) of the extracts were the variables selected as responses of the extraction process. The experimental data were processed using PCA and one-way ANOVA.

The general conclusions of the thesis and some research perspectives are presented in chapter 3

PURPOSE AND STRUCTURE OF THE THESIS

In accordance with the title, the doctoral thesis aimed at assessing the quality of food products and the efficiency of extraction of bioactive compounds from plants using chemometric methods.

The thesis is structured in 2 main parts, *i.e.*, **1. CRITICAL STUDY OF LITERATURE DATA** and **2. ORIGINAL CONTRIBUTIONS**, to which general conclusions, research perspectives, the list of publications, and the bibliography are added.

The first part of the thesis contains relevant information from the specialized literature regarding chemometric methods, *i.e.*, PCA (Principal Component Analysis), CA (Cluster Analysis), LDA (Linear Discriminant Analysis), PLS (Partial Least Squares) and MLR (Multiple Linear Regression), the quality evaluation of honey and the extraction of bioactive compounds from edible plants.

The ORIGINAL CONTRIBUTIONS section, in which the results obtained from the author's own research are presented, is structured into two chapters: one concerning the characterization and classification of honey based on physicochemical parameters and chemometrics, and the other concerning the effect of the extraction solvent on the composition and antioxidant activity of edible plant extracts.

In chapter **2.1. Characterization and classification of Romanian acacia honey based on physicochemical parameters and chemometrics**, the results of the physicochemical analysis for samples of pure acacia honey (P), indirectly adulterated honey (I), obtained by feeding bees with 3 different industrial syrups, and directly adulterated honey (D), prepared by mixing P honey with the same syrups, are presented. The study aimed at measuring the relevant physicochemical parameters of the honey samples and to discriminate pure from adulterated honey based on these parameters, using PCA and LDA as chemometric tools. A total of 23 honey samples (P, I, D) were analyzed, and the following parameters were determined: contents of moisture, ash, 5-hydroxymethylfurfural (HMF), reducing sugars (RS) and sucrose, free acidity (FA), diastase activity (DA), and $\Delta\delta^{13}C = \delta^{13}C_H - \delta^{13}C_P$ were the physicochemical parameters considered in the multivariate analysis.

Chapter **2.2. The effect of the extraction solvent on the composition and antioxidant activity of edible flower extracts** is structured in 2 subchapters, one referring to the extracts obtained from the aerial parts of chicory (*C. intybus*), basil (*O. basilicum*) and sage (*S. officinalis*) plants, the other to the extracts obtained from the flowers of cornflower (*C. cyanus*), pot marigold (*C. officinalis*), hibiscus (*H. sabdariffa*) and chamomile (*M. chamomilla*).

The extracts were obtained by maceration with agitation, using ethanol, methanol and two mixtures of ethanol and methanol (containing 40% and 60% ethanol) as extraction solvents. The total polyphenol content (TPC), total flavonoid content (TFC) and percentage inhibition (PI) of the extracts were the variables selected as responses of the extraction process. The experimental data were processed using PCA and one-way ANOVA.

Chapter **3** presents the general conclusions of the thesis and some research perspectives regarding the evaluation of food quality and the extraction of bioactive compounds from edible plants.

2.1. Characterization and classification of Romanian acacia honey based on its physicochemical parameters and chemometrics

2.1.1. Materials and methods

2.1.1.1. Honey samples.

The research was developed in collaboration with a beekeeper from Vâlcea county of Romania, who agreed to participate to the study with 12 hives (H1–H12), each of them containing 3 colonies of *Apis mellifera carpatica*. Three acacia honey types were prepared and analyzed, i.e., authentic or pure (P), indirectly (I) adulterated by bee-feeding with sugar syrups, and directly (D) adulterated by mixing P honey with the same syrups. Three types of industrial syrups (S1–S3 in Table 2.1.), which are commonly employed for bee-feeding, were used for adulterating. Data on the experiments performed to obtain pure and adulterated honey are summarized in Table 2.2.

Table 2.1. Industrial syrup composition.

Sirop	Solid phase concentration (%)	Solid phase composition (%)				
		Fructose	Glucose	Sucrose	Maltose	Others
S1	73	39	31	30	0	0
S2	75	50	32	0	15	3
S3	82	14	43	43	0	0

Table 2.2. Experimental runs.

No.	Honey type	Code	Description	Hives	Honey samples
1	Pure (P)	P	No bee-feeding	3(H1-H3)	5
2	Directly (D) adulterated	D1	P honey mixed with S1		3
3		D2	P honey mixed with S2		3
4		D3	P honey mixed with S3		3
5	Indirectly (I) adulterated	I1	Bee-feeding with S1	3(H4-H6)	3
6		I2	Bee-feeding with S2	3(H7-H9)	3
7		I3	Bee-feeding with S3	3(H10-H12)	3

P honey was produced in 3 hives (H1–H3) without bee-feeding with sugar syrups, whereas I adulterated honey was obtained in 9 hives (H4–H12) as follows: H4–H6 hives were fed with S1, H7–H9 with S2, and H10–H12 with S3. 1 L of sugar syrup was fed in each hive (H4–H12) once every 3 days, between April 1st and May 5th, 2017. Honey was collected from each hive according

to Romanian standard SR 784-139 and following an “averagetaking” protocol²². D adulterated honey was prepared by mixing P honey with S1–S3 syrups (1/1 mass ratio). According to data presented in Table 2.2., 23 samples of honey were physicochemically analyzed. Prior to analysis, all samples were homogenized for 10 min using a mixer.

2.1.1.2. Physicochemical analysis

Physicochemical parameters in terms of moisture content, ash content, FA, RS content, sucrose content, DA, and HMF content were determined based on Romanian standard SR 784-3. This standard was harmonized with Official methods of analysis of Association of Official Analytical Chemists (AOAC) (AOAC, 1990) and Harmonized methods of the European honey commission (Bogdanov & Lüllmann, 1997).

Moisture content was measured at 20 °C with an Atago Digital Refractometer RX-5000i (Atago, USA).

Ash content was evaluated as follows: a honey sample (5 g) was desiccated in a platinum dish, kept in a thermostat at 80 °C for 4 h, and further calcined at 550 °C in a laboratory furnace (Nabertherm, Germany).

FA was determined by the titrimetric method, *i.e.*: 10 g of honey sample was dissolved in 75 mL of CO₂- free water in a 250 mL beaker. The solution was magnetically stirred and titrated to pH 8.3 by adding 0.05 N NaOH solution. The pH was measured using a Mettler Toledo SevenMulti pH meter S40 (Mettler Toledo, Canada).

RS were evaluated by reducing Soxhlet’s modification of Fehling’s solution by titration at boiling point against a solution of reducing sugars in honey in the presence of methylene blue as indicator. The difference in concentrations of invert sugar before and after the hydrolysis was multiplied by 0.95 to obtain the apparent sucrose content.

DA was evaluated using a buffered solution of honey and soluble starch incubated at 40 °C. 1 mL volumes of this solution were taken at regular intervals (5 min) and their absorbance was measured at 660 nm in a Perkin Elmer Luminescence Spectrophotometer LS-50B (Perkin Elmer, UK).

For HMF determination, 5 g of honey sample was mixed with 25 mL distilled water. After clarifying with Carrez reagents (I and II), the solution was diluted to 100 mL with distilled water. Absorbance of this clarified solution was measured at 284 and 336 nm in a Perkin Elmer Luminescence Spectrophotometer LS-50B (Perkin Elmer, UK) using as a reference the same solution containing 0.2% (m/v) sodium bisulphate.

The analysis of ¹³C/¹²C stable isotope ratio for honey and protein fraction extracted from honey was carried out according to the Official Methods of Analysis 998.1243 (AOAC, 2005). The values of ¹³C/¹²C ratio were determined using a Thermo Scientific FlashEA 1112 HT elemental analyzer (EA) coupled to a Delta V Continuous Flow Isotope Ratio Mass Spectrometer (CF-IRMS) (Thermo Fisher Scientific, Waltham, MA, USA) and expressed depending on PDB internal standard as $\delta^{13}C_H$ and $\delta^{13}C_P$. The proteins extraction involved mixing 10–12 g of honey sample with 4 mL of distilled water in a 50 mL centrifuge tube. After addition of 2 mL of 2/3 N sulphuric acid and 2 mL of 10% sodium tungstate solutions, the tube was heated to 80 °C until protein precipitated. The supernatant was removed after centrifuging and rinsing with 50 mL of distilled

water. After drying in an oven (75 °C) for 6 h, the protein sample (200 µg) was placed into a tin capsule for analysis.

All reagents used for physicochemical analyzes were analytical grade and were purchased from Merck (Darmstadt, Germany).

2.1.1.3. Statistical analysis

Univariate (one-way ANOVA) and multivariate (PCA and LDA) analyzes of physicochemical parameters were performed using Statistica 12 (StatSoft, Inc).

2.1.2. Results and discussions

2.1.2.1. Experimental results

Table 2.3 contains mean, standard deviation, and variation range of characteristic physicochemical parameters of pure and adulterated honey (23 samples). Characteristic value of moisture, ash, RS, sucrose, and HMF contents, FA, and DA were compared with limit levels specified in the national standard SR 784-340, whereas the values of $\delta^{13}C_H$, $\delta^{13}C_P$, and $\Delta\delta^{13}C = \delta^{13}C_H - \delta^{13}C_P$ were compared with those reported in the related literature

Table 2.3. Limit level, mean, standard deviation, and range (min–max) for characteristic physicochemicals parameters of honey samples.

Parameter (u.m) (limit)	Sample						
	P	I1	I2	I3	D1	D2	D3
Moisture content (%) (max. 20%)	16,8±0,36 16,3–17,2	22,2±0,35 21,9–22,6	18,1±0,17 18,0–18,3	16,1±0,33 15,9–16,5	20,8±0,28 20,6–21,1	19,7±0,30 19,5–20,1	17,1±0,13 17,0–17,2
Ash content (% × 10 ³) (max. 0,5%)	8,1±0,13 8,0–8,3	10±0,10 10,0–10,2	20±0,15 20,1–20,4	4±0,03 4,00–4,05	2±0,02 2,01–2,05	4±0,03 4,01–4,07	8±0,02 7,98–8,02
Free acidity (FA) (meq/kg) (max. 40 meq/kg)	0,75±0,02 0,72–0,77	6,50±0,10 6,4–6,6	4,03±0,12 3,9–4,1	6,03±0,21 5,8–6,2	5,00±0,20 4,8–5,2	5,03±0,06 5,0–5,1	5,03±0,12 4,9–5,1
RS content (%) (min. 70%)	70,5±1,48 68,7–72,5	66,5±0,81 65,7–67,3	65,3±0,58 64,8–66,0	58,1±0,48 57,5–58,5	66,0±1,05 65,0–67,1	66,5±0,68 65,8–67,2	65,3±0,61 64,8–66,0
Sucrose content (%) (max. 5%)	3,79±0,04 3,75–3,85	14,7±0,22 14,5–15,0	6,81±0,05 6,76–6,85	7,60±0,08 7,51–7,67	6,17±0,09 6,08–6,25	6,65±0,08 6,59–6,74	6,33±0,06 6,28–6,39
Diastase activity (DA) (Gothe units /g) (min. 6.5 Gothe units /g)	13,9±0,25 13,6–14,2	5,01±0,01 5,00–5,02	5,04±0,03 5,01–5,07	5,02±0,09 4,94–5,11	6,41±0,11 6,30–6,51	6,44±0,08 6,38–6,53	6,43±0,10 6,30–6,50
HMF content (mg/kg) (max. 15 mg/kg)	1,21±0,03 1,18–1,25	23,0±0,13 22,9–23,2	40,9±0,38 40,6–41,3	25,7±0,31 25,4–26,0	18,5±0,18 18,3–18,7	24,9±0,29 24,6–25,2	20,2±0,20 20,0–20,4
$ \delta^{13}C_H $ (‰)	23,7±0,59 23,0–24,4	26,3±0,20 26,1–26,5	12,4±0,12 12,3–12,5	14,9±0,15 14,7–15,0	24,5±0,50 24,0–25,0	18,6±0,55 18,0–19,1	17,2±0,25 17,0–17,5
$ \delta^{13}C_P $ (‰)	24,1±0,52 23,5–24,8	29,7±0,25 29,5–30,0	23,5±0,45 23,0–23,9	20,3±0,70 19,6–21,0	27,6±0,65 27,0–28,3	25,2±0,75 24,5–26,0	25,2±0,80 24,4–26,0
$\Delta\delta^{13}C$ (‰) (0–1‰)	0,46±0,09 0,4–0,6	3,43±0,06 3,4–3,5	11,1±0,46 10,7–11,6	5,43±0,55 4,9–6,0	3,13±0,15 3,0–3,3	6,67±0,21 6,5–6,9	7,97±0,55 7,4–8,5

Moisture content is a relevant parameter as it affects the viscosity, density, taste, flavour, colour, crystallization, and fermentation of honey (Al-Farsi et al., 2018; Cengiz et al., 2018; da Silva et al., 2016; Gomes et al., 2010). A high water content can accelerate the crystallization as well as produce fermentation during the storage (Al-Farsi et al., 2018; Cengiz et al., 2018; Gomes et al., 2010; Mārghitaş et al., 2009; Saxena et al., 2010). It is observed that only I1 and D1 samples contain more moisture than the regulated limit (max. 20%). The mean values of moisture content for I (18.8%) and D (19.2%) samples are similar and higher than the mean value of P samples (16.8%).

Ash or mineral content is an indicator affecting the colour and flavour of honey. Usually, the higher the ash content, the darker the colour and the stronger the flavour (da Silva et al., 2016). The mean values of ash content for P (0.0081%), I (0.0113%), and D (0.0047%) samples are much lower than the imposed limit (max. 0.5%). There were no differences in colour and flavour among the samples.

FA is mainly due to the presence of organic acids in equilibrium with lactones or internal esters and inorganic ions, e.g., chloride, sulphate, phosphate, and it can heavily influence the honey taste (Al-Farsi et al., 2018; da Silva et al., 2016; Gomes et al., 2010; Naila et al., 2018; Özcan et al., 2006). An increase in FA can occur over time as effect of acid formation (e.g., gluconic acid

from glucose, formic and levulinic acids from HMF) as well as in the case of fermentation (by producing acetic acid from ethylic alcohol resulted in the fermentation process in the presence of honey yeasts) (da Silva et al., 2016; Saxena et al., 2010). All samples have values of FA much lower than the regulated limit (max. 40 meq/kg). The mean values of FA for I (5.5 meq/kg) and D (5.0 meq/kg) samples are similar and about 7 times higher than the mean value of P samples (0.75 meq/kg).

All adulterated samples have values of sucrose content higher than the regulated limit (max. 5%). The mean value of sucrose content for I samples (9.71%) is higher than those of D and P samples (6.38% and 3.79%). On the other hand, all adulterated samples have values of RS (fructose and glucose) content lower than the imposed limit (min. 70%). The mean value of RS content for I samples (63.3%) is lower than those of D and P samples (65.9% and 70.5%). Accordingly, the honey samples adulterated by bee-feeding contain more sucrose which has not been converted to fructose and glucose.

DA and HMF content are indicators of honey freshness. A high quality honey is characterized by high values of DA and low level of HMF. In the case of heating or storage for a long time, DA decreases and HMF content increases (HMF can be produced by fructose and glucose decomposition) (Cengiz et al., 2018; da Silva et al., 2016; Kukurová et al., 2008; Saxena et al., 2010). Moreover, a higher value of HMF content can indicate an adulteration with inverted sugar syrup, because HMF can be formed by heating sucrose solutions to obtain inverted syrup, whereas a low level of DA can be an effect of an indirect adulteration. All adulterated samples have values of HMF content higher and of DA lower than the regulated limits (max. 15 mg/kg and min. 6.5 Gothe units/g). The mean value of HMF content for I samples (29.9 mg/kg) is higher than those of D and P samples (21.2 mg/kg and 1.21 mg/kg), whereas the mean value of DA for I samples (5 Gothe units/g) is lower than those of D and P samples (6.5 Gothe units/g and 13.9 Gothe units/g).

The values of $\delta^{13}C_H$ and $\delta^{13}C_P$ ($-24.4 \div -23.0$ and $-24.8 \div -23.5$ for P, $-26.5 \div -12.3$ and $-30.0 \div -19.6$ for I, $-25.0 \div -17.0$ and $-28.3 \div -24.4$ for D honey) are within the ranges reported by other researchers (Cengiz et al., 2018; Çinar et al., 2014; Guler et al., 2007, 2014; Kukurová et al., 2008; Padovan et al., 2003; Simsek et al., 2012; Tosun, 2013; Wu et al., 2017). Levels of $\Delta\delta^{13}C = \delta^{13}C_H - \delta^{13}C_P$ for I and D samples ($3.4 \div 11.6$ and $3.0 \div 8.5$) are more than 5 times higher than those for P samples ($0.4 \div 0.6$). These results indicate that P honey is pure (values of $\Delta\delta^{13}C$ are in the range $[-1, 1]$), whereas I and D samples are adulterated.

The data summarized in Table 2.3 highlight that some physicochemical parameters of adulterated honey are within the limit levels specified in the national standard. Consequently, only checking these parameters is not sufficient to detect the adulteration. Physicochemical analysis methods coupled with chemometrics could be an effective alternative to discriminate between pure and adulterated honey.

2.1.2.2. Statistical analysis

One-way ANOVA was used to determine the effect of honey type, *i.e.*, pure (P), directly adulterated (D1–D3), and indirectly adulterated (I1–I3), on the physicochemical parameters considered in the experimental research. Data summarized in Supplementary Table 2.4. ($F_{cr} = 2.741$, $F \geq 52.5$, and p value $\leq 1.2 \times 10^{-9}$) reveal that the influence of honey type on all physicochemical parameters is statistically significant.

Table 2.4. One-way ANOVA results for pure and adulterated honey samples

Variable	F_{cr}	F	p
Moisture content (%)	2,741	189,57	6,0E-14
Ash content (%)		12386	2,1E-28
Free acidity (FA) (meq/kg)		983,36	1,3E-19
RS content (%)		52,541	1,2E-09
Sucrose content (%)		3804,2	2,6E-24
Diastase activity (DA) (Gothé units /g)		2245,9	1,8E-22
HMF content (mg/kg)		10455	8,0E-28
$ \delta^{13}C_H $ (‰)		509,87	2,4E-17
$ \delta^{13}C_P $ (‰)		75,246	8,0E-11
$\Delta\delta^{13}C = \delta^{13}C_H - \delta^{13}C_P$ (‰)		395,56	1,8E-16

PCA and LDA were applied to discriminate between pure and adulterated honey samples based on their physicochemical parameters (independent variables or predictors). Eight independent variables were considered in the multivariate analysis, *i.e.*, moisture, ash, RS, sucrose, and HMF contents, FA, DA, and $\Delta\delta^{13}C$.

PCA results referring to eigenvalues, explained variance of principal components (PCs), and factor (PC) coordinates of variables are presented in Table 2.5. Tabulated data highlight that the eigenvalues corresponding to PC1 (4.32), PC2 (1.78), and PC3 (1.32) are greater than 1 and these first three PCs explain 92.8% (54.0% + 22.3% + 16.5%) of the total variance. Depending on their coordinates in the first three PCs (factor loadings), the most important variables are as follows: (i) DA (0.99), HMF content (− 0.91), FA (− 0.91), $\Delta\delta^{13}C$ ($lv = -0.73$), RS content (0.69), and sucrose content (− 0.68) for PC1, (ii) moisture content (0.71) for PC2, and (iii) ash content (0.70) for PC3. Only PC1 and PC2 were retained in the analysis, because the cumulative percentage of total variance explained by them (76.3%) was higher than 70%

Table 2.5. PCA results in terms of eigenvalues and explained variance for pure and adulterated honey samples

Principal component	Eigenvalue		% Total variance	
	PC	Cumulative	PC	PC
PC1	4,320	4,320	54,00	54,00
PC2	1,782	6,102	22,28	76,28
PC3	1,322	7,424	16,52	92,80
PC4	0,428	7,852	5,354	98,15
PC5	0,115	7,967	1,432	99,59
PC6	0,018	7,985	0,230	99,82
PC7	0,014	7,999	0,169	99,99
PC8	0,001	8,000	0,015	100,0

Projections of variables on the factor-plane PC1-PC2 are shown in Fig. 1. Depending on their loading (l_v) in the first two PCs, the most important variables are as follows: DA ($l_v=0.98$), HMF content ($l_v=-0.89$), FA ($l_v=-0.87$), $\Delta\delta^{13}C$ ($l_v=-0.68$), RS content ($l_v=0.63$), and sucrose content ($l_v=-0.61$) for PC1, as well as moisture content ($l_v=0.75$), $\Delta\delta^{13}C$ ($l_v=-0.66$), sucrose content ($l_v=0.60$), and ash content ($l_v=-0.58$) for PC2. Accordingly, based on the sign and magnitude of variable loading, PC1 can discriminate between honey samples with high values of DA and RS content along with low levels of FA, $\Delta\delta^{13}C$, HMF and sucrose contents and other samples. On the other hand, PC2 can discriminate between samples with high values of moisture and sucrose contents along with low levels of $\Delta\delta^{13}C$ and ash content and the others.

Based on the sign and magnitude of factor loadings, PC1 can discriminate between pure honey samples with high values of DA and RS content along with low levels of FA, $\Delta\delta^{13}C$, HMF and sucrose contents and adulterated samples. Projections of cases on the factor-plane PC1-PC2 highlight a good discrimination between P, D, and I honey groups on the PC1 direction.

PC1 coordinates of P samples ($3.5 \div 3.9$) correspond to higher values of DA ($13.6 \div 14.2$ Gothe units/g) and RS content ($68.7 \div 72.5\%$) along with lower levels of FA ($0.75 \div 0.77$ meq/kg), $\Delta\delta^{13}C$ ($0.4 \div 0.6\%$), HMF content ($1.18 \div 1.25$ mg/kg), and sucrose content ($3.75 \div 3.85\%$). Concurrently, PC1 coordinates of D1-D3 samples ($-0.6 \div 0$) and I1-I3 samples ($-1.9 \div -1.4$) correspond to lower values of DA ($6.30 \div 6.53$ and $4.94 \div 5.11$ Gothe units/g) and RS content ($64.8 \div 67.2$ and $57.5 \div 67.3\%$) as well as to higher levels of FA ($4.8 \div 5.2$ and $3.9 \div 6.6$ meq/kg), $\Delta\delta^{13}C$ ($3 \div 8.5$ and $3.4 \div 11.6\%$), HMF content ($18.3 \div 25.2$ and $22.9 \div 41.3$ mg/kg), and sucrose content ($6.08 \div 6.74$ and $6.76 \div 15\%$).

On the other hand, PC2 can discriminate between the samples adulterated with S1 syrup (with PC2 coordinates of $2.1 \div 2.3$ for I1 and $1.2 \div 1.5$ for D1 samples), having high values of moisture content ($21.9 \div 22.6\%$ and $20.6 \div 21.1\%$, respectively) and those adulterated with S2 and

S3 syrups (with PC2 coordinates of $-2.5 \div 0.5$), which had lower levels of moisture content ($15.9 \div 20.1\%$).

Accordingly, PC1 is mostly related to the adulteration and PC2 to the type of syrup used to adulterate the honey. It appears in the bi-plot (Fig. 1) that the separation on PC1 direction is comparable in magnitude to that on PC2 direction. However, it must be considered that PC1 accounts for 54.0% of the total variance, while PC2 only 22.3%.

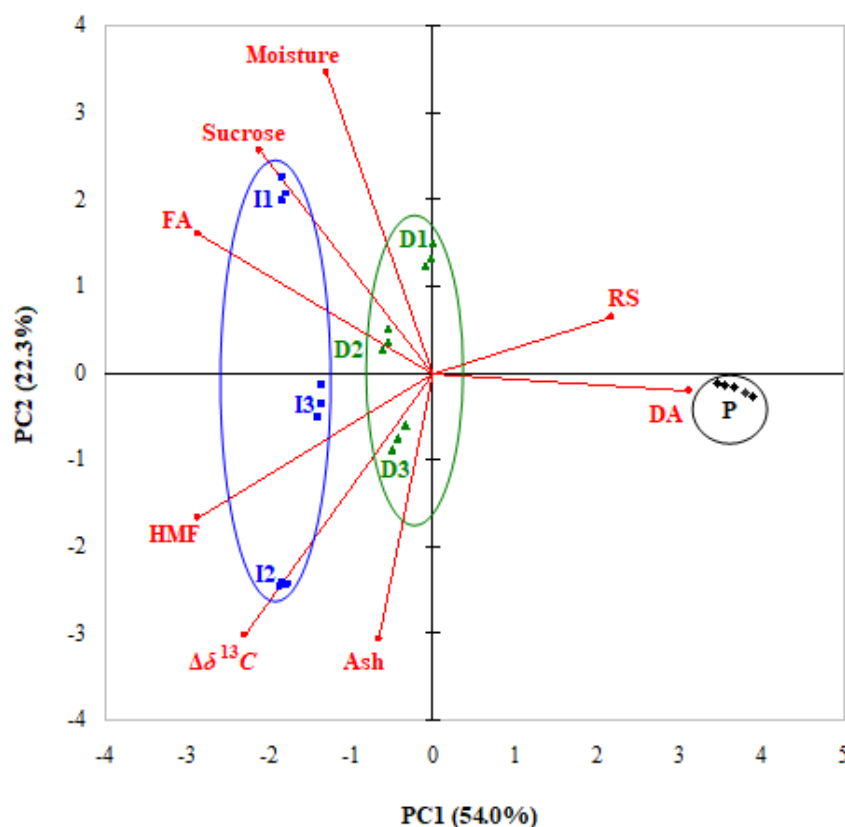


Figure 2.1. PCA bi-plot (projections of samples and variables on the factor-plane PC1–PC2).

In order to obtain the variables with higher discriminative power and their corresponding discriminant and classification functions, LDA was applied using forward stepwise (FS) method (8 steps, tolerance=0.01, F to enter=1, F to remove=0) and considering P, D, and I honey as predefined groups. The most important discriminative physicochemical parameters determined by FS method were DA, HMF, sucrose, and RS contents. The first linear discriminant (LD1) function explains 99.2% of the discriminative power, whereas the second (LD2) accounts for 0.8%, the eigenvalues corresponding to LD1 and LD2 functions being of 1409.7 and 11.7, respectively. Accordingly, LD1 can be considered as the most relevant function. Values of standardized coefficients of physicochemical parameters for LD1 and LD2 functions are summarized in Table 2.6. Tabulated data highlight that LD1 function is positively correlated with RS content, negatively correlated with DA, sucrose and HMF contents, as well as DA has the most significant effect. On the contrary, LD2 function is negatively correlated with RS content, positively correlated with DA, sucrose and HMF contents, as well as DA has an insignificant effect.

Table 2.6. LDA results in terms of variable standardized coefficients for LD1 and LD2 functions

No.	Variable name	Standardized coefficients	
		LD1	LD2
1	RS content	-0,947	2,410
2	Sucrose content	0,810	-2,989
3	DA	1,215	-0,428
4	HMF content	0,558	-2,598

Projections of training honey samples on the factor-plane LD1–LD2 are presented in Fig. 2.2. P honey samples are plotted much further to the right in the scatterplot than D and I ones, LD1 scores being as follows: $41.58 \div 45.12$ for P, $-11.44 \div -9.49$ for D, and $-19.03 \div -17.22$ for I samples. Accordingly, LD1 function separates all three honey classes (groups). Taking into account the factor loadings represented in LDA bi-plot (Fig. 2), LD1 function mostly discriminates between pure and adulterated honey, the most discriminative predictor being DA ($13.6 \div 14.2$, $6.30 \div 6.53$, and $4.94 \div 5.11$ Gothe units/g for P, D, and I honey classes). On the other hand, LD2 function discriminates between D and I honey types (LD2 scores of $1.67 \div 5.54$ for D and $-3.63 \div -2.56$ for I samples), the most discriminative predictor being sucrose content ($6.08 \div 6.74\%$ for D and $6.76 \div 15\%$ for I).

LD1 function accounts for 98.7% of the discriminative power, consequently the most significant discrimination is possible for all three honey types by this function. Mean values of factor scores for each group (centroids) and 95% confidence ellipses for training set as well as projections of validation honey samples on the factor-plane LD1–LD2 are also shown in Fig. 2. It is observed that all points corresponding to LD1 and LD2 scores for validation set are included in the ellipses corresponding to their group.

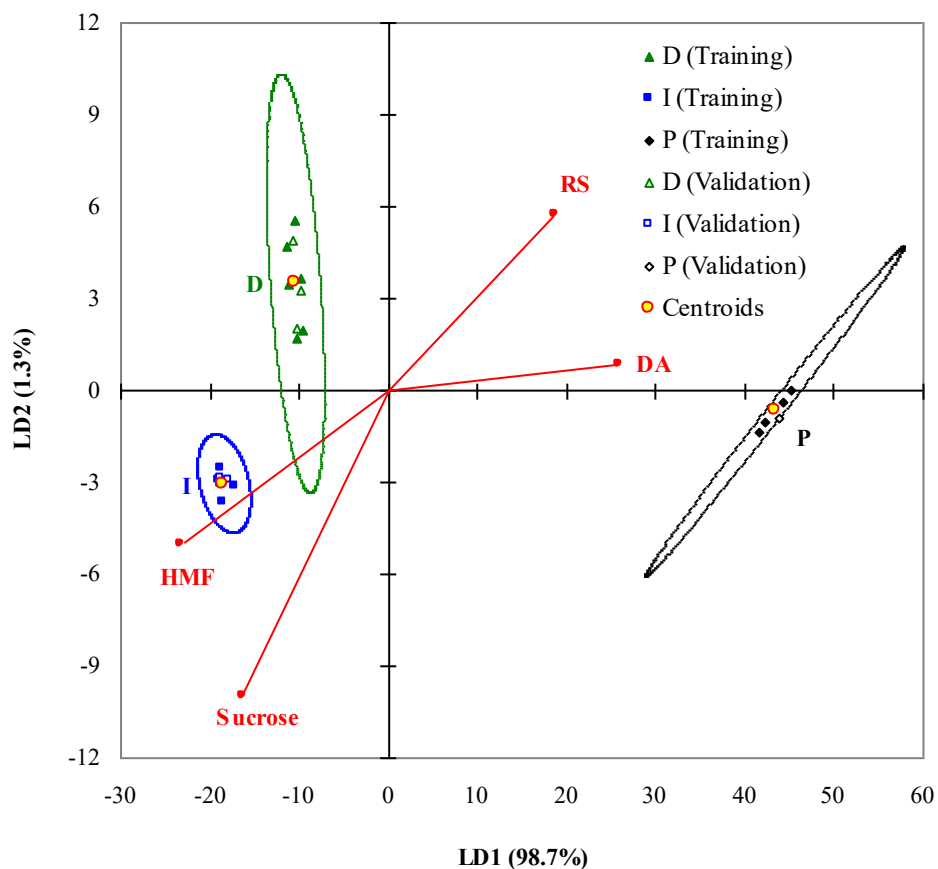


Figure 2.2. LDA bi-plot (projections of samples and the most important discriminative variables on the factorplane LD1–LD2).

Honey samples (cases) were classified into the group to which they were closest. Mahalanobis distances between each case and the group centroids were calculated. According to Bayes rule, the probability that a case belongs to a group, i.e., posterior classification probability (PP), was determined depending on squared Mahalanobis distance (SMD) and a priori classification probability (APP). APP values were assumed to be proportional to the number of samples in each group ($APPP = 0.25$, $APPD = 0.375$, and $APPI = 0.375$). A case can be classified based on largest value of PP . Prior and posterior classification and levels of SMD and PP for training (T) and validation (V) sets are summarized in Supplementary Table 2.7.

Table 2.7. LDA results for honey samples P, D, and I from the training (T) and validation (V) datasets.

No.	Data set	Classification		Square of the Mahalanobis distance			Posterior probability			Classification function score		
		<i>a priori</i>	posterior	SMD_P	SMD_I	SMD_D	PP_P	PP_I	PP_D	CFS_P	CFS_I	CFS_D
1	T	P	P	4,01	3698,8	2804,6	1	0	0	3938,8	2091,3	2538,9
2		P	P	6,60	3608,8	2730,8	1	0	0	3864,7	2063,6	2503,0
3		P	P	6,73	4052,8	3100,2	1	0	0	4214,3	2191,1	2667,9
4		P	P	3,94	3951,2	3016,1	1	0	0	4134,7	2161,0	2629,0
5		I	I	3843,5	5,96	122,10	0	1	0	-1304,8	613,8	556,2
6		I	I	3868,6	7,00	117,68	0	1	0	-1301,3	629,4	574,5
7		I	I	3900,5	5,84	120,54	0	1	0	-1328,6	618,6	561,8
8		I	I	3876,5	6,78	113,77	0	1	0	-1298,5	636,3	583,3
9		I	I	3816,3	6,34	111,19	0	1	0	-1332,4	572,6	520,5
10		I	I	3678,4	6,67	95,38	0	1	0	-1231,9	603,8	559,9
11		D	D	3031,9	111,21	4,69	0	0	1	-573,1	887,1	940,8
12		D	D	2930,7	140,26	6,24	0	0	1	-457,6	937,5	1004,9
13		D	D	2885,9	90,68	5,42	0	0	1	-515,1	882,4	925,5
14		D	D	2800,9	108,02	5,62	0	0	1	-430,7	915,6	967,3
15		D	D	2977,1	98,89	2,53	0	0	1	-560,4	878,6	927,2
16		D	D	2855,7	119,79	2,27	0	0	1	-450,8	917,1	976,3
17	V	P	P	3,06	3883,8	2964,1	1	0	0	4074,6	2134,1	2594,4
18		I	I	3864,8	6,25	117,69	0	1	0	-1305,1	624,1	568,8
19		I	I	3880,9	5,91	117,44	0	1	0	-1311,9	625,5	570,2
20		I	I	3758,3	5,84	101,98	0	1	0	-1286,7	589,5	541,8
21		D	D	2950,3	125,56	4,08	0	0	1	-498,3	914,0	975,2
22		D	D	2890,0	93,57	4,47	0	0	1	-507,1	891,0	936,0
23		D	D	2849,3	115,78	2,39	0	0	1	-458,6	908,1	965,2

Moreover, linear classification functions based on linear discriminant functions were determined for each honey group. Table 2.8. contains characteristic coefficients of classification functions, i.e., c_{ij} and c_j , where i and j denote independent variable and group, respectively. These linear classification functions can be used directly to classify cases, i.e., a case is assigned to the group for which it has the highest value of classification function score (CFS). Values of CFS determined from raw data for both T and V sets are also summarized in Supplementary Table 2.7.. Classification (confusion) matrices presented in Supplementary Table 2.9. reveal that 100% of honey samples were correctly assigned to their original group for both T and V sets.

Table 2.8. LDA results in terms of characteristic coefficients of classification functions.

$i \backslash j$	P	D	I
RS content	-8,08	13,71	10,79
Sucrose content	8,60	-14,40	-9,05
DA)	620,55	189,98	145,39
HMF content	1,71	-5,49	-3,29
c_j	-4041,17	-958,86	-614,72

Table 2.9. Classification matrix

Set	Grup	P	D	I	Total	% Corect	Set	Grup	P	D	I	Total	% Corect
T	P	4	0	0	4	100	V	P	1	0	0	1	100
	D	0	6	0	6	100		D	0	3	0	3	100
	I	0	0	6	6	100		I	0	0	3	3	100
	Total	4	6	6	16	100		Total	1	3	3	7	100

2.1.3. Conclusions

The paper aimed at measuring various physicochemical parameters of honey samples and discriminating the pure and adulterated honey based on these parameters using PCA and LDA as chemometric tools. Acacia pure (P) and indirectly (I) adulterated honey, obtained by bee-feeding with 3 different industrial sugar syrups, were produced in a small apiary in the Vâlcea county of Romania, whereas directly (D) adulterated honey was prepared by mixing P honey with the same syrups. Honey samples were analysed in terms of moisture, ash, HMF, RS (glucose and glucose), and sucrose contents, FA, DA, $\delta^{13}C_H$, and $\delta^{13}C_P$.

Adulteration determined an increase in water content (by *cca.*10 %), FA (by *cca.* 7%), sucrose content (2.6 and 1.7 times higher for I and D samples, respectively), HMF content (about 25 and 18 times higher for I and D samples, respectively), and $\Delta\delta^{13}C=\delta^{13}C_H-\delta^{13}C_P$ (about 17 and 15 times higher for I and D samples, respectively), as well a decrease in RS content (by *cca.*10 %) and DA (2.8 and 2.1 times lower for I and D samples, respectively) than the mean value of P samples. For the types and dosage of sugar syrups used in this study, indirect adulteration had effects similar to those produced by direct adulteration. The values of DA, HMF, sucrose, and RS contents for D and I honey samples were not within the ranges imposed by the national standard.

Moisture, ash, RS, sucrose, and HMF contents, FA, DA, and $\Delta\delta^{13}C$, were the physicochemical parameters considered in the multivariate analysis. According to PCA, the 8-dimensional feature space was reduced to a 2-dimensional one, where the directions of new axes were defined by PC1 and PC2 eigenvectors. PC1, explaining 54.0% of total variance, was dominated by DA, HMF content, FA, $\Delta\delta^{13}C$, RS and sucrose contents, whereas PC2, accounting for 22.3% of total variance, was dominated by moisture content. Three well separated honey groups (P, D, and I) on the PC1 direction were obtained by projecting the honey samples on the factor-plane PC1–PC2.

Starting from these predefined groups, forward stepwise LDA revealed that the most important discriminative physicochemical parameters were DA, HMF, sucrose, and RS contents. Linear discriminant functions and classification functions were built based on a training dataset and then these models were validated using a validation dataset. Cases were classified based on the largest values of posterior classification probability and classification function score. Characteristic confusion matrices of both training and validation sets indicated that 100% of honey samples were correctly assigned to their original group. Accordingly, the evaluation of physicochemical parameters using PCA and LDA was very effective to discriminate between pure, directly and indirectly adulterated honey. Based on the physicochemical parameters in terms of DA, HMF, sucrose, and RS contents, any sample of acacia honey could be simply and quickly classified as pure, directly or indirectly adulterated by means of classification functions obtained

by applying LDA. However, the study has some limitations, e.g., a small number of acacia honey samples, a relative similarity of them (all coming from the same producer and being produced within the same few months). The analysis could be extended using numerous samples of different types of honey collected from several producers over a longer period.

2.2. EFFECT OF EXTRACTION SOLVENT ON THE COMPOSITION AND ANTIOXIDANT ACTIVITY OF EDIBLE FLOWER EXTRACTS

2.2.1. Effect of extraction solvent on the composition and antioxidant activity of extracts of basil (*O. basilicum*), chicory (*C. intybus*) and sage (*S. officinalis*)

2.2.1.1. Materials and methods

2.2.1.1.1. Plant material

Dried aerial parts of *O. basilicum*, *C. intybus*, and *S. officinalis* were provided by Fares (Orastie, Hunedoara, Romania), Stef Mar (Ramnicu Valcea, Valcea, Romania), and Dacia Plant (Bod, Brasov, Romania), respectively. The dried plant material was ground in a grinder.

2.2.1.1.2. Chemicals

Ethanol, methanol, Folin-Ciocalteu reagent (FCR), 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, aluminum chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$), and quercetin were purchased from Merck (Darmstadt, Germany). Sodium carbonate (Na_2CO_3), sodium nitrite (NaNO_2), sodium hydroxide (NaOH), and ascorbic acid were purchased from VWR International (Vienna, Austria). Distilled water (DW) was obtained using a Milli-Q (Millipore, Merck, Italy) water purification system (Millipore, Merck). All chemicals were of analytical grade.

2.2.1.1.3. Extraction procedure

Extracts of edible flowers were prepared through maceration under stirring of dried and ground plant material, using ethanol, methanol, and two mixtures of ethanol and methanol as extraction solvents. Extraction process was performed for 24 h at four levels of ethanol concentration in the extraction solvent (0%, 40%, 60%, and 100%) and a single level of solid/liquid ratio (1/10 g/mL), process temperature (30 °C), and stirring speed (250 rpm). Each extract was filtered using filter paper.

2.2.1.1.4. Determination of total phenolic content (TPC) of plant extracts

TPC of plant extracts was determined by the Folin-Ciocalteu method with some modifications (Agbo et al., 2015). 1 mL of extract was mixed with 9 mL of DW in a 25 mL flask, then 2.5 mL of diluted FCR (1/10 mL FCR/mL DW) was added to the flask. The mixture was stirred for 5 min, then 10 mL of 7.5% Na_2CO_3 solution was added and made up to the mark with DW. This mixture was kept in the dark for 90 min at ambient temperature. The values of sample absorbance were read at a wavelength of 760 nm with a UV/Vis spectrophotometer (Specord 250 Plus, Analytik Jena, Germany). TPC of each sample was determined from the calibration curve and expressed as mg GAE (gallic acid equivalents)/g DM (dry matter). The determinations were performed in five replicates.

2.2.1.1.5. Determination of total flavonoid content (TFC) of plant extracts

TFC of plant extracts was determined by the colorimetric method with $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ solution (Agbo et al., 2015). 1 mL of extract was mixed with 4 mL of DW in a 10 mL flask, then 0.30 mL of 5% NaNO_2 was added. The mixture was stirred for 5 min, then 0.30 mL of 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ solution was added and, after another 5 min of stirring, 2 mL of 1 M NaOH solution was added to the mixture and made up to the mark with DW. The values of sample absorbance were read at a wavelength of 510 nm with the UV/Vis spectrophotometer Specord 250 Plus. *TFC* of each sample was determined from the calibration curve and expressed as mg QE (quercetinequivalents)/g DM. The determinations were performed in five replicates.

2.2.1.1.6. Evaluation of antioxidant activity of plant extracts

Evaluation of antioxidant activity of plant extracts was performed according to the DPPH free radical scavenging assay (Singh et al., 2015). 1 mL of extract was mixed with 1 mL of 0.1 mM DPPH methanolic solution in a flask. The mixture was kept in the dark for 30 min at ambient temperature, and then the values of sample absorbance were read at a wavelength of 517 nm with the UV/Vis spectrophotometer Specord 250 Plus. The results were expressed as percentage of inhibition (*PI*) defined by equation (1), where A_0 is the absorbance of DPPH solution without extract sample and A_1 the absorbance of DPPH solution with extract sample.

$$PI = \frac{A_0 - A_1}{A_0} \times 100 \quad (2)$$

2.2.1.1.7. Data processing

The values of response variables (*TPC*, *TFC*, and *PI*) obtained at different levels of ethanol concentration in the extraction solvent (0–100%) were processed applying principal component analysis (PCA). One-way analysis of variance (ANOVA) was used to assess whether the mean values of the response variables were significantly different ($p < 0.05$) or not. The strength of the linear correlations between different response variables was evaluated based on the Pearson correlation coefficient (r). Statistical analysis was performed using XLSTAT version 2019.1.

2.2.1.2. Results and discussion

A data matrix with 15 rows (5 replicates for each type of flower) and 12 columns (number of variables, *i.e.*, *TPC*0, *TPC*40, *TPC*60, *TPC*100, *TFC*0, *TFC*40, *TFC*60, *TFC*100, *PI*0, *PI*40, *PI*60, and *PI*100) was used in PCA. Only the first two principal components (PCs) had eigenvalues > 1 (10.20 for PC1 and 1.79 for PC2). PC1 and PC2 explained 99.9% (85.0% + 14.9%) of the total variance, and only these two PCs were further considered in the multivariate analysis. The results shown in Table 2.10., Fig. 2.3., and Fig. 2.4. indicate the following:

- depending on significant levels of factor loadings (highlighted in bold in Table 2.10.), the most important variables are *TPC*0, *TPC*40, *TPC*60, *TPC*100, *TFC*0, *TFC*40, *TFC*60, *PI*0, *PI*40, *PI*60, and *PI*100 for PC1 as well as *TFC*100 for PC2;
- the extracts obtained from common sage (*S. officinalis*) had higher values of *TPC*0–100, *TFC*0–60, and *PI*0–100 than those prepared from chicory (*C. intybus*) and basil (*O. basilicum*) (discrimination on PC1 between *S. officinalis* samples and the other samples in Fig. 1); with one exception, the data presented in Fig. 2 confirm this finding, *i.e.*, the mean values of *TPC*0–100, *TFC*0–60, and *PI*0–100 for *S. officinalis* (*SO*) samples (*TPC*0–100_{m,SO} = 46.31–61.77 mg

GAE/mg DM, *TFC0–60m,SO* = 24.44–34.92 mg QE/mg DM, and *PI0–100m,SO* = 80.45–89.35%) were generally significantly higher ($p < 0.05$) than those for *C. intybus* (*CI*) samples (*TPC0–100m,CI* = 24.98–31.41 mg GAE/mg DM, *TFC0–60m,CI* = 9.32–12.51 mg QE/mg DM, and *PI0–100m,CI* = 70.28–72.81%) and *O. basilicum* (*OB*) samples (*TPC0–100m,OB* = 31.70–35.76 mg GAE/mg DM, *TFC0–60m,OB* = 19.16–26.02 mg QE/mg DM, and *PI0–100m,OB* = 80.45–89.35%); only *TFC60m,SO* (24.44 mg QE/mg DM) was significantly lower than *TFC60m,OB* (26.02 mg QE/mg DM);

- the extracts obtained from *O. basilicum* had lower values of *TFC100* than those prepared from the other plants (discrimination on PC2 between *O. basilicum* samples and the other samples in Fig. 1); the data presented in Fig. 2 confirm this finding, *i.e.*, the mean value of *TFC100* for *O. basilicum* samples (*TFC100m,OB* = 0.97 mg QE/mg DM) was significantly lower than *TFC100m,SO* = 24.40 mg QE/mg DM and *TFC100m,CI* = 14.85 mg QE/mg DM;

Table 2.10. Factor loadings

No.	Name	Symbol	PC1	PC2
1	Total phenolic content for extraction with 100% methanol (0%Et)	<i>TPC0</i>	0,989	0,144
2	Total phenolic content for extraction with 60% methanol + 40% ethanol (40%Et)	<i>TPC40</i>	0,944	0,325
3	Total phenolic content for extraction with 40% methanol + 60% ethanol (60%Et)	<i>TPC60</i>	0,944	0,327
4	Total phenolic content for extraction with 100% ethanol (100%Et)	<i>TPC100</i>	0,998	0,050
5	Total flavonoid content for extraction with 100% methanol (0%Et)	<i>TFC0</i>	1,000	0,013
6	Total flavonoid content for extraction with 60% methanol + 40% ethanol (40%Et)	<i>TFC40</i>	0,997	-0,077
7	Total flavonoid content for extraction with 40% methanol + 60% ethanol (60%Et)	<i>TFC60</i>	0,744	-0,668
8	Total flavonoid content for extraction with 100% ethanol (100%Et)	<i>TFC100</i>	0,513	0,858
9	Antioxidant activity for extraction with 100% methanol (0%Et)	<i>PI0</i>	0,906	-0,422
10	Antioxidant activity for extraction with 60% methanol + 40% ethanol (40%Et)	<i>PI40</i>	0,977	0,210
11	Antioxidant activity for extraction with 40% methanol + 60% ethanol (60%Et)	<i>PI60</i>	0,967	-0,252
12	Antioxidant activity for extraction with 100% ethanol (100%Et)	<i>PI100</i>	0,960	-0,278

Significant values are highlighted in bold.

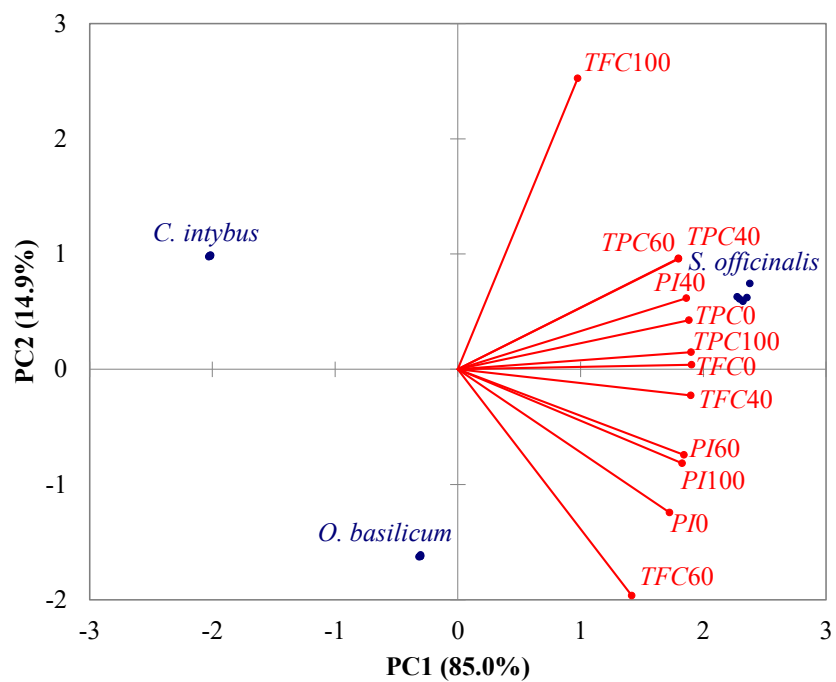


Figura 2.3. Projections of variables (TPC0–100, TFC0–100, and PI0–100) and samples (*C. intybus*, *O. basilicum*, and *S. officinalis*) on PC1 and PC2.

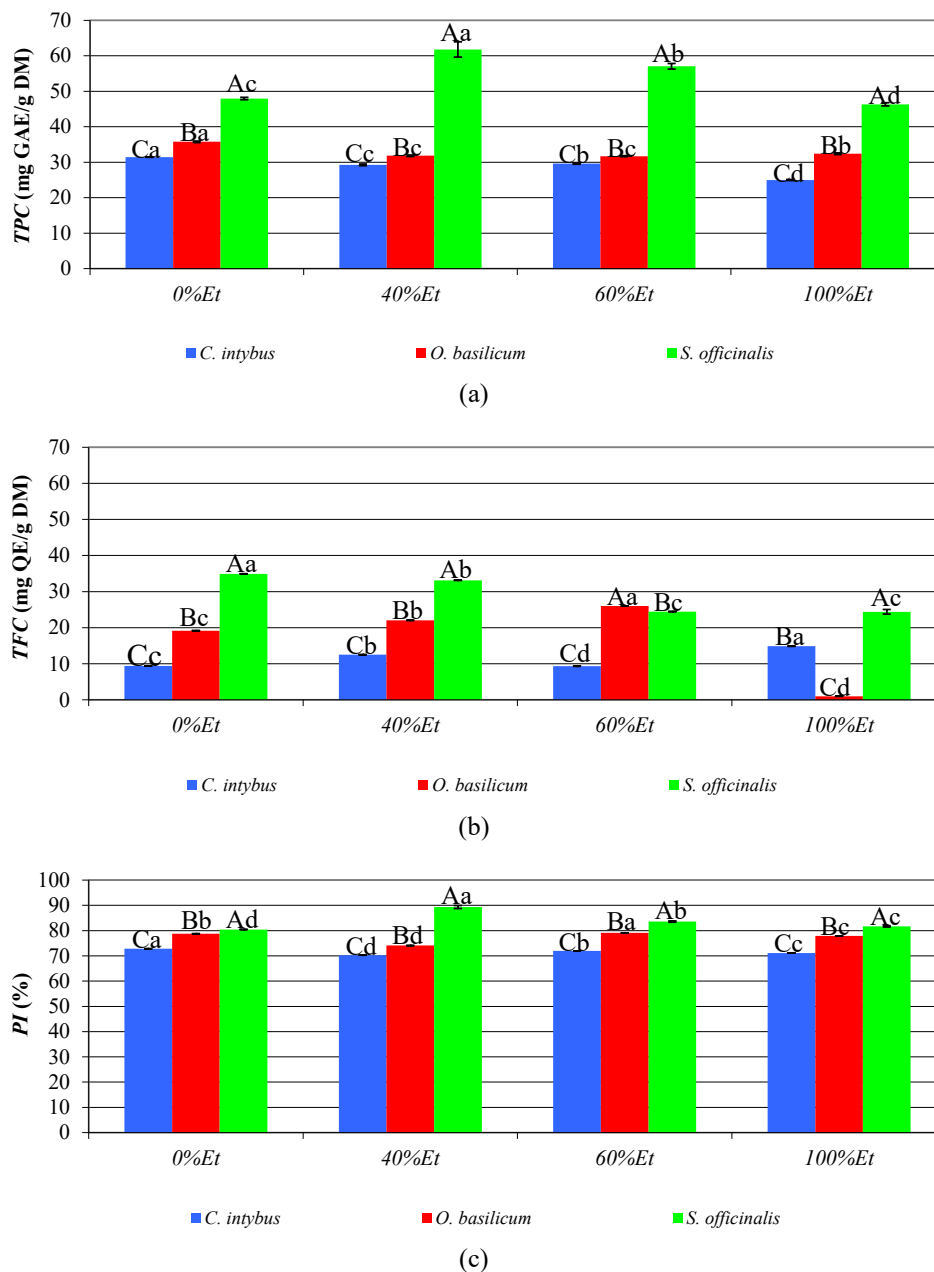


Fig. 2. Mean values $\pm$ SD of total polyphenol content (TPC) (a), total flavonoid content (TFC) (b), and percentage of inhibition (PI) (c) of extracts of edible flowers obtained at different levels of ethanol concentration in a solution consisting of ethanol and methanol (0%, 40%, 60%, and 100%); different uppercase letters indicate significant differences ($p < 0.05$) among flower type for each treatment; different lowercase letters indicate significant differences among treatments for each flower type.

Except for the non-significant correlations between *TFC*60 and *TPC*40, *TFC*60 and *TPC*60, *TFC*100 and *TFC*40, *TFC*100 and *TFC*60, *TFC*100 and *PI*0, *TFC*100 and *PI*60, and *TFC*100 and *PI*100 ($-0.191 \leq r \leq 0.486$), the other correlations between variables were significant ($0.525 \leq r \leq 0.999$) (Table 2.11.).

Table 2.11. Correlation matrix

Variabl	TPC	TPC4	TPC6	TPC10	TFC	TFC4	TFC6	TFC10	PI0	PI40	PI60	PI100
TPC0	1											
TPC40	0,98	1										
TPC60	0,98	0,997	1									
TPC100	0,99	0,956	0,959	1								
TFC0	0,99	0,947	0,949	0,999	1							
TFC40	0,97	0,915	0,916	0,991	0,99	1						
TFC60	0,64	0,486	0,484	0,709	0,73	0,793	1					
TFC100	0,63	0,763	0,764	0,556	0,52	0,446	-0,191	1				
PI0	0,83	0,718	0,718	0,883	0,90	0,936	0,957	0,103	1			
PI40	0,99	0,992	0,991	0,985	0,98	0,958	0,588	0,681	0,79	1		
PI60	0,92	0,831	0,831	0,953	0,96	0,984	0,888	0,280	0,98	0,89	1	
PI100	0,91	0,815	0,816	0,944	0,95	0,979	0,900	0,255	0,98	0,88	0,99	1

Values in bold of correlation coefficient (r) are different from 0 with a significance level $\alpha = 0.05$.

Moreover, the data shown in Fig. 2.4. highlight the following aspects:

- for *S. officinalis* extract samples, higher values of *TPC* and *PI* were obtained at ethanol concentrations (*cet*) of 40% (61.67 ± 2.14 mg GAE/mg DM and $89.35 \pm 0.63\%$) and 60% (57.04 ± 0.78 mg GAE/mg DM and $83.56 \pm 0.26\%$) compared to *cet* = 0% (47.93 ± 0.34 mg GAE/mg DM and $80.45 \pm 0.04\%$) and *cet* = 100% (46.31 ± 0.47 mg GAE/mg DM and $81.73 \pm 0.26\%$); higher values of *TFC* were obtained at *cet* = 0% (34.92 ± 0.07 mg QE/mg DM) and *cet* = 40% (33.13 ± 0.05 mg QE/mg DM) compared to *cet* = 60% (24.44 ± 0.03 mg QE/mg DM) and *cet* = 100% (24.40 ± 0.61 mg QE/mg DM); accordingly, operating at *cet* = 40% is more advantageous;

- for *O. basilicum* extract samples, the highest value of *TPC* was obtained at *cet* = 0% (35.76 ± 0.20 mg GAE/mg DM) and the highest values of *TFC* and *PI* were obtained at *cet* = 60% (26.02 ± 0.05 mg QE/mg DM and $79.12 \pm 0.02\%$);

- for *C. intybus* extract samples, higher values of *TPC* were obtained at *cet* = 0% (31.41 ± 0.06 mg GAE/mg DM), *cet* = 40% (29.28 ± 0.24 mg GAE/mg DM), and *cet* = 60% (29.62 ± 0.16 mg GAE/mg DM) compared to *cet* = 100% (24.98 ± 0.16 mg GAE/mg DM); higher values of *TFC* were obtained at *cet* = 100% (14.85 ± 0.05 mg QE/mg DM) and *cet* = 40% (12.51 ± 0.03 mg QE/mg DM) compared to *cet* = 0% (9.41 ± 0.03 mg QE/mg DM) and *cet* = 60% (9.32 ± 0.07 mg QE/mg DM); higher values of *PI* were obtained at *cet* = 0% ($72.81 \pm 0.01\%$) and *cet* = 60% ($71.99 \pm 0.01\%$) compared to *cet* = 40% ($70.28 \pm 0.02\%$) and *cet* = 100% ($71.15 \pm 0.01\%$);

- extract samples prepared from *S. officinalis* generally had significantly higher mean values of *TPC* (46.31–61.77 mg GAE/mg DM), *TFC* (24.40–34.92 mg QE/mg DM), and *PI* (80.45–89.35%) compared to those obtained from *O. basilicum* and *C. intybus* (only *TFC*60m, *SO* = 24.44 mg QE/mg DM was lower than *TFC*60m, *OB* = 26.02 mg QE/mg DM); these values are consistent with data reported in the related literature (Moshari-Nasirkandi et al., 2024; Pop et al., 2015).

Methanol and methanol solutions generally extract a greater amount of bioactive compounds. The solvent can then be removed from the extract, resulting in a powder, which can then be dissolved in an environmentally friendly solvent, e.g., ethanol

2.2.1.3. Conclusions

Extracts of common sage (*S. officinalis*), chicory (*C. intybus*), and basil (*O. basilicum*) were obtained through maceration, using ethanol, methanol, and two mixtures of ethanol and methanol (containing 40% and 60% ethanol) as extraction solvents. Total polyphenol content (*TPC*), total flavonoid content (*TFC*), and percentage of inhibition (*PI*) of edible flower extracts were determined. The experimental data, which were processed using PCA and one-way ANOVA, indicate the following relevant aspects:

- generally, the extracts obtained from common *S. officinalis* had significantly higher mean values of *TPC*0–100 (46.31–61.77 mg GAE/mg DM), *TFC*0–60 (24.44–34.92 mg QE/mg DM), and *PI*0–100 (80.45–89.35%) than those prepared from *C. intybus* (24.98–31.41 mg GAE/mg DM, 9.32–12.51 mg QE/mg DM, and 70.28–72.81%) and *O. basilicum* (31.70–35.76 mg GAE/mg DM, 19.16–26.02 mg QE/mg DM, and 80.45–89.35%);
- the extracts obtained from *O. basilicum* had significantly lower mean values of *TFC*100 (0.97 mg QE/mg DM) than those prepared from *S. officinalis* (24.40 mg QE/mg DM) and *C. intybus* (14.85 mg QE/mg DM);
- the correlations between *TPC*, *TFC*, and *PI* were generally significant ($0.525 \leq r \leq 0.999$).

3. GENERAL CONCLUSIONS AND RESEARCH PERSPECTIVES

The doctoral thesis entitled **CHEMOMETRIC METHODS USED FOR THE EVALUATION OF THE QUALITY OF FOOD PRODUCTS AND THE EFFICIENCY OF THE EXTRACTION OF BIOACTIVE COMPOUNDS FROM PLANTS** is structured in 2 main parts, *i.e.*, a **CRITICAL STUDY OF LITERATURE DATA**, which contains relevant aspects about chemometric methods, the evaluation of the quality of honey and the extraction of bioactive compounds from edible plants, and a part of **ORIGINAL CONTRIBUTIONS**, in which the results obtained in the own research are presented. This part is structured in 2 chapters, one chapter regarding the characterization and classification of honey based on physicochemical parameters and chemometrics, the other regarding the effect of the extraction solvent on the composition and antioxidant activity of edible plant extracts.

In chapter **2.1. Characterization and classification of Romanian acacia honey based on physicochemical parameters and chemometrics**, the results of the physicochemical analysis for samples of pure acacia honey (P), indirectly adulterated honey (I), obtained by feeding bees with 3 different industrial syrups, and directly adulterated honey (D), prepared by mixing P honey with the same syrups, are presented. The study aimed at measuring the relevant physicochemical parameters of the honey samples and to discriminate pure from adulterated honey based on these parameters, using PCA and LDA as chemometric tools.

A total of 23 honey samples (P, I, D) were analyzed, and the following parameters were determined: contents of moisture, ash, 5-hydroxymethylfurfural (HMF), reducing sugars (RS) and sucrose, free acidity (FA), diastase activity (DA), and $\Delta\delta^{13}C = \delta^{13}C_H - \delta^{13}C_P$ were the physicochemical parameters considered in the multivariate analysis.

The adulteration of honey samples determined the increase in the average values of water content (by approximately 10%), free acidity (FA) (by approximately 7 times), sucrose content (by 2.6 times, respectively 1.7 times for samples I and D), HMF content (by approximately 25 times, respectively 18 times for samples I and D) and $\Delta\delta^{13}C = \delta^{13}C_H - \delta^{13}C_P$ (by approximately 17 times, respectively 15 times for samples I and D), as well as a decrease in the average values of sugar content (by approximately 10%) and diastase activity (by 2.8 times, respectively 2.1 times for samples I and D) compared to the average values of the variables for samples P. The values obtained for diastase activity (DA) and HMF, RS and sucrose contents for honey samples D and I did not fall within the ranges imposed by the national standard.

For the types and doses of sugar syrups used in this study, indirect adulteration had effects similar to those produced by direct adulteration.

The physicochemical parameters considered in the multivariate analysis were: moisture, ash, RS, sucrose and HMF contents, FA, DA and $\Delta\delta^{13}C$. According to PCA, the eight-dimensional feature space was reduced to a two-dimensional one, in which the directions of the new axes were defined by the eigenvectors PC1 and PC2.

PC1 and PC2 explained 76.3% (54.0% + 22.3%) of the total variation. The relevant variables on the PC1 axis were DA, HMF, FA, $\Delta\delta^{13}C$ and RS and sucrose contents, and the important ones on the PC2 axis were moisture content, $\Delta\delta^{13}C$ and sucrose and ash contents. Three well-separated honey groups (P, D and I) on the PC1 direction were obtained by designing the honey samples on the PC1-PC2 factorial design.

Starting from these predefined groups, stepwise LDA indicated that the most important discriminatory physicochemical parameters were DA and the contents of HMF, RS and sucrose. Linear discrimination and classification functions were built based on a training data set, and then these models were validated using a validation data set. Cases were classified based on the highest values of the posterior classification probability and the score of the classification function. The classification matrices for the training and validation data sets indicated that 100% of the honey samples were correctly assigned to their original group.

Consequently, the evaluation of physicochemical parameters using PCA and LDA was very effective in discriminating between pure (P) and directly (D) and indirectly (I) adulterated honey. Based on the physicochemical parameters, i.e., DA and the contents of HMF, sucrose and RS, any acacia honey sample could be simply and quickly classified as pure or adulterated (directly or indirectly) using the classification functions obtained by applying LDA.

However, the study has some limitations, e.g., a small number of acacia honey samples, their relative similarity (all coming from the same producer and being produced in the same period). The analysis could be extended using more samples of different types of honey collected from more producers over a longer period.

Chapter 2.2. The effect of the extraction solvent on the composition and antioxidant activity of edible flower extracts is structured in 2 subchapters, one referring to the extracts obtained from the aerial parts of chicory (*C. intybus*), basil (*O. basilicum*) and sage (*S. officinalis*) plants, the other to the extracts obtained from the flowers of cornflower (*C. cyanus*), pot marigold (*C. officinalis*), hibiscus (*H. sabdariffa*) and chamomile (*M. chamomilla*). The extracts were obtained by maceration with agitation, using ethanol, methanol and two mixtures of ethanol and methanol (containing 40% and 60% ethanol) as extraction solvents. The total polyphenol content (*TPC*), total flavonoid content (*TFC*) and percentage inhibition (*PI*) of the extracts were the variables selected as responses of the extraction process. The experimental data were processed using PCA and one-way ANOVA.

The results presented in subchapter **2.2.1. The effect of extraction solvent on the composition and antioxidant activity of basil (*O. basilicum*), chicory (*C. intybus*) and sage (*S. officinalis*)** extracts highlighted the following relevant aspects:

- generally, the extracts obtained from sage had significantly higher mean values of *TPC*_{0–100} (46.31–61.77 mg GAE/mg DM), *TFC*_{0–60} (24.44–34.92 mg QE/mg DM), and *PI*_{0–100} (80.45–89.35%) than those prepared from chicory (24.98–31.41 mg GAE/mg DM, 9.32–12.51 mg QE/mg DM, and 70.28–72.81%) and basil (31.70–35.76 mg GAE/mg DM, 19.16–26.02 mg QE/mg DM, and 80.45–89.35%)
- the extracts obtained from basil had significantly lower mean values of *TFC*₁₀₀ (0.97 mg QE/mg DM) than those prepared from sage (24.40 mg QE/mg DM) and chicory (14.85 mg QE/mg DM);
- the correlations between *TPC*, *TFC*, and *PI* were generally significant ($0.525 \leq r \leq 0.999$).

Metanolul și soluțiile de metanol extrag în general o cantitate mai mare de compuși bioactivi. Solventul poate fi eliminat apoi din extract, rezultând o pulbere, care, ulterior, poate fi dizolvată într-un solvent ecologic, e.g., etanol.

The results presented in subchapter 2.2.2. **The effect of extraction solvent on the composition of cornflower (*C. cyanus*), marigold (*C. officinalis*), hibiscus (*H. sabdariffa*) and chamomile (*M. chamomilla*)** extracts indicated the following aspects:

- the extracts obtained from marigold had significantly higher mean values of *TPC*0–100 (49,13–62,36 mg GAE/mg DM), *TFC*0, *TFC*40 și *TFC*100 (32,46–34,72 mg QE/mg DM) than those prepared from cornflower (27,26–35,38 mg GAE/mg DM, and 6,46–9,58 mg QE/mg DM), chamomile (30,62–33,84 mg GAE/mg DM, and 7,64–9,98 mg QE/mg DM) and hibiscus (33,16–35,03 mg GAE/mg DM, and 12,00–13,64 mg QE/mg DM);

- average value of the *TFC*60 variable for hibiscus extract (26,76 mg QE/mg DM) was significantly higher than the average values of the *TFC*60 variable for marigold extracts (18,22 mg QE/mg DM), cornflower (5,93 mg QE/mg DM) and chamomile (5,87 mg QE/mg DM);

- *TPC*0, *TPC*40, *TPC*60, *TPC*100, *TFC*0, *TFC*40 și *TFC*100 were directly correlated very strongly ($0,966 \leq r \leq 0,999$); except for the significant correlation between *TFC*60 și *TFC*0 ($r = 0,480$), the correlations between *TFC*60 and the other variables were insignificant ($0,262 \leq r \leq 0,405$);

- for marigold extracts, the average *TPC* value obtained from ethanol extraction (62,36 mg GAE/mg DM) was significantly higher than the average values obtained in extractions with the other solvents (49,13–52,69 mg GAE/mg DM); average *TFC* value obtained from ethanol extraction (2,46 mg QE/mg DM) was up to 7% lower than the highest average *TFC* values obtained in methanol extractions (34,51 mg QE/mg DM), respectively solvent containing 40% ethanol (34,72 mg QE/mg DM); thus, ethanol is an effective solvent for the extraction of phenolic compounds from marigold flowers;

- for chamomile extracts, the average *TFC* value obtained from ethanol extraction (9,98 mg QE/mg DM) was significantly higher than the average values obtained in extractions with the other solvents (5,87–8,57 mg QE/mg DM); thus, ethanol is an effective solvent for the extraction of flavonoids from chamomile flowers.

The research undertaken so far can be continued/deepened in future studies. ***The main objectives/research directions that I continue to pursue*** relate to:

- quality assessment of other food products (oils, wines) based on specific analyses and chemometrics;
- - extraction of bioactive compounds, *e.g.*, phenolic compounds (phenolic acids, flavonoids, coumarins, lignans, tannins), alkaloids, glycosides, terpenoids (monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids, tetraterpenoids), from other edible plants using various conventional/unconventional techniques and experimental programming;
- - evaluation of the antioxidant and/or antimicrobial activity of the obtained extracts and their integration into cosmetic formulations and/or food products.

PAPER LIST

Papers published in ISI journals:

1. **Donise, A.C.**, Pârvulescu, O.C., Crăciun, M.E., Popescu, G.E., Drăghici-Popa, A.M, Tudor, C.A. Effect of extraction solvent on the composition and antioxidant activity of edible flower extracts. *U.P.B. Sci. Bull. Series B* **2025**, 87(4) (accepted). **IF (2024): 0.3**.
2. Crăciun, M.E., Pârvulescu, O.C., **Donise, A.C.**, Dobre, T., Stanciu, D.R. Characterization and classification of Romanian acacia honey based on its physicochemical parameters and chemometrics. *Scientific Reports* **2020**, 10(1), 20690. **IF (2020): 4.379**. <https://doi.org/10.1038/s41598-020-77685-9>

Oral (O)/poster (P) presentations at international conferences/symposia:

1. **Donise, A.C.**, Crăciun, M.E., Cristea, C. *Microbial incidence in food products of animal origin*. 23rd Romanian International Conference on Chemistry and Chemical Engineering (RICCCE 23), 4-7 September 2024, Constanta - Mamaia, Romania (P).
2. **Donise, A.C.**, Mirică, O., Crăciun, M.E., Pârvulescu O.C. *Statistical approach on sensory quality of croissants stored in improper conditions*. 16th International Conference of Constructive Design and Technological Optimization in Machine Building Field (OPROTEH), 25-27 May 2021, Bacau, Romania (P).
3. Popescu, G.E., **Donise, A.C.**, Păun, A., Crăciun, M.E. *Quality control of Metaxa cognac*. The Virtual Eurachem Workshop 2020 - "Quality Assurance for Analytical Laboratories in the University", 14-15 July 2020 (O).
4. **Donise, A.C.**, Popescu, G.E., Păun, A., Crăciun, M.E. *Determination of chemical and nutritional characteristics of edible flowers*. The Virtual Eurachem Workshop 2020 - "Quality Assurance for Analytical Laboratories in the University", 14-15 July 2020 (P).
5. Crăciun, M.E., Pârvulescu, O.C., **Donise, A.C.** *Detection of adulterated honey based on Romanian standardized methods*. 11th International Conference of Applied Sciences (CISA 2019), 22-24 May 2019, Bacau, Romania (P).
6. Marinică, A.D., Savu, A.V., **Donise, A.C.**, Crăciun, M.E. *Enzymatic dosing on honey*. IasiCHEM 2018, 26 October 2018, Iasi, Romania (P).

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