“Dunărea de Jos” University of Galati

Doctoral School of Fundamental Sciences and Engineering

DOCTORAL THESIS

BIOTECHNOLOGICAL RESEARCH ON THE USE OF HONEY IN THE FOOD INDUSTRY

PhD Student,

Giorgiana- Valentina BLAGA

Scientific coordinator:

Prof.dr.ing. Camelia VIZIREANU

Series I 1: Biotechnology Nr. 13

GALATI

2021

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(Doctoral thesis summary)

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CONTENT

Introduction

Nutrition education, along with food safety and security, are priorities that guarantee the future of the nation. Increasing awareness of the impact of food on the body resulted in a change in food preferences. Thus, consumers are in a constant search for products that bring many benefits to the body. Functional foods are excellent food options, because they improve the quality of life (Domínguez Díaz et al., 2019). Functional foods have been promoted in the food industry and products containing a suitable amount of bioactive compounds have been developed that significantly reduce the occurrence of diseases (Al-Mamary et al., 2002).

Bee products are recognized as valuable nutrient resources since antiquity. Bee products, honey, pollen, beebread and royal jelly represents a source of main nutrients such as: proteins, sugars, fatty acids, minerals, vitamins and organic acids (Dezmirean et al., 2011). Honey is the most common bee product and is one of the most complex products from biological point of view, in the composition of which are valuable compounds for the human body. The nutritional and therapeutic properties of honey as a functional food are offered by its varied composition.

Also in this category of functional foods we find germinated cereals. The germination process improves the nutritional quality of cereals, increases the content and availability of essential nutrients and also decreases the content of antinutrients (Marton et al., 2010; Patil and Khan, 2011; Wu et al., 2013). Bars are the fastest and easiest way to secure our food, an alternative to the busy schedule of consumers. Honey, sprouted sorghum, dehydrated fruits are ingredients that can increase the nutritional value and bring benefits to the health of consumers.

The Ph.D. thesis entiled ”**Biotechnological research on the use of honey in the food industry**” focused on the study of different types of honey (chestnut, sunflower, acacia, lime, rape, spring, mint, raspberry, buckwheat, honeydew and polyfloral) and soryz flour, under physico-chemical, biochemical and rheological aspect and also the development of an innovative product.

The main aim of the thesis was fundamental knowledge exploitation regarding the honey and flour germinated soryz flour for the development of a product with added nutritional value destined for people who are intolerant to gluten, but also for those who want a healthy diet.

In this context, the work plan of the doctoral program revealed the following specific objectives:

- collection of honey samples directly from beekeepers who certified their botanical and geographical origin;
- analysis of the composition of honey samples with different botanical and geographical origins, by physico-chemical methods;
- the study of biologically active properties of honey samples by assessing the total polyphenol content, total flavonoid content and antiradical capacity;
- FT-IR spectrometry analysis of honey samples;
- evolution of the rheological behavior of honey by evaluating different rheological parameters;

- investigation of the presence of azole compounds and fungicide residues from honey samples;
- the germination process of soryz grains and the physical-chemical characterization of soryz flour;
- development and the physical-chemical characterization of a functional product intended for people with food intolerance.

The PhD thesis follows two main parts such as:

I.DOCUMENTARY STUDY has 5 chapters which presents the most recent data from the literature regarding the evolution of beekeeping in Romania, the impact of bees on the environment, the functionality of honey, aspects related to celiac disease and the functional product concept and the importance of germinated grains in the daily diet.

II.THE EXPERIMENTAL PART includes the original investigations that were carried out durring the doctoral research and is structured in 8 chapters, as follows:

Chapter 6, entitled **Physico-chemical characterization of honey**, presents the results obtained following the identification and quantification of the most important parameters of honey, such as: water, pH, ash, electrical conductivity, refractive index and color.

Chapter 7, entitled **Study of antioxidant activity of honey**, presents the results obtained from the evaluation of the antioxidant capacity by the following methods: determination of the total polyphenol content by Folin- Ciocâlteu method, determination of the total flavonoid content and the method using the DPPH reagent.

Chapter 8, entitled **FT-IR spectrometry analysis of honey**, presents the identification of the bands characteristic of the specific frequencies of the main components of honey.

Chapter 9, entitled **Rheological behavior of honey**, presents the rheological profiles of honey samples analyzed by various tests such as: oscilatory stress sweep, time sweep and stepped sweep.

Chapter 10, entitled **The determination of fungicide residues from honey**, presents the identification of fungicide residues in honey samples with the high performance liquid chromatography coupled with high resolution mass spectrometry Q Exactive Orbitrap MS.

Chapter 11, entitled **The germination process of soryz grains and the physical-chemical characterization**, presents the characterization of the germination process and the physico-chemical characterization of native and germinated soryz grains.

Chapter 12, entitled **Obtainment of a functional product**, presents the technology developed to obtain bars based on germinated soryz flour, buckwheat honey, dried fruits, almonds and beebread.

Chapter 13, entitled **General conclusions**, shows the main conclusions that were drawn based on the experiments that followed the obtainment of a functional product for people with food intolerances.

The thesis includes 154 pages, which includes 31 figures and 19 tables. The documentary study represents 20% and the experimental part represent 80%.

The research activities of the PhD thesis were carried out within the MoRAS Research Center-Chromatography and Imagistic Laboratory and within the Master Research Laboratory, from the Faculty of Food Science and Engineering, ”Dunărea de Jos” University Galati.

Throughout the PhD stage, the PhD student was involved in the following projects research teams: POSCCE-ID 1815, SMIS code 48745, MORAS with the title ”Romanian Center for modeling recirculating aquaculture systems”; PN-III-P2-2.1-BG-2016-0143 with the title ”Solutions for Multicerealier Grinding”; ”AUF- Organisation de séminaires doctoraux - appel à projets” 2016 and Contract no.9 PCCDI/2018 with the title ” Complex system for full recovery of agricultural species with energy and food potential” (VALINTEGR).

The thesis was conducted under the scientific coordinationof the scientific committee that has the following members:

- Prof.dr.eng. Camelia Vizireanu- PhD supervisor;
- Prof.dr.eng. Daniela Borda;
- Prof.dr.eng. Iuliana Aprodu;
- Conf.dr.eng. Daniela Istrati.

6. Physico-chemical characterization of honey

Honey contains mainly fructose (38%), glucose (31%), water, amino acids, minerals, enzymes, vitamins and small amounts of protein (Alvarez-Suarez et al., 2010). Depending on the source of nectar or polen used by the bees, honey can be floral (monofloral and polyfloral) and honeydew (animal or vegetable). The composition of honey is influenced by botanical and geographical origin and climatic conditions (Baroni et al., 2015; Solayman et al., 2016). It is a complex natural product, which presents a multitude of mythological, historical and traditional references (Mădaș et al., 2012). In a first stage, this importance is evaluated and confirmed, by determining the physico-chemical parameters. Most of the physico-chemical methods used in honey analysis are mainly developed for quality control and honey authenticity. However, some of them, such as mineral content, electrical conductivity, pH, color and sugar content allow the identification of the botanical origin of honey (Rouff, 2006; Vanhanen et al., 2011).

The analytical methods used for honey classification are mostly the same as the ones used for routine honey control. The methods are validated and improved by the International Honey Commission (Bogdanov et al., 1997).

6.1. Materials and methods

Eightteen honey samples from different geographic areas in Romania were analyzed: 2 samples from Oltenia region, 12 samples from Moldova region and 4 samples from Transilvania region (Table 6.1.). The sample were purchased from beekeepers who certified their botanical and geographical origin. All samples were harvested in 2015. All honey samples were stored in in glass containers, under refrigeration.

Investigations in this chapter are followed:

- Moisture determination
- pH determination
- Ash determination
- Electrical conductivity determination
- Refractive index determination
- Colour determination

Table 6.1. Geographical and botanical origin of the honey samples used in the study

Type of honey	Geographical origin
Chestnut (CI;1)	Iasi
Sunflower (FSI;2)	
Honeydew (MI;3)	
Mint (MtI;7)	
Spring (PrI;4)	
Rape (RI;5)	
Acacia (SI;8)	
Linden (TI;6)	

Raspberry (ZmI;9)	
Buckwheat (HSb;10)	Sibiu
Honeydew (MSb;13)	
Rape (RSb;11)	
Acacia (SSb;12)	
Polyfloral (PV;14)	Valcea
Salcâm (SV;15)	
Sunflower (FS;16)	Galati
Polyfloral (P;18)	
Acacia (S;17)	

6.2. Results and disscusion

The maximum moisture content is 20 % according to the international regulations of quality (Codex Alimentarius, 2001). The acacia honey from Sibiu had the lowest moisture content (12%) and the highest was found in the buckwheat honey (21.64%). However, the samples showed normal values for our country, since Mărghitaș (2008) reported values between 13.30% and 22.40%; moreover, Bogdanov and Martin (2002) indicate values between 13.60% and 23%.

The pH of honey is acid, between 3.5 and 5.5 for blossom honey and 4.5 – 6.1 for honeydew. Its determination can be correlated with other parameters of authenticity to detect the adulteration of honey. The pH values varied between 3.16 – 4.42, in according to the european and international standards.

In relation to the ash content, honeydew had a higher content than floral honey. In addition, quite high values were obtained in the case of chestnut and raspberry honey, while the lowest values were two acacia honeys (acacia Iasi and acacia Sibiu).

In correlation with the ash content, electrical conductivity (EC), an indicator used in quality control of honey but also for the differentiation of floral honey from honeydew (Karabagias et al., 2014; Pita-Calva et al., 2017), has high values for honeydew and raspberry honey. EC for the rest of the monofloral and polyfloral honey, was below 0.8 mS/cm, being a value required monofloral honey.

The values of the refractive index were between 1.4792 – 1.5002. A sample of honeydew had the highest refractive index value but also the highest amount of sugar. The lowest value was for rape from Sibiu (Table 6.2.).

In the present study, honeydew showed values of the colour parameter L * ≤50, presenting a brown color. All acacia honey showed a light yellow color, so high values could be observed in terms of brightness compared to other samples. More intense shades of yellow were identified in the sunflower, polyflora and mint honey which had high values of the colour parameter b * (Table 6.2.). L^* colour parameter ranged from 33.94 to 94.04, a^* from -1.25 to 15.39 and b^* from 6.06 to 66.19.

Table 6.2. Physico-chemical characteristics of the analyzed honey samples

Type of honey	Moisture (%)	pH	Ash (%)	EC (mS/cm)	IR(%)	L*	a*	b*
CI	13.50±0.27^{e,f,g,h}	3.70±0.00^{e}	0.51±0.00^{b}	0.43±0.00^{f}	1.4911±0.0001^{f}	81.64±0.51^{f}	1.53±0.01^{e}	47.46±1.90^{d}
FS	13.83±0.54^{d,e,f,g,h}	3.33±0.00^{h,i}	0.15±0.01^{d,e,f}	0,35±0.00^{h}	1.4883±0.0002^{g}	76.95±0.11^{h}	3.11±0.01^{d}	66.19±0.15^{a}
FSI	13.77±0.49^{e,f,g,h}	3,45±0,00^{g}	0,16±0,03^{d,e,f}	0,41±0,00^{g}	1,4865±0,0001^{h,i}	80,41±0,04^{g}	-1,25±0,01^{f}	58,56±0,12^{b}
HSb	21.64±0.03^{a}	3.30±0.00^{h,i}	0.13±0.00 ^{d,e,f,g,h}	0.49±0.00^{e}	1.4865±0.0001^{g,h}	33.94±0.03^{o}	10.88±0.21^{b,c}	6.06±0.22^{l}
MI	13.35±0.50^{f,g,h,i}	3.96±0.00^{d}	0.64±0.01^{a}	1.01±0.00^{c}	1.5001±0.0001^{a}	59.37±0.16^{m}	15.39±0.79^{a}	44.72±0.19^{e}
MSb	15.31±0.05^{c}	4.10±0.00^{c}	0.70±0.00^{a}	1.10±0.00^{a}	1.4955±0.0001^{b,c}	53.32±0.30^{n}	10.22±0.03^{c}	23.75±0.16^{i}
MtI	13.26±0.26^{g,h,i}	3.29±0.01^{i}	0.30±0.02^{c}	0.33±0.00^{i}	1.4945±0.0002^{c,d,e}	80.41±0.04^{g}	-1.75±0.11^{f,g,h}	58.56±0.12^{b}
P	19.91±0.01^{b}	3.16±0.00^{j}	0.12±0.02 ^{d,e,f,g,h}	0.33±0.00^{i}	1.4802±0.0000^{k}	75.49±0.04^{i}	-1.44±0.01^{f,g}	60.06±0.04^{b}
PV	14.44±0.47^{c,d,e,f,g,h}	4.42±0.00^{b}	0.10±0.01 ^{e,f,g,h}	0.12±0.00^{o}	1.4961±0.0001^{b}	68.41±0.21^{k}	10.11±0.22^{c}	56.19±0.05^{c}
PrI	13.22±0.38^{g,h,i}	3.37±0.00^{h}	0.10±0.00 ^{e,f,g,h}	0.24±0.00^{k}	1.4999±0.0001^{a}	89.19±0.21^{d}	-2.71±0.04^{l,j}	40.45±0.47^{f}
RI	14.81±0.12^{c,d,e,f}	3.52±0.00^{f,g}	0.14±0.01 ^{d,e,f,g}	0.31±0.00^{j}	1.4842±0.0001^{j}	72.33±0.04^{j}	2.78±0.02^{d}	34.94±0.09^{g}
RSb	15.28±0.32^{c,d}	3.50±0.00^{f,g}	0.04±0.00^{h}	0.15±0.00^{n}	1.4795±0.0004^{k}	85.25±0.22^{e}	-1.98±0.15^{f,g,h,i}	23.94±0.30^{i}
S	14.26±0.09^{c,d,e,f,g,h}	3.40±0.00^{g}	0.18±0.03^{d,e}	0.16±0.00^{m}	1.4934±0.0006^{d,e}	91.33±0.23^{c}	-2.52±0.08^{h,i}	16.41±0.59^{j}
SI	14.91±0.83^{c,d,e}	3.46±0.00^{g}	0.05±0.01^{g,h}	0.12±0.00^{o}	1.4949±0.0004^{b,c,d}	94.04±0.35^{a}	-1.63±0.06^{f,g,h}	9.37±0.30^{k}
SSb	12.00±0.13^{i}	3.56±0.00^{f}	0.08±0.02^{f,g,h}	0.18±0.00^{l}	1.4933±0.0008	92.69±0.01^{b}	-2.30±0.01^{g,h,i}	16.64±0.02^{i}
SV	14.57±0.40^{c,d,e,f,g}	3.56±0.00^{f}	0.14±0.03^{d}	0.10±0.00^{p}	1.4937±0.0005^{d,e}	92.87±0.14^{b}	-1.89±0.00^{f,g,h,i}	10.73±0.05^{k}
TI	14.18±0.31^{c,d,e,f,g,h}	4.07±0.00^{c}	0.20±0.00^{d}	0.70±0.00^{d}	1.4855±0.0006^{i,j}	82.00±0.07^{f}	-3.62±0.02^{j}	31.61±0.39^{h}
ZmI	13.07±0.17^{h,i}	4.51±0.00^{a}	0.51±0.07^{b}	1.05±0.00^{b}	1.4963±0.0006^{b}	62.26±0.18^{l}	11.43±0.44^{b}	46.99±0.26^{d}

* encodings are indicated in table 6.1.

Different letters in columns denote significant differences, P < 0.05.

6.3. Partial conclusions

All honey samples showed good quality parameters fulfilling the imposed limits of EU Council Directive 2001/110. The physico-chemical parameters analyzed for the 18 honey samples are as follows:

- moisture values varied between 12.00 % and 21.64%;
- pH values was between 3.33 and 4.51, therefore in accordance to the recommended legal limits for fresh honey;
- ash content ranged from 0.04% to 0.70%; honeydew had the highest ash content and the lowest were at two samples of acacia honey (Iasi and Sibiu);
- electrical conductivity, an important parameter for the classification and botanical authentification of honey, showed values below 0.8 mS/cm for monofloral honey, while honeydew had values higher than 0.8 mS/cm;
- IR values was between 1.4792 – 1.5002; in term of colour, L^* ranged from 33.94 to 94.04, a^* ranged from-1.25 to15.39 and b^* ranged from 6.06 to 66.19.

7. Study of antioxidant activity of honey

Honey is one of the most complete food due to its therapeutic (Blasa et al., 2007), antioxidant (Lachman et al., 2010), antimicrobial (Escuredo et al., 2012), antitumoral (Jaganathan et al., 2011; Piljac-Zegarac et al., 2009), anti-inflammatory (Van Den Berg et al., 2008), antiviral (Watanabe et al., 2014) and antiulcer activities (Vandamme et al., 2013). Research into the role of apitherapic products in the prevention and treatment of human diseases has intensified (Nasuti et al., 2006).

7.1. Materials and methods

Investigations followed:

- Determination of the total polyphenol content by Folin- Ciocâlteu method
- Determination of the total flavonoid content
- Determination of antioxidant activity using the DPPH reagent

7.2. Results and discussion

The total polyphenolic content of analyzed honey samples showed a significant variation, with values between 26.73 mg EAG/100g to 264.17 mg EAG/100g (Figure 7.1). Of all the types of honey analyzed, buckwheat honey had the highest content of polyphenols (264.17 mg EAG/100g). High values were also recorded in a polyfloral honey from Galati (112.13 mg EAG/100g) and in a honeydew honey from Sibiu (110.30 mg EAG/100g). The research results showed that there are variations in the total polyphenol content depending on the floral and geographical origin of honey.

The flavonoid contents, were ranged from 6.59 mg Q/100g to 84.75 mg Q/100g (Figure 7.2). Buckwheat honey is noted as being the richest in total flavonoid content. Honeydew, sunflower and chestnut honeys they also presented a higher content of total flavonoids. Lower values of flavonoids content were found in rape, linden and spring honeys.

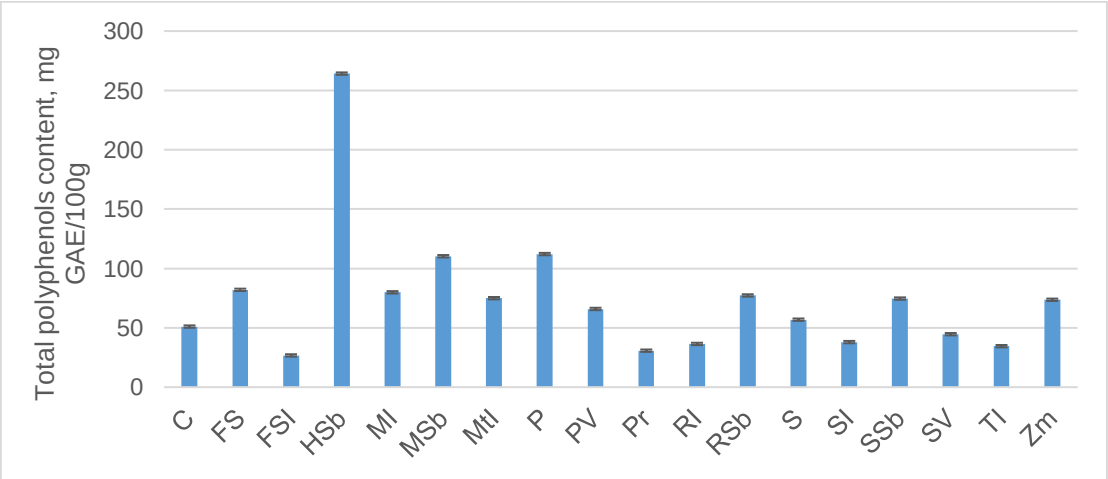

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Figure 7.1. The total polyphenols content in analyzed honey samples

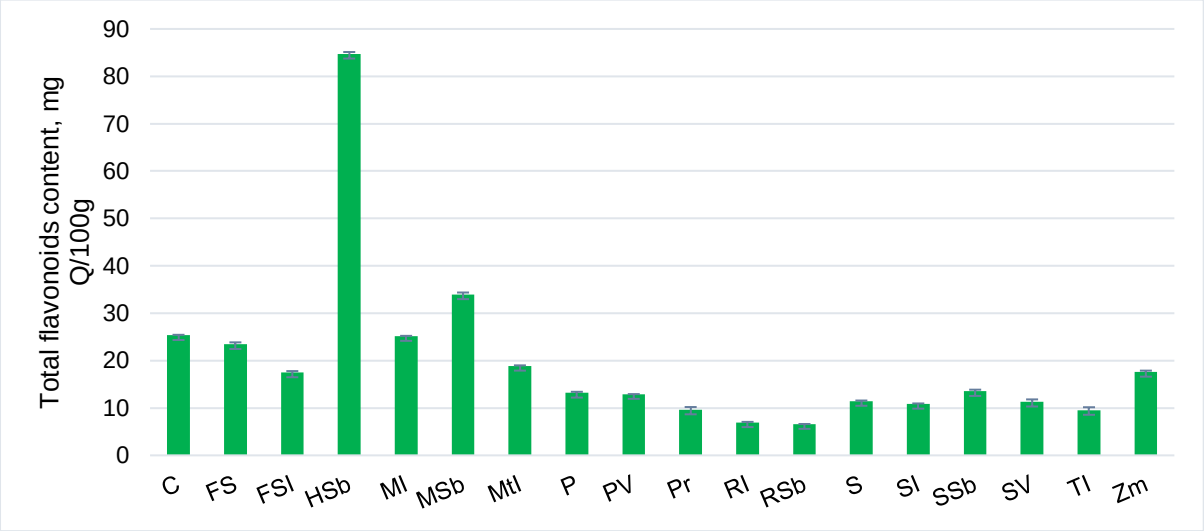

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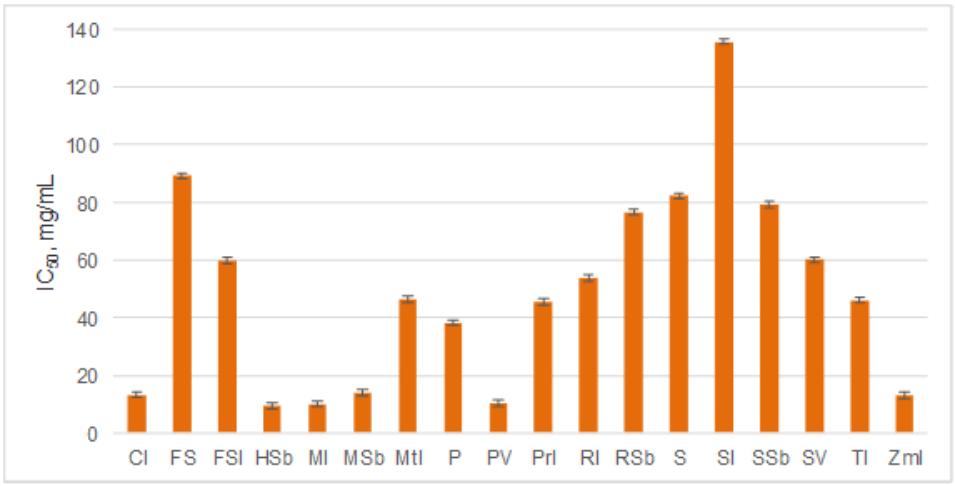
Figure 7.2. The total flavonoids content in analyzed honey samples

The results of antioxidant activity were expressed as IC_{50}. Buckwheat honey was revealed to other types of honey, having the highest antioxidant capacity (40.90%). Honeydew honey from Iasi also had a high content (37.26%). On the opposite side there was acacia honey from Iasi (4.65%) and a sunflower honey (4.33%). IC_{50} values varied between 9.44 mg/mL (buckwheat honey) – 135.72 mg/mL (acacia honey).



* encodings are indicated in table 6.1.

Figure 7.3. IC_{50} values in analyzed honey samples

7.2.4. Partial conclusions

The total content of phenolic compounds ranged from 26.73 mg EAG/100g to 264.17 mg EAG/100g. Regarding the percentage of DPPH free radical inhibition, buckwheat honey stood out compared to the other samples (40.90%). The same situation was recorded for flavonoids and polyphenols content. The results of this study showed that the honey samples analyzed are a good source of antioxidants. Honeydew were differentiated from other honey samples. The variation of the antioxidant activity of the analyzed honey samples was due to different botanical and geographical origins.

8. FT-IR spectrometry analysis of honey

In order to improve the analytical methods for honey quality control, it was considered necessary to develop simple and precise methods for routine analyzes of honey. (Jandrić et al., 2015). In this context, Fourier transform infrared spectroscopy (FT-IR) has proven to be a useful method (Kelly et al., 2006; Boffo et al., 2012) to evaluate different food constituents, especially when used in combination with multivariate chemometric methods, thus improving the speed of analysis, and is therefore neither time-consuming nor expensive,
by using expensive reagents (Anjos et al., 2015; Kasprzyk et al., 2018).

8.1. Materials and methods

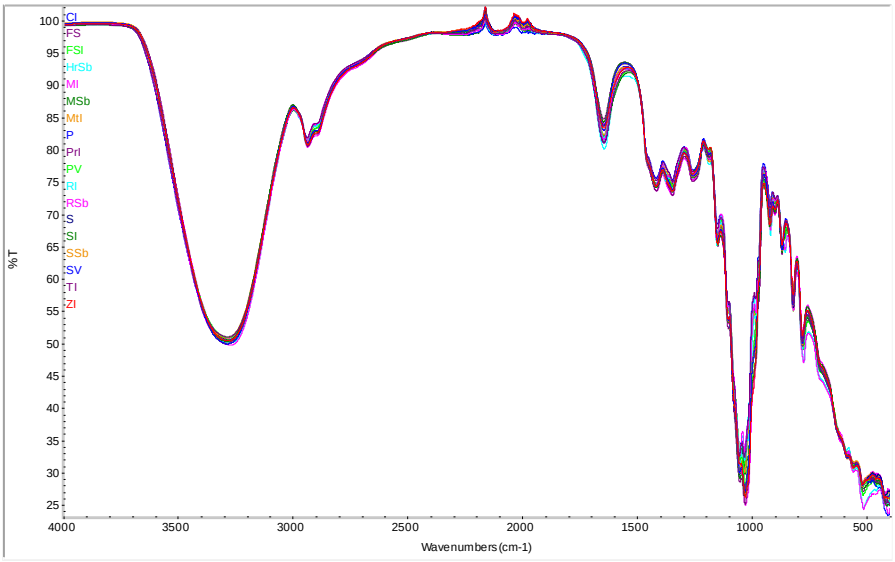
The spectra for each sample were obtained using a *Thermo Scientific Nicolet iS50 FT-IR* spectrophotometer. The samples were analyzed using the ATR technique (attenuated total reflection), with a cell equipped with a diamond crystal. The spectra were recorded from 4000 cm^{-1} to 400 cm^{-1}, with a resolution of 4 cm^{-1}. Data collection was performed using Omnic 9.2 (*Nicolet iS50*) software. For the interpretation of the results were taken into account the frequency characteristics of the functional groups of the organic compounds present in the honey, taken from the literature.

8.2. Results and discussion

The bands characteristic of water molecules at 3280 cm^{-1} (OH stretch) and 1641 cm^{-1} (OH deformations) (Kasprzyk et al., 2018). Band of 2932 cm^{-1} was attributed according to Tewari and Irudayaraj (2004) and Gallardo-Velazquez and colab., 2009 to the C - H bonds in carboxylic acids and NH3 in free amino acids. These compounds are found in honey in low concentrations. The bands in the range 1500 - 750 cm^{-1} represent the region characteristic of the important components of honey, being also the most suitable region to quantify the content of sugar and organic acids in honey. Sucrose, glucose and fructose have characteristic bands in the region between 1500 and 900 cm^{-1} (Wang et al., 2010).

According to the literature, the following peaks are important for the characterization of saccharides (Anjos et al., 2015; Sivakesava și Irudayaraj, 2001):

- the peak at 918 cm^{-1} corresponds to the C–H bending of the carbohydrate.
- the peaks at 1043 cm^{-1} and 1254 cm^{-1} correspond to the C–O stretch in the C–OH group as well as the C–C stretch in the carbohydrate structure;
- the small peak at 1110 cm^{-1} corresponds to stretching of the C–O band of the C–O–C linkage;
- the peak at 1321 cm^{-1} is due to O–H bending of the C–OH group;
- The peak at 1411 cm^{-1} is a combination of O–H bending of the C–OH group and C–H bending of the alkenes.



* encodings are indicated in table 6.1.

Figura 8.1. The overlapping ATR - FTIR spectra of all analyzed honey samples (18), recorded from 4000 cm^{-1} to 400 cm^{-1}

8.3. Partial conclusions

Based on the analysis of IR absorption frequencies in the ATR-FTIR spectra of the analyzed honey samples, recorded from 4000 cm^{-1} la 400 cm^{-1}, it was observed that the bands from 1413 cm^{-1}, 1343 cm^{-1}, 1254 cm^{-1}, 1100 cm^{-1}, 1050 cm^{-1} and 918 cm^{-1} are specific to the groups in the carbohydrate structure. The bands in the region 1500 – 750 cm^{-1} represent the IR absorption region of most of the main components of honey, being useful in the quantification of sucrose and organic acids in honey. Sucrose, glucose and fructose shows characteristic bands in the region between 1500 - 900 cm^{-1} (1413 cm^{-1}, 1343 cm^{-1}, 1254 cm^{-1}, 1100 cm^{-1}, 1050 cm^{-1}, 918 cm^{-1}). Also, in the spectra of honey samples, the bands characteristic of water molecules were identified between 3280 cm^{-1} - 1641 cm^{-1}.

9. Rheological behavior of honey

Studies on the rheological behavior of honey, as well as on other fluid foods, are important in view of technological operations, such as: handling, storage, processing, quality control and sensory analysis (Yoo 2004; Yanniotis et al., 2006). Moreover, the rheology of honey was correlated with its chemical composition (Gómez-Díaz et al. 2009). It is understood that the honey in its liquid form, has a Newtonian behavior.

9.1. Materials and methods

The rheological properties were studied using a *AR 2000ex* (*TA Instruments, Ltd.*) rheometer. The experimental data were obtained using the *TA Data Analysis software V4.8.3* software.

The rheometric tests applied were:

a. *oscilatory stress sweep*;

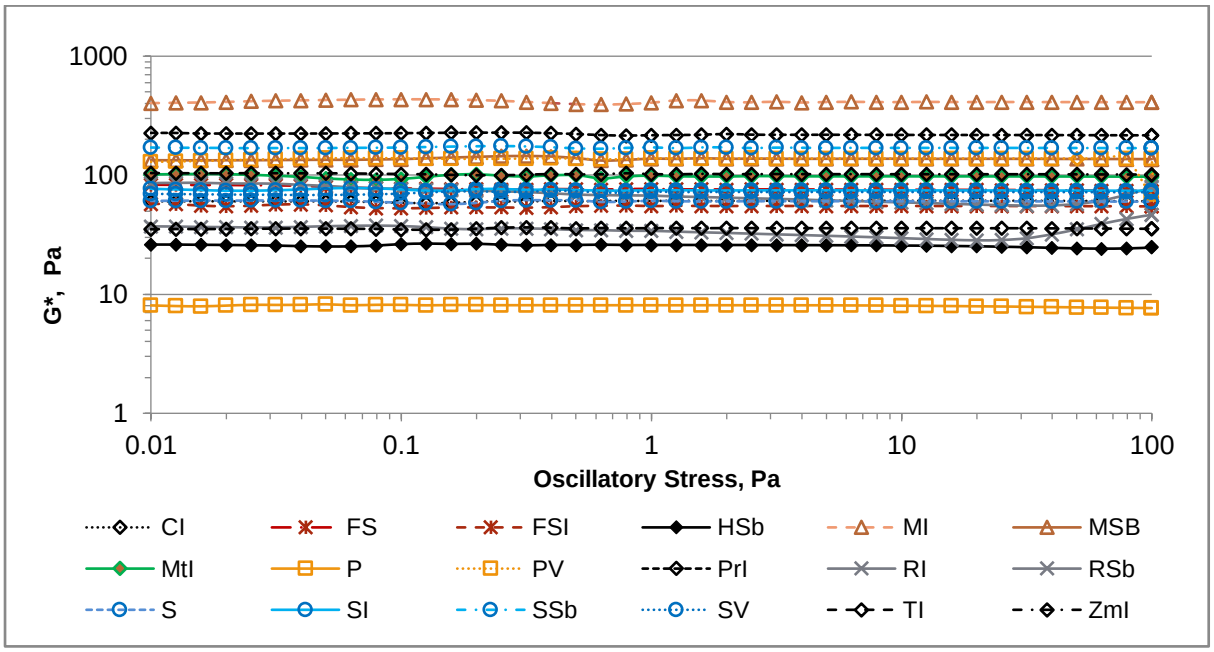
b. *time sweep*;

c. *stepped flow*.

In order to determine some rheological parameters as well as to quantify the phenomenon of thixotropy, the data obtained were analyzed using two mathematical models: *Newtonian* și *Herschel-Bulkley*.

9.2. Results and discussion

The rheological behavior of the honey samples during the dynamic oscillatory tests to the applied strain (*stress sweep*), was exposed in Figure 9.1. as an evolution of the G* complex module.

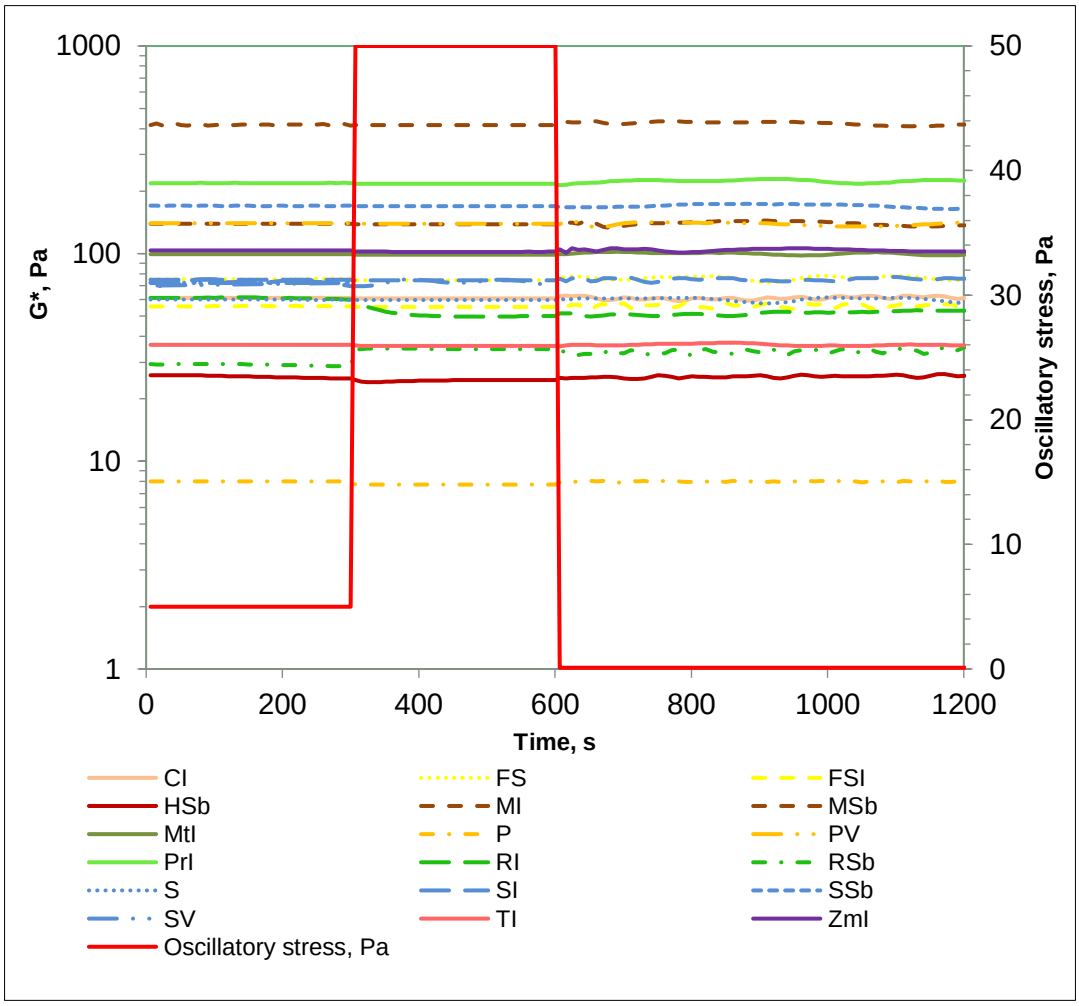


* encodings are indicated in table 6.1.

Figure 9.1. Rheological behavior of honey samples analyzed during dynamic oscillation stress sweep test

G* values remained fairly constant for the entire tested stress domain, with values ranging from 7.66 to 434.65 Pa, which demonstrates their Newtonian behavior. Rape Sb and polyfloral Valcea honey samples, both crystallized, showed an increase of the G* module.

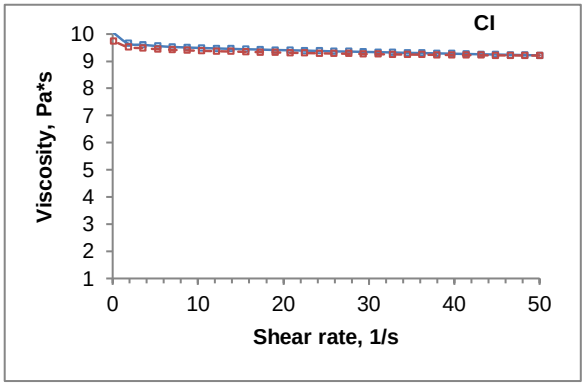
During dynamic oscillatory shear test under constant strain (*time sweep)*, when a strain of 50 Pa was applied, the honey samples showed a Newtonian behavior, while after the discharge, the G* values showed a slight increase in the previously accumulated forces of the sample, however these differences were statistically insignificant ($p>0.05$), confirming the Newtonian behavior of the analized honey samples.

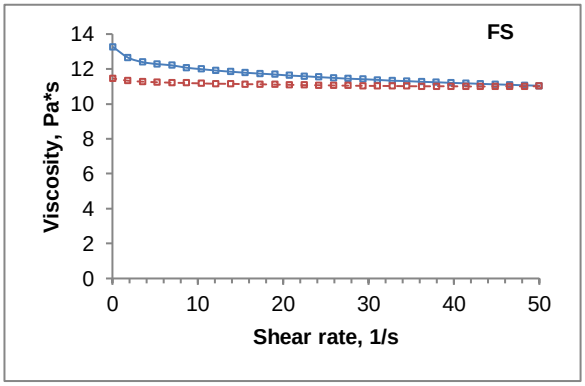


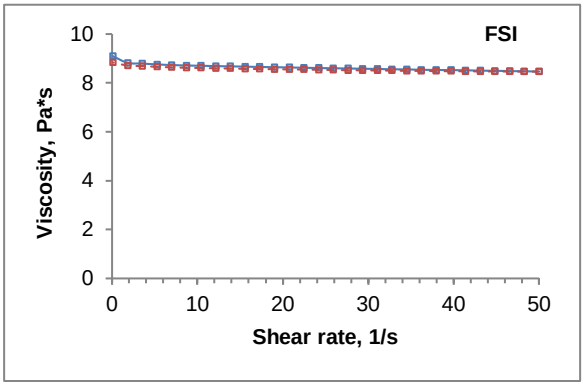
* encodings are indicated in table 6.1.

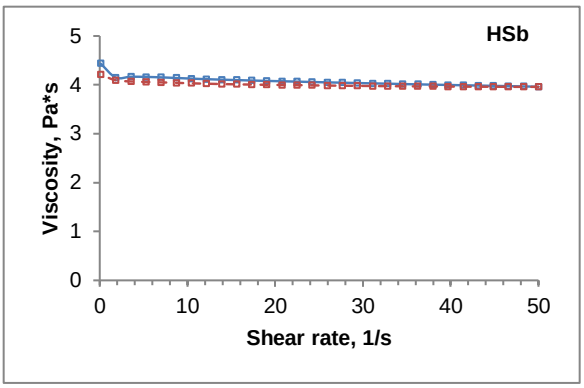
Figure 9.2. The rheological behavior of the honey samples during the dynamic oscillating shear test under constant strain

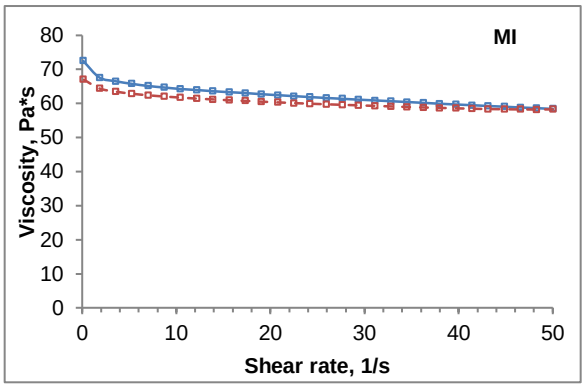
The rheological behavior of the samples during the forced flow was represented in figure 9.3 as the variation of the dynamic viscosity on the shear rate.

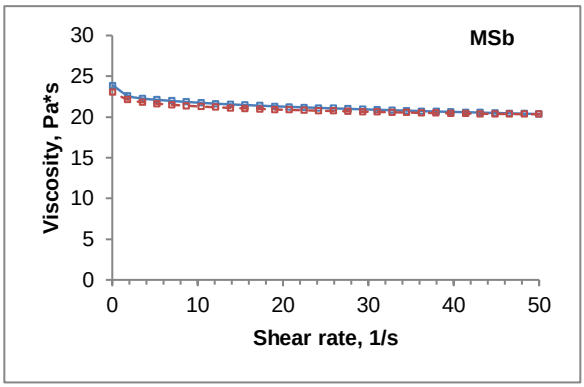


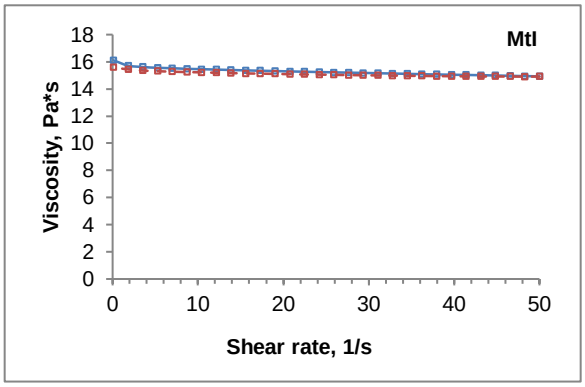


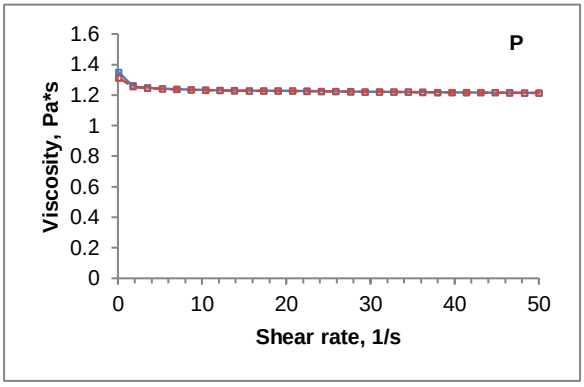

FSI
Viscosity, Pa*s
Shear rate, 1/s

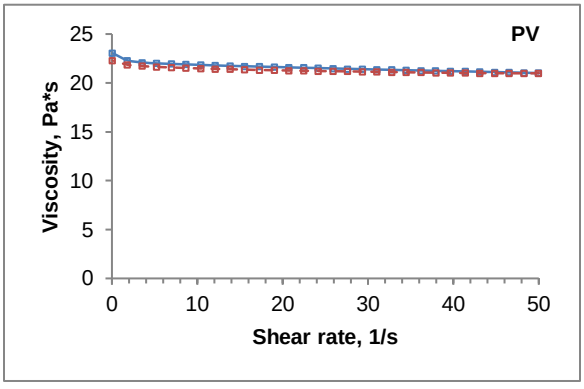

HSb
Viscosity, Pa*s
Shear rate, 1/s

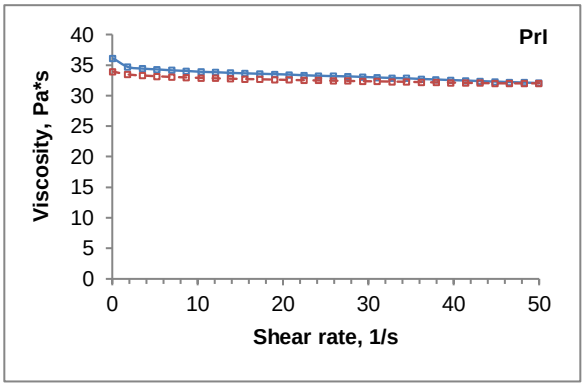

MI
Viscosity, Pa*s
Shear rate, 1/s


MSb
Viscosity, Pa*s
Shear rate, 1/s


MtI
Viscosity, Pa*s
Shear rate, 1/s


P
Viscosity, Pa*s
Shear rate, 1/s


PV
Viscosity, Pa*s
Shear rate, 1/s


PrI
Viscosity, Pa*s
Shear rate, 1/s

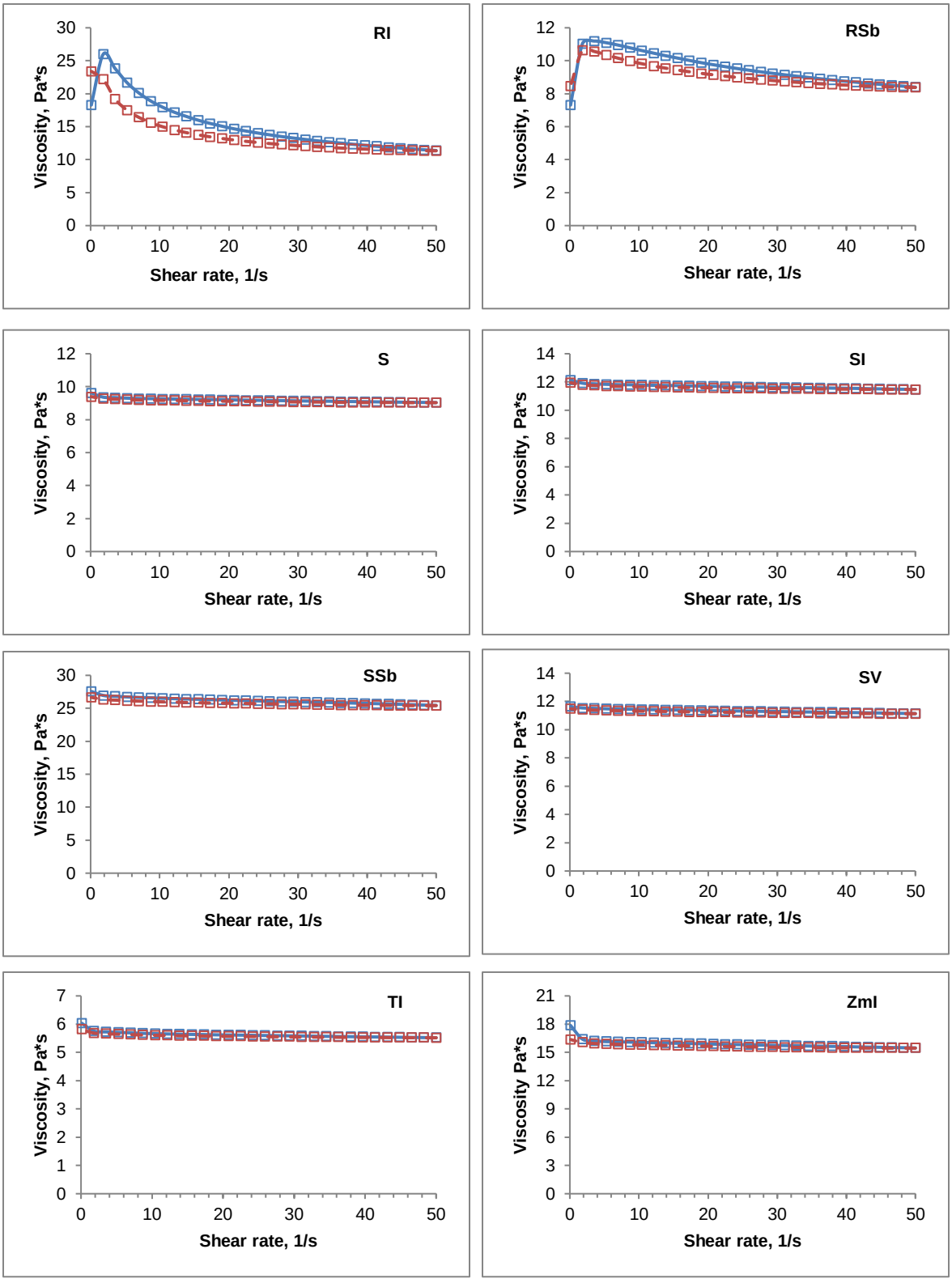


Figure 9.3. The rheological behavior of honey samples analyzed in forced flow conditions

Regarding the flow curve for rape honey, the thixotropy phenomenon was observed with a hysteresis area value of 443.5 Pa/s (rape Sibiu) and 1276.25 Pa/s (rape Iași) (Table 9.1).

Obtained data showed different viscosities for tested honey samples. Honeydew Iași (78.33 Pa*s) and spring honey (39.45 Pa*s) had the highest viscosity, at the opposite pole being chestnut, buckwheat, linden, sunflower, rape and acacia honey.

After the application of the mathematical model Herschel Bulkley, was identified the presence of the lowest yield stress at the rape Iaşi (Table 9.1). By comparing the results obtained from the two applied mathematical models: Newtonian (Table 9.2) and Herschel-Bulkley (Table 9.1), the Herschel-Bulkley model proved to be the most suitable for the flow curves in this study.

Table 9.1. Herschel-Bulkley mathematical model applied to the analyzed honey samples

Type of honey	Mathematical model								
	Herschel-Bulkley								
	Up curve				Down curve				Thixotropy, Pa/s
	Yield stress, Pa	Viscosity, Pa*s	Rate index	Error	Yield stress, Pa	Viscosity, Pa*s	Rate index	Error	
CI	ND	10.15±0.08^{c,d,e,f}	0.98±0.00^{a}	0.63±0.01	ND	9.66±0.07^{l}	0.99±0.00^{a}	0.28±0.02	75.38±12.47^{b}
FS	ND	13.97±2.47^{c,d,e,f}	0.94±0.04^{a,b}	1.13±0.48	0.15±0.02^{b}	11.38±0.01^{k}	0.99±0.00^{a}	0.77±0.33	406.15±311.62^{b}
FSI	ND	9.20±0.08^{d,e,f}	0.98±0.00^{a}	0.77±0.16	0.11±0.00^{b}	8.84±0.02^{m}	0.99±0.00^{a}	0.34±0.05	64.36±1.22^{b}
HSb	0.02±0.00^{b}	4.43±0.23^{e,f}	0.97±0.01^{a,b}	1.14±0.34	0.06±0.00^{b}	4.13±0.01^{o}	0.99±0.00^{a}	0.25±0.07	57.99±34.80^{b}
MI	ND	78.33±5.08^{a}	0.93±0.01^{a,b}	2.57±0.45	ND	68.88±0.00^{a}	0.96±0.00^{c}	0.91±0.01	1756.50±202.94^{a}
MSb	ND	24.93±0.30^{b,c,d}	0.95±0.00^{a,b}	0.97±0.01	ND	22.81±0.25^{d}	0.97±0.00^{b}	0.42±0.05	294.55±34.58^{b}
MtI	ND	16.58±0,44^{c,d,e,f}	0.97±0.00^{a}	0.93±0.26	0.22±0.02^{b}	15.67±0.11^{h}	0.99±0.00^{a}	0.36±0.01	171.65±10.96^{b}
P	0.03±0.00^{b}	1.26±0.00^{f}	0.99±0.00^{a}	0.38±0.04	0.03±0.00^{b}	1.26±0.01^{p}	0.99±0.00^{a}	0.36±0.08	ND
PV	0.39±0.07^{a}	23.33±0.01^{b,c,d,e}	0.97±0.00^{a}	1.21±0.10	0.35±0.00^{b}	22.23±0.08^{e}	0.99±0.00^{a}	0.43±0.04	263.10±3.39^{b}
PrI	ND	39.45±0.76^{b}	0.95±0.00^{a,b}	1.91±0.13	0.53±0.03^{b}	34.42±0.10^{b}	0.98±0.00^{a}	0.68±0.09	687.75±58.05$^{a.b}$
TI	ND	5.95±0.07^{d,e,f}	0.98±0.00^{a}	0.50±0.04	0.08±0.00^{b}	5.72±0.03^{n}	0.99±0.00^{a}	0.21±0.01	33.91±5.03
RI	ND	39.37±18.94^{b}	0.70±0.12^{c}	1.64±0.84	6.59±1.32^{a}	20.26±0.06^{f}	0.85±0.01^{e}	4.96±0.96	1276.25±1169.20$^{a.b}$
RSb	ND	15.54±3.03^{c,d,e,f}	0.85±0.04^{b}	5.43±2.04	ND	12.59±0.10^{i}	0.90±0.01^{d}	1.60±0.50	443.95±348.25^{b}
S	ND	9.85±0.00^{c,d,e,f}	0.98±0.00^{a}	0.74±0.21	0.11±0.01^{b}	9.51±0.18^{l}	0.99±0.00^{a}	0.31±0.03	62.27±7.59^{b}
SI	ND	12.47±0.18^{c,d,e,f}	0.98±0.00^{a}	0.87±0.2	0.14±0.00^{b}	11.95±0.01^{j}	0.99±0.00^{a}	0.29±0.00	93.85±3.08^{b}
SSb	0.39±0.05^{a}	28.54±0.25^{b,c}	0.97±0.00^{a,b}	1.65±0.04	0.37±0.03^{b}	26.89±0.18^{c}	0.99±0.00^{a}	0.41±0.04	398.15±13.08^{b}
SV	ND	12.09±0.13^{c,d,e,f}	0.98±0.00^{a}	0.84±0.24	0.11±0.00^{b}	11.60±0.00$^{j.k}$	0.99±0.00^{a}	0.31±0.02	92.49±5.18^{b}
ZmI	ND	17.56±0.28$^{c.d,e,f}$	0.97±0.00^{a,b}	0.88±0.12	0.25±0.01^{lb}	16.27±0.03^{g}	0.99±0.00^{a}	0.41±0.01	198.35±45.33^{b}

* encodings are indicated in table 6.1.

Different letters in columns denote significant differences, P < 0.05.

Tabel 9.2. Newtonian mathematical model applied to the analyzed honey samples

Type of honey	Mathematical model				
	Newtonian				
	Up curve		Down curve		Thixotropy, Pa/s
	Viscosity, Pa*s	Error	Viscosity, Pa*s	Error	
CI	9.29 ±0.03^{h}	4.22±0.28	9.24±0.02^{j}	2.55±0.05	75.38±12.47^{b}
FS	11.26±0.29^{g}	11.56±7.78	11.03±0.13^{i}	2.08±0.43	406.15±311.62^{b}
FS I	8.53±0.03^{h}	4.01±0.06	8.49±0.03^{k}	2.41±0.01	64.36±1.22^{b}
HSb	4.01±0.03^{j}	5.86±2.59	3.97±0.01^{m}	2.35±0.00	57.99±34.80^{b}
MI	59.99±0.31^{a}	14.42±0.96	58.87±0.20^{a}	8.291±0.08	1756.50±202.94^{a}
MSb	20.70±0.06^{d}	9.25±0.46	20.51±0.06^{e}	6.09±0.20	294.55±34.58^{b}
Mt I	15.07±0.10^{e}	5.06±0.21	14.96±0.08^{g}	2.75±0.01	171.65±10.96^{b}
P	1.22±0.00^{k}	2.27±0.18	1.22±0.00^{n}	2.023±0,04	ND
PV	21.25±0.02^{d}	5.67±0.02	21.08±0.02^{d}	3.21±0.14	263.10±3.39^{b}
PrI	32.62±0.10^{b}	8.76±0.72	32.19±0.07^{b}	4.01±0.03	687.75±58.05$^{a.b}$
TI	5.54±0.03^{j}	3.46±0.32	5.52±0.02^{l}	2.13±0.04	33.91±5.03^{b}
RI	12.54±0.98^{f}	70.16±31.30	11.85±0.40^{h}	39.24±1.05	1276.25±1169.20$^{a.b}$
RSb	8.89±0.33^{h}	34.91±11.87	8.62±0.15^{k}	21.72±0.27	443.95±348.25^{b}
S	9.22±0.17^{h}	3.46±0.03	9.17±0.17^{j}	2.19±0.03	62.27±7.59$^{a.b}$
SI	11.57±0.01$^{f.g}$	3.94±0.03	11.51±0.01$^{h.i}$	2.28±0.03	93.85±3.08^{b}
SSb	25.78±0.11^{c}	6.20±0.30	25.52±0.10^{c}	3.13±0.17	398.15±13.08^{b}
SV	11.23±0.00^{g}	3.83±0.16	11.17±0.00^{i}	2.25±0.01	92.49±5.18^{b}
ZmI	15.64±0.01^{e}	54.52±0.64	15.51±0.01^{f}	2.89±0.04	198.35±45.33^{b}

* encodings are indicated in table 6.1.

Different letters in columns denote significant differences, P < 0.05.

9.3. Partial conclusions

The rheometric tests applied to the honey samples were: *oscilatory stress sweep*, time sweep and stepped flow.

G* values remained fairly constant for the entire tested stress domain, with values ranging from 7.66 to 434.65 Pa, which demonstrates their Newtonian behavior. Rape Sb and polyfloral Valcea honey samples, both crystallized, showed an increase of the G* module.

Regarding the flow curve for rape honey, the thixotropy phenomenon was observed with a hysteresis area value of 443.5 Pa/s (rape Sibiu) and 1276.25 Pa/s (rape Iasi).

The Herschel-Bulkley model has proven to be the best model that define the rheological behavior of honey samples studied. The honey samples showed a Newtonian behavior when applying high shear speeds, higher than 15 - 20 rot/s.

10. Fungicide residues determination in honey

Agricultural contamination with pesticides and antibiotics is a complex issue that needs to be fully addressed. Pe de o parte vorbim de contaminarea solului ce poate conduce la contaminarea culturilor și a pânzei freatice, iar pe de altă parte despre contaminarea produselor animale datorată consumului de hrană contaminată. Honey, a widely consumed product both as food and medicine, its contamination may carry serious health hazards. Honey and other bee products are polluted by pesticides, heavy metals, bacteria and radioactive materials. Pesticide residues can cause genetic mutations and cellular degradation and the presence of antibiotics might increase resistant human or animal's pathogens. (Al-Waili et al., 2012).

The aim of this study was to investigate the presence of azole compounds and fungicide residues in different types of honey from different geographic areas in Romania.

10.1. Materials and methods

A comprehensive list of 25 compounds including 24 azole compounds and fungicides, but also amitraz, which is commonly used as non-systemic acaricide and insecticide in beekeeping, was selected in the current study.

All standards used were purchased from Sigma–Aldrich (Germany). Organic solvents methanol and acetonitrile, HPLC grade, were purchased from Merck Romania; formic acid (98%), Tris(hydroxymethyl)aminomethane, acetic acid, ammonium acetate, ammonia, ultrapure water (LC-MS grade) were purchased from Merck (Romania). All reagents showed high degrees of analytical purity.

In the present work, a Q Exactive high-performance benchtop quadrupole-Orbitrap LC–MS/MS was used to identify the fungicide residues in honey samples. Screening of the samples for the selected contaminants was followed by an MS/MS analysis that allowed targeted ion fragmentation (t- MS^2) and endorsed in achieving high sensitivity and selectivity thus enabling confirmation.

Table 10.1. Working conditions for extraction and instrumental analysis

Sample pre-treatment	**LC-MS analysis**
1.5 g honey is disolved in 15 mL hot ultrapure water Filtrate (150nm filter).	Q-Exactive Orbitrap coupled to an U-HPLC chromatograph
Ajust pH at 8.5 (Tris solution 0.2 M)	Ion source - HESI (Heated Electrospray)
SPE - Strata X, 200 mg, 6 mL^{-1}	Full scan acquisition of m/z 130 – 1000 Resolution in full scan 70 000 FWHM Resolution in MS-MS analysis 17500 FWHM
Precondition- 6 mL MeOH followed by 6 mL H_2O	Column Accucore C_{18}, 150 x 2.1 mm, 2.6 µm)
Wash step- 6 mL H_2O followed by 6 mL MeOH / H_2O 20%	Mobile phase: Eluent A: 100 µL/L of formic acid in H_2O Eluent B: 100 µL/L of formic acid in MeOH
Elution- 6 mL MeOH	Flow: 400 µL/min
Evaporation	
Redissolved in 50 µL MeOH and 200 µL H_2O	

The validation of the quantitative method was based on the 2002/657/EC European Decision and SANTE/11813/2017. For confirmatory analysis of the identity, the criteria described in EU 2002/657/EC were applied, including the detection of at least three product ions. Principal component analysis (PCA) was performed for the matrix containing 18 honey samples and 11 target pesticides residues, while the maximum decrease in the residual variance was considered as criteria in selecting the relevant number of components.

Table 10.2. Characteristics of the target compounds.

Compound	Family	MRL* µg/kg	Molecular formula	Rt (min)	Exact mass	Monitored ions (m/z)
Thiabendazole	Benzimidazole	50	$C_{10}H_7N_3S$	4.02	201.0361	202.0434 $[M+H]^+$
Flubendazole	Benzimidazole	-	$C_{16}H_{12}FN_3O_3$	6.81	313.0863	314.0936 $[M+H]^+$
Carbendazim	Benzimidazole	1000	$C_9H_9N_3O_2$	3.80	191.0695	192.0768 $[M+H]^+$
Griseofulvin	Mitotic inhibitor	-	$C_{17}H_{17}ClO_6$	6.68	352.0714	353.0787 $[M+H]^+$
Enilconazole (imazalil)	Azole antifungal	50	$C_{14}H_{14}Cl_2N_2O$	5.95	296.0483	297.0556 $[M+H]^+$
Epoxiconazole	Triazole antifungal	50	$C_{17}H_{13}ClFN_3O$	8.34	329.0731	330.0804 $[M+H]^+$
Flutriafol	Triazole antifungal	50	$C_{16}H_{13}F_2N_3O$	6.73	301.1027	302.1100 $[M+H]^+$
Myclobutanil	Triazole antifungal	50	$C_{15}H_{17}ClN_4$	8.01	288.1142	289.1215 $[M+H]^+$
Difenoconazole	Conazole antifungal	50	$C_{19}H_{17}Cl_2N_3O_3$	9.31	405.0647	406.0720 $[M+H]^+$
Hexaconazole	Conazole antifungal	-	$C_{14}H_{17}Cl_2N_3O$	9.05	313.0749	314.0822 $[M+H]^+$
Penconazole	Conazole antifungal	50	$C_{13}H_{15}Cl_2N_3$	8.75	283.0643	284.0716 $[M+H]^+$
Propiconazole	Conazole antifungal	50	$C_{15}H_{17}Cl_2N_3O_2$	8.89	341.0698	342.0771 $[M+H]^+$
Paclobutrazol	Conazole antifungal	-	$C_{15}H_{20}ClN_3O$	7.81	293.1295	294.1368 $[M+H]^+$
Prochloraz	Conazole antifungal	-	$C_{15}H_{16}Cl_3N_3O_2$	8.83	375.0308	376.0381 $[M+H]^+$
Tebuconazole	Conazole antifungal	50	$C_{16}H_{22}ClN_3O$	8.81	307.1451	308.1524 $[M+H]^+$
Bromuconazole	Conazole antifungal	-	$C_{13}H_{12}BrCl_2N_3O$	8.66	374.9541	375.9614 $[M+H]^+$
Cyproconazole	Conazole antifungal	50	$C_{15}H_{18}ClN_3O$	8.14	291.1138	292.1211 $[M+H]^+$
Fluquinconazole	Conazole antifungal	20	$C_{16}H_8Cl_2FN_5O$	8.15	375.0090	376.0163 $[M+H]^+$
Flusilazole	Conazole antifungal	50	$C_{16}H_{15}F_2N_3Si$	8.57	315.1003	316.1076 $[M+H]^+$
Metconazole	Conazole antifungal	50	$C_{17}H_{22}ClN_3O$	9.05	319.1451	320.1524 $[M+H]^+$
Prothioconazole	Conazole antifungal	50	$C_{14}H_{15}Cl_2N_3OS$	9.02	343.0313	344.0386 $[M+H]^+$
Triticonazole	Conazole antifungal	10	$C_{17}H_{20}ClN_3O$	8.25	317.1295	318.1367 $[M+H]^+$
Fenbuconazole	Conazole antifungal	50	$C_{19}H_{17}ClN_4$	8.47	336.1142	337.1215 $[M+H]^+$
Metalaxyl	Phenylamide	50	$C_{15}H_{21}NO_4$	6.81	279.1471	280.1544 $[M+H]^+$
Amitraz	Insecticide	20	$C_{19}H_{23}N_3$	3.77	239.1892	294.1970 $[M+H]^+$

10.2. Results and discussion

Due to the high complexity of honey composition, one of the main objectives of this work was to develop a robust extraction method which reduces interferences of the matrix, enhancing detection sensitivity. ultrasonic assisted extraction with a mixture of dichlormethane:ethyl acetate (1:3) as solvent was compared with SPE method. the SPE method was the method that gave the best recoveries for the selected analytes, so our extraction was based on it.

The proposed method allowed the simultaneous detection and quantification of all target compounds with acceptable recoveries, LOD lower than 5 lg $kg^{-}1$ for all target analytes and LOQ

as low as 10 lg kg-1 for most of the analytes, except flubendazole and griseofulvin. All target compounds were detected in the honey samples in the ESI (+) ionization mode.

Detection limits (LODs) were lower than 5 µg kg-1 for all analytes indicating a good sensitivity of the method. With LOQs lower than 15 µg kg^{-1} and below MRL for all compounds the analytical method could be considered adequate for contaminants quantification in honey.

Table 10.3. Validation parameters for SPE extraction in combination with LC-HRMS analysis

Selected compounds	LOD µg kg^{-1}	LOQ µg kg^{-1}	R^2	Recovery %	RSD (%)	Matrix effect (%)	Average Δppm	SD Δppm
Thiabendazole	1.05	3.2	0.978	99	12.5	-13.20	0.75	1.65
Flubendazole	4.60	13.2	0.977	87	15.7	-11.40	0.80	0.86
Carbendazim	1.70	5.2	0.980	110	6.2	+16.20	0.23	0.63
Griseofulvin	4.20	12.6	0.986	80	5.3	-5.50	0.30	0.75
Enilconazole	0.80	2.4	0.990	94	4.8	-17.10	0.54	0.39
Epoxiconazole	1.40	4.2	0.994	102	6.9	+11.20	0.56	0.53
Flutriafol	2.80	8.3	0.985	85	12.7	-0.16	0.80	0.47
Myclobutanil	1.80	5.2	0.992	92	7.2	-30.10	0.95	0.32
Difenoconazole	2.90	8.8	0.972	65	16.2	-5.40	0.25	0.57
Hexaconazole	0.80	2.1	0.988	97	7.4	+18.70	0.51	0.59
Penconazole	0.80	2.5	0.990	100	6.2	+23.50	0.30	1.74
Propiconazole	0.60	1.5	0.992	99	8.1	+19.70	0.23	0.23
Paclobutrazol	2.90	8.6	0.986	88	9.2	-1.80	0.70	0.64
Prochloraz	3.06	9.2	0.982	95	5.2	-4.80	0.35	0.55
Tebuconazole	0.90	2.7	0.989	105	10.1	+16.40	0.41	0.32
Bromuconazole	1.30	3.9	0.994	92	4.1	-55.00	1.02	0.77
Cyproconazole	0.60	1.7	0.990	89	12.7	-11.80	1.11	0.41
Fluquinconazole	1.00	2.9	0.999	86	11.9	-10.30	0.41	0.52
Flusilazole	0.93	2.7	0.993	101	2.8	+23.80	0.12	0.45
Metconazole	1.80	5.4	0.990	104	9.1	+28.20	0.51	0.37
Prothioconazole	2.20	6.5	0.984	66	15.1	-30.40	0.87	0.85
Triticonazole	1.90	5.8	0.987	88	6.2	+3.10	0.75	1.35
Fenbuconazole	1.60	5.0	0.991	109	4.8	+26.40	0.82	0.50
Metalaxyl	0.60	1.8	0.990	80	5.1	-7.20	0.18	0.23
Amitraz	1.30	4.0	0.987	85	4.8	-9.10	0.60	0.36

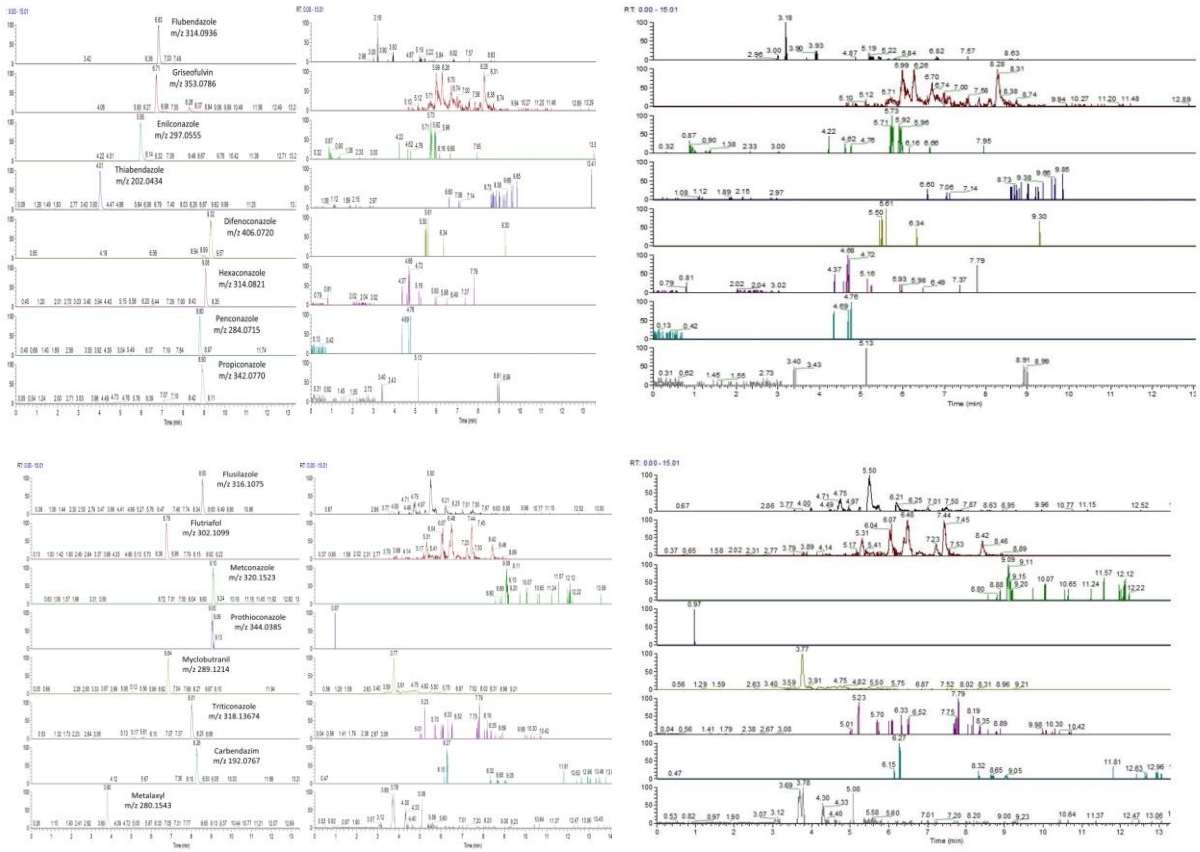


Figure 10.1. Chromatograms of the selected compounds in spiked honey at concentration of 5 µg kg-1 (on the left) compared to the chromatograms of a blank honey sample (on the right). No interferences were observed.

The transitions of at least three product ions were monitored. (Table 10.4.)

Table 10.4. Ions used for LC–MS/MS confirmation with Q-Exactive Orbitrap in targeted ion fragmentation (t-MS^2) mode. Collision energy was set at 45 eV.

Compounds	**Calculated exact mass of the precursor ion**	**Confirmation transition ions m/z**			
	[M+H]+	1	2	3	4
Carbendazim	192.0768	161.05428	160.0507	132.0557	127.9560
Enilconazole	297.0550	255.0099	200.9869	158.9765	109.0764
Metalaxyl	280.1544	220.13321	192.1385	160.1123	148.1121
Tiabendazole	202.0434	176.03528	175.0325	158.07129	131.0603
Cyproconazole	292.1211	237.1008	234.0648	201.0801	177.0468
Hexaconazole	314.0821	245.04579	184.9919	158.9763	125.0154
Penconazole	284.0715	172.99202	160.9732	158.9754	70.04073
Propiconazole	342.0770	204.9816	186.9711	158.9762	69.0706
Tebuconazole	308.1524	249.0782	179.0613	125.0153	139.0308
Metconazole	320.1524	177.04636	163.0308	139.0308	107.0858
Flusilazole	316.1076	187.0587	185.0762	165.0701	171.04359

Overall results in terms of the compound detected, concentration levels and distribution of the contaminant residues in the investigated honey samples are summarized in Table 10.5..

A detectable level of residues was found in 15 samples (83%), 11 compounds (44%), azole compounds and fungicides: carbendazim, imazalil, hexaconazole, penconazole, tebuconazole, flusilazole, thiabendazol, cyproconazole, metconazole, propiconazole și metalaxyl. The most abundant was enilconazole (imazalil) detected in fourteen samples of various honey, followed by metalaxyl in six samples, penconazole and propiconazole in five samples, hexaconazole and tebuconazole in four samples each. Fungicides less frequently detected were flusilazole, carbendazim, thiabendazole and cyprocopnazole.

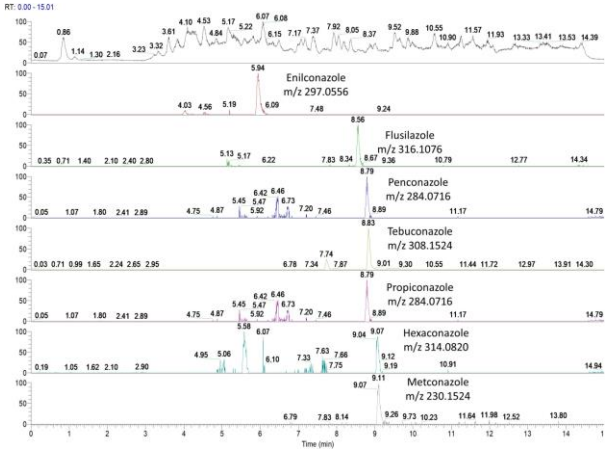


Figure 10.2. HRMS chromatograms of the detected contaminants in rape honey sample collected from Iasi. Chromatograms were extracted from TIC using a 10 ppm window.

The measured quantities ranged from 1.7-7.2 µg kg^{-1}, far below MRLs. In the current study it was possible to quantify smaller amounts of flusilazole residue in honey with a LOQ value of 2.8 µg kg^{-1}.

The results obtained from the principal component analysis are presented in the figure 10.3. From the Bi-plot of the first two principal components (PC1 and PC2) it can be noticed that 29 % of the total variation is explained by PC2 and carbendazim is mostly present in sunflower honey from Moldova counties (Galati and Iasi) (Figure 10.3.).

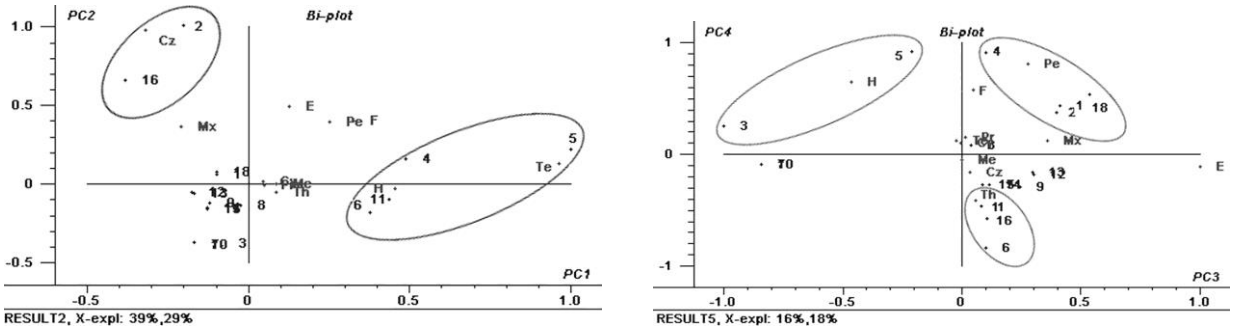


Figure 10.3. Bi-plots of the principal components: PC1, PC2, PC3 and PC4, resulted from the PCA analysis using normalized Quatrimax rotation of the pesticide residues in honey. The sample codes are specified in Table 2, while fungicides are coded as follows: Cz—carbendazim; thiabendazole—Th; E—enilconazole; Mx—metalaxil; Cz—cyproconazole; F—flusilazole; Pe—penconazole; Te—tebuconazole; H—hexaconazole; Me—metconazole.

In the same graph the first component (PC1) is highly influenced by the presence of tebuconazole in rape honey from Iasi and Sibiu and linden honey but also spring honey from Iasi (Figure 10.3.).

Furthermore, tebuconazole and carbendazim were found in the honey samples, with a highest concentration of 7,2 μg kg^{-1} and 5,4 μg kg^{-1}, respectively. Propiconazole and metconazole were detected in concentration lower than LOQ (1,5 μg kg^{-1} and 1,8 μg kg^{-1}, respectively). Co-occurrence of more than two compounds was observed in 39% of the analyzed samples; the frequency of more than four compound mixtures was 22%.

In the Figure 3, the variation explained by PC3 is strongly influenced by the presence of penconazole in sunflower, spring and chestnut honey from Iasi and includes 16% of the total variation. PC4 is strongly influenced by the cluster formed of rape and honeydew from Ias¸i where hexaconazole is present and by the cluster formed of linden, sunflower from Galati and rape from Sibiu where thiabendazole is present.

In three honey samples representing 17% (mint honey Iasi, buckwheat honey Sibiu and polyfloral honey Valcea) none of the compounds has been identified.

Amitraz, an acaricide that can be used by beekeepers, was not found in any analyzed sample most likely because its instability in the natural acidic condition of honey.

Compared to the conventional LC-MS/MS methods, our study uses a simplified sample preparation and reduces the time of the LC separation step with superior sensitivity.

Carbendazim, used to treat sunflower during growth, caused residues presence of about 5 μg kg^{-1} in both honey samples from Iasi and Galati. Residues of fungicides used in spring for fruit tree spraying (propiconazole, penconazole, metalaxyl) were found in samples of honeydew, spring, polyfloral. Rape honey was found to be contaminated with imazalil, propiconazole and tebuconazole. In the sample of rape honey collected from Ias¸i county seven contaminants were present, tebuconazole and imazalil being the most abundant (Figure 4). Six compounds were detected in the spring honey collected from Iasi County among which: imazalil, flusilazole, tebuconazole, cyproconazole and hexaconazole were identifed.

No correlation between the possible sources of the pollution can be done since the specific location of the bee hives was not clearly disclosed by the producers.

10.3. Partial conclusions

The proposed multi-residue method analysis using the Q Exactive Orbitrap MS technology and confirmation by MS/ MS for 25 fungicides has been successfully applied for 18 honey samples produced in Romania. An SPE method was optimized for the compound extraction. The achieved LOQ values were lower than the MRLs for all target compounds demonstrating the method applied is adequate for the detection of traces of pesticides and has potential to be applied in long-term surveillance of the honey matrix. The method was used to analyze various honey samples collected from different geographic regions in Romania in different seasons. The data obtained in this study indicate that a total of 83% of the honey samples collected in the regions under study, showed concentrations below the MRLs of at least one of the monitored compounds. The results obtained indicated that the tested honey samples are safe for human consumption.

11. The germination process and physico-chemical characterization of soryz grains (Sorghum oryzoidum)

Soryz, a hybrid of sorghum, was obtained from studies carried out in 1987-1988 by the researcher Gheorghe Moraru, at the Institute for Scientific Research for Corn and Sorghum in the Republic of Moldova. It is the result of the crossing of wild types of common sorghum (Sorghum bicolor) with Sudan grass (Sorghum sudanense), a forage plant related to sorghum. Since 2010, the cultivation and exploitation of soryz is carried out at the Institute of Plant Protection and Organic Agriculture of the Academy of Sciences of Moldova.

Soryz name comes from the combination of the latin words Sorghum oryzoidum, as soryz grains are similar to rice (Dremlyuk și Vereshinsky, 2000; Moraru, 1995).

11.1. Materials and methods

Soryz was obtained in 2016, at the Institute of Plant Protection and Organic Agriculture of the Academy of Sciences from the Republic of Moldova.

For the germination of soryz, the stages were followed and the conditions are presented in the Table 11.1.

Table 11.1. Steps and conditions applied for obtaining germinated soryz

Treatment	Condition	Time
Disinfection	Ethanol 70%	5 min
Wash	Ultrapure water	25 min
Germination	24 ± 1°C, under dark	48 h
Dry	46 ± 2°C, convection oven	24

Investigations followed:

- Determination of ash content
- Determination of moisture
- Determination of lipid content
- Determination of fiber content
- Determination of total protein content
- Determination of total phenolic content
- Determination of total flavonoid content
- Determination of antioxidant activity
- Determination of gluten
- FTIR analysis
- Confocal microscopy analysis

11.2. Results and discussion

The results of the physico-chemical parameters before and after the germination of the sorghum grains are presented in table 11.2.

Table 11.2. Physico-chemical parameters before and after soryz germination

Samples	Moisture (%)	Ash (%/d.w.)	Protein (%/d.w.)	Fat (%/d.w.)	Fiber (%/d.w.)
Sng	7.73±0.13	1.10±0.04	6.68±0.01	3.05±0.04	1.08±0.00
Sg 24 h	7.50±0.01	0.96±0.03	7.35±0.07	2.98±0.12	1.31±0.00
Sg 48 h	6.64±0.07	0.94±0.01	8.11±0.61	2.94±0.19	2.45±0.76

Sng- soryz grains; Sg 24 h- germinated soryz after 24 h; Sg 48 h- germinated soryz after 48 h

During germination, a decrease in lipid content is observed. The protein content increased from 6.68%/d.w in soryz native, at 8.11%/d.w to germinated soryz after 48 hours.

Based on the results presented in table 11.2. an increase in fiber content could be observed from 1.08%/d.w. at 2.45% d/w.. The ash content decreases with germination.

The results obtained from the determination of the total polyphenol content are shown in figure 11.2.. The highest amount of polyphenols (7.5 mgEqAG/100mg extract) was recorded in germinated soryz after 24 hours. In native soryz the total polyphenol content was 2.5 mgEqAG /100mg extract. Germination led to an increase in polyphenol content.

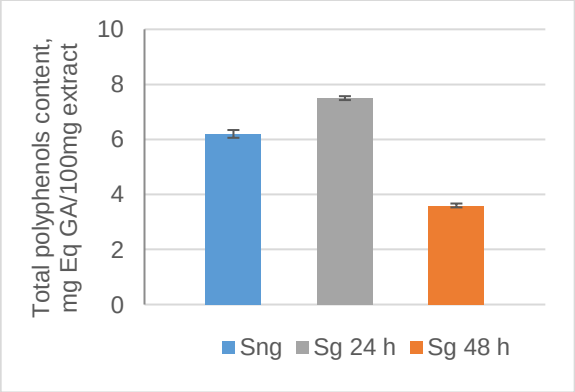


Figure 11.2. Total polyphenol content

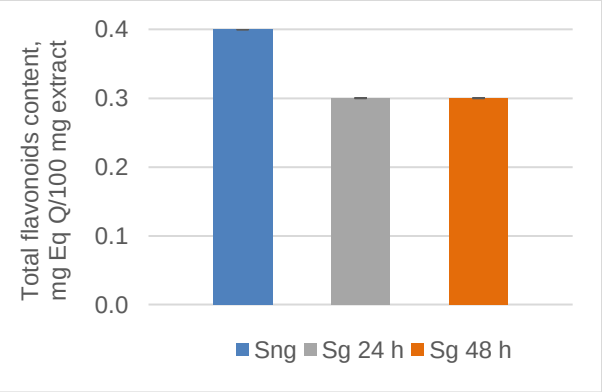


Figure 11.3. Total flavonoid content

The highest amount of flavonoids was in the case of native soryz (0.4mg EqQ/100mg extract), followed by germinated soryz after 48 hours (0.3 mg EqQ/100mg extract). Figure 11.3 shows the highest concentration of flavonoids (quercetin equivalent) in the native soryz samples.

The antioxidant activity of the samples was expressed as IC_{50}, representing the equivalent amount of sample that neutralizes 50% of the free radical. The analyzes were performed at an interval of 20 and 50 minutes from the addition of the DPPH solution. Antioxidant capacity increases with concentration.

From table 11.3. it is observed that the percentages of inhibition of the free radical DPPH for the determination of the antioxidant activity after 20 minutes from the incubation were 62.8%, 61.3% and 63.4% for the native and germinated soryz after 24 h and 48 h respectively (Sng, Sg 24 h, Sg 48 h).

Also, after 50 minutes from incubation, the inhibition percentages increased with values between 1- 3%, which shows that the compounds in the extracts are stable over time and continue their inhibitory action after a longer time.

Table 11.3. Percentages of DPPH inhibition of methanolic extracts of native and germinated soryz after 20 and 50 minutes of incubation

Samples	DPPH			
	Inhibition percentage %		IC_{50} µg/mL	
	20 min	50 min	20 min	50 min
Sng	62.8	65.6	45.5	47.5
Sg 24 h	61.3	62.2	32.5	41
Sg 48 h	63.4	65.9	50	55

By reference to IC_{50} for native and germinated soryz after 20 minutes of incubation, were identified the following values: 45.5, 32.5 and 50 µg/mL (Sng, Sg 24h and Sg 48 h). After 50 minutes of reaction were obtained the following results: 47.5, 41 and 55 µg/mL. A lower IC_{50} value indicates an increased antioxidant activity, which shows that the soryz germinated after 24 h showed the highest antioxidant activity, as well as in the case of polyphenol content.

The results obtained showed the absence of toxic subfractions of prolamins. According to the literature and in the present study, the gluten content was less than 20 ppm. Because soryz is gluten free, can be also included in the diet of people suffering from gluten intolerance.

In the interpretation of the results obtained from FTIR analysis, were taken into account the characteristic frequencies of the functional groups from the organic compounds present in soryz, from the specialized literature. The Mid-IR region between 4000 cm^{-1} – 500 cm^{-1} presents information resulting from molecular vibrations.

FTIR spectra showed a broad absorption band specific to polysaccharides at 1200 - 800 cm^{-1}. A strong absorption band was also observed to peak at 994 cm^{-1} region fingerprint polysaccharide. The bands in the range 1645.54 – 1539.09 cm^{-1} represent the region characteristic of the protein content. The peak at 1744 cm^{-1} (C = O stretch) corresponds to the carbonyl group of flavonoid molecules. The bands in the range 3296.76- 3291.58 corresponds to the OH stretch.

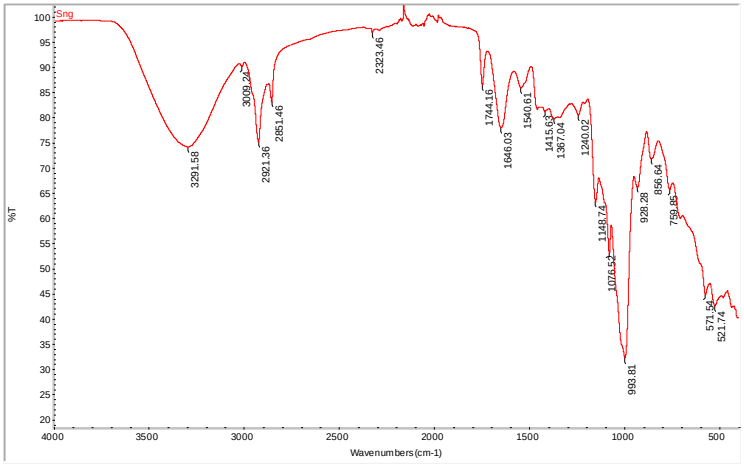


Figure 11.4. ATR - FTIR spectra of the native soryz sample, between 4000 cm^{-1} - 500 cm^{-1}

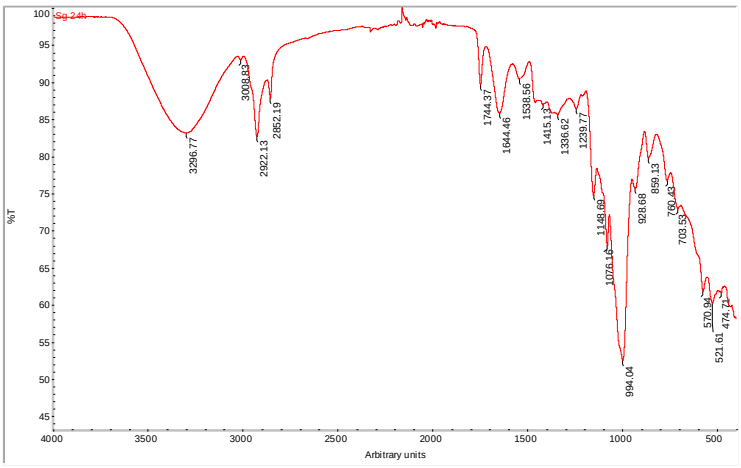


Figure 11.5. ATR - FTIR spectra of the germinated soryz sample, after 24 h, between 4000 cm^{-1} -500 cm^{-1}

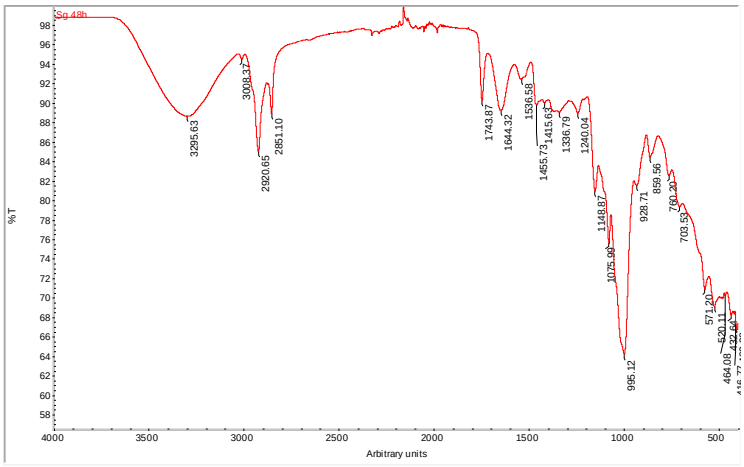
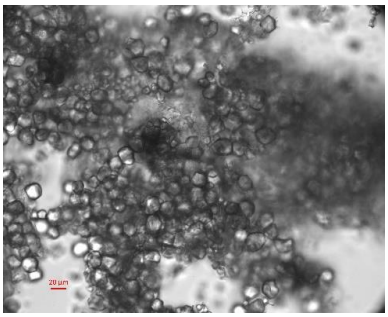


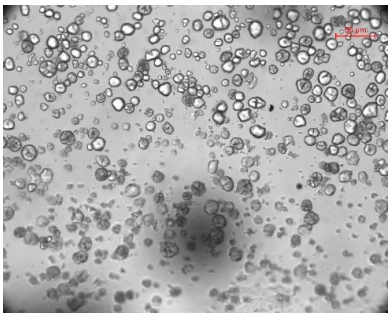
Figure 11.6. Spectrele ATR - FTIR spectra of the germinated soryz sample, after 48 h, between 4000 cm^{-1} - 500 cm^{-1}

In the endosperm of the grain is observed the presence of starch granules of polygonal shape of approximately 10 - 20 µm, with the lobed and central hilum (Figure 11.7.a). The pollen of the species is in a spherical shape, slightly irregular with protuberances and echinulations, with dimensions between 10 - 50 µm (Figure 11.7.b). Parenchymal cells are observed, with thin cellulosic walls, without intercellular spaces and with a rich cellular content.

In the case of germinated soryz samples (Figure 11.8.c) is visualized the top of the root is visualized with meristematic tissue with undifferentiated cells, with approximately equal sides and which through repeated divisions generate somatic cells that will differentiate. As the cells begin to differentiate, they take on a polygonal shape. The cell wall undergoes secondary changes, thickens, small intercellular spaces and vacuoles with different bioactive compounds resulting from cellular metabolism appear. (Figure 11.8.e). The process of differentiation causes morphological and structural changes in the cells and cells get bigger. (Figure 11.8.d). The cytoplasm is rich in cell constituents, namely the vacuole (V) filled with bioactive compounds that correlate with antioxidant capacity.

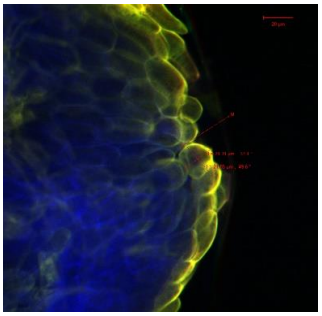


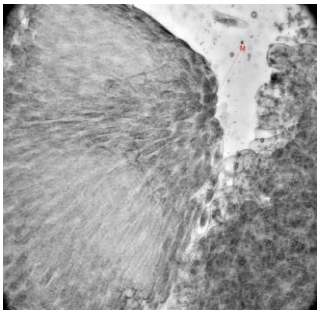
a) endosperm soryz



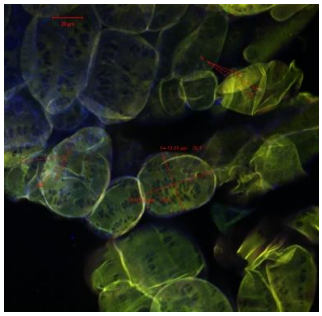
b) pollen soryz

Figure 11.7. Microscopic images with confocal laser scanning of germinated soryz samples

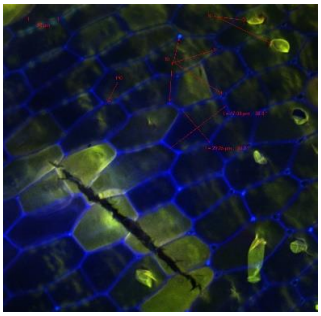




c) root meristem

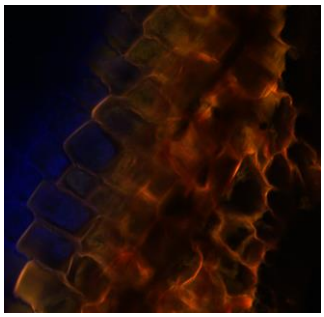


d) differentiated cells

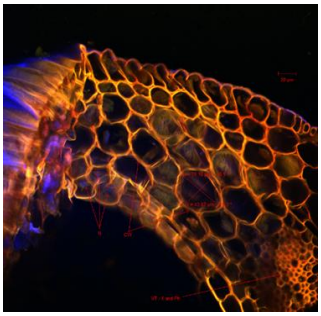


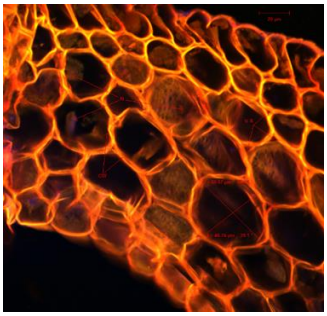
e) parenchyma cell

Figura 11.8. Microscopic images with confocal laser scanning of germinated soryz samples after 24 h



f) cross section through the root





g) cross section through the root

Figura 11.9. Microscopic images with confocal laser scanning of germinated soryz samples after 48 h

Cell differentiation is already observed in the stem, thus distinguishing the epidermal cells covered by the cuticle (Figure 11.9.g) and globular cells with intercellular spaces with highlighted nuclei. The central cylinder with wooden leading vessels and fluent conducting vessels is also observed.

11.3. Partial conclusions

The germination of sorghum determined the increase of the proteins and fibers content, polyphenolic compounds and the antioxidant capacity.

FTIR spectroscopy was a useful tool for identifying specific functional groups in a molecule based on absorption bands at characteristic frequencies.

Due to its gluten content of less than 20 ppm, soryz can be introduced into the diet of people with gluten intolerance.

The germination process has favorable effects on nutritional and biological value, so catabolism products are more digestible.

12. Obtaining a functional product

Celiac disease has an overall prevalence of approximately 1.00% (Rubio-Tapia et al., 2012), and regarding the prevalence of celiac disease in Romania, different studies indicated different percentages from 2.22% (Dobru et al., 2003) to 3.90% (Cev et al., 2010) even up to 9.20% (Gabriel et al., 2011).

Currently, interest in gluten-free products has grown increasingly more. One option in this sense on the world market consists in the manufacture of gluten-free cereal bars. They are among the most consumed foods because the consumer focuses on health, comfort and are convenient in terms of accessibility, storage and handling. (Palazzolo, 2003).

The aim of the research was to develop a functional product: a bar based on germinated soryz flour, buckwheat honey, beebread and fruits.

12.1. Materials and methods

The raw materials used to obtain the bar were: almonds, dates, cranberries, golden berries, chokeberry, goji, beebread. Buckwheat honey was purchased directly from the beekeeper (Hamba, Sibiu). Soryz was purchased from the Republic of Moldova.

Germinated soryz flour was obtained by grinding germinated soryz grains at a laboratory mill (Mlynek Laboratory JNY Tip WZ/2).

Investigations in this chapter are followed:

- Determination of ash content
- Determination of dry matter
- Determination of lipid content
- Determination of protein content
- Determination of fiber content
- Determination of gluten
- Determination of carbohydrate content
- Water activity (aw)
- Determination of antioxidant activity
- FTIR analysis
- Texture analysis
- Colorimetric determination
- Confocal microscopy analysis of beebread
- Sensory analysis

12.3. Results and discussion

12.3.1. Characterization of beebread

The results of the physico-chemical analyzes are presented in the table 12.1.

Table 12.1. Beebread composition

Parameters	Beebread
Moisture %	9.66±0.12
Fat g/100g	5.88±0.22
Proteins %	19.26±0.85
Carbohydrates %	62.36±0.45
Fiber %	2.84±0.40
Mineral substances g/100g	2.40±0.10

Beebread presented a total polyphenol content of 1937.50 mg ferulic acid (FA)/100g and a totatl flavonoids content of 388.50 mg q quercitin (Q)/100g.

Analyzing the results presented in table 12.2 it can be seen that the beebread had a DPPH activity of 89.25 %.

Table 12.2. Antioxidant activity, total polyphenols and flavonoids content of beebread

Beebread	TPC, mg AF/100g	TFC, mg Q/100g	DPPH,%
	1937.50±0.71	388.50±0.71	89.25±0.35

L* colour parameter had a value of 4166, a* 11.74 and b* 28.02. Values were directly propotional to the color of beebread.

More intense bands appear in the IR spectrum of the beebread than in the analyzed honey samples, characteristic of the functional groups which are found in various classes of bioactive compounds such as: proteins, saccharides, polyphenols and flavonoids. Peak 1735.90 cm^{-1}, has an intense band corresponding to the carbonyl ketone groups, which are found in amino acids but also in polyphenols and flavonoids. The intense band from 1644.71cm^{-1} is characteristic of N-H bonds, found in amide and amino groups, also present in amino acids, proteins and peptides. The band 1123.03 - 1050.92 corresponds to the aliphatic and aromatic alkoxy groups present in glycosides but also in carbohydrates. The intense band from 719.25 cm^{-1} belongs to the C-H bonds, present in all classes of organic compounds.

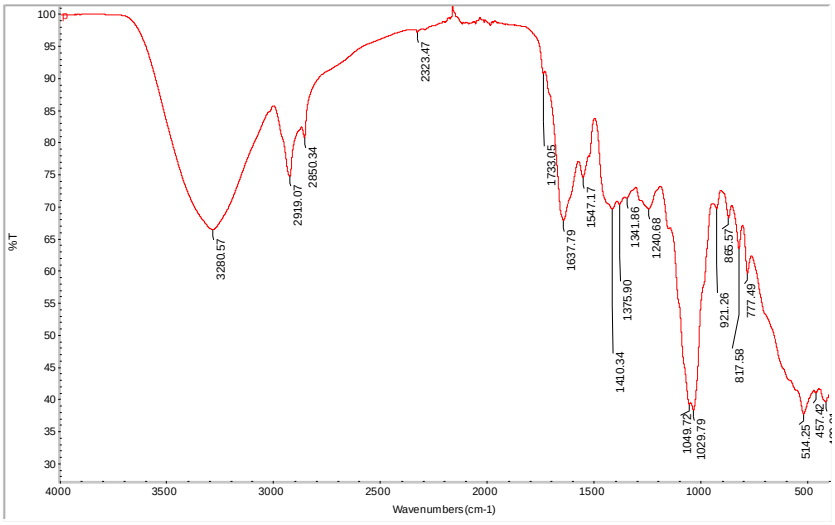


Figure 12.3. FTIR spectra of beebread

Following the analysis, the floral source of the beebread sample was identified, as well as the frequency of occurrence of pollen grains. Thus, in the beebread sample, *Robinia pseudoacacia* pollen (2) was dominant and *Helianthus annuus* (1) was present with a medium frequency.

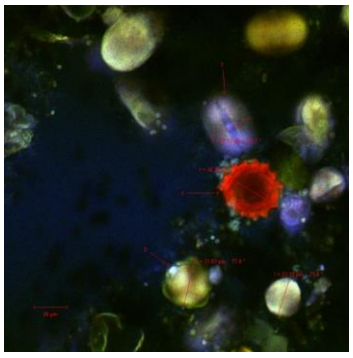


Figure 12.4. Microscopic images with confocal laser scanning of the beebread sample

According to the results presented in Table 12.4. no significant differences were identified between the two products in terms of chemical composition. According to the EC regulation no.1924/2006, the high fiber content of both bars, allows the inscription on the label "Rich in fiber".

Table 12.4. Physico-chemical characterization of bars

Parameters	Bar I	Bar II
Moisture %	10.70±0.22	10.84±0.30
Fat g/100g	2.95±0.34	1.08±0.11
Proteins %	8.34±0.78	8.65±0.29
Fiber %	7.81±1.35	7.77±1.22
Mineral substances g/100g	1.44±0.11	1.26±0.10
A_w	0.47±0.00	0.48±0.01

Regarding the antioxidant capacity can be seen in Figure 12.5. that the bars show a decrease in total polyphenol content during storage. Bar I had the highest content of polyphenols (577.50 mg FA / 100g) compared to bar II (460.20 mg FA / 100g).

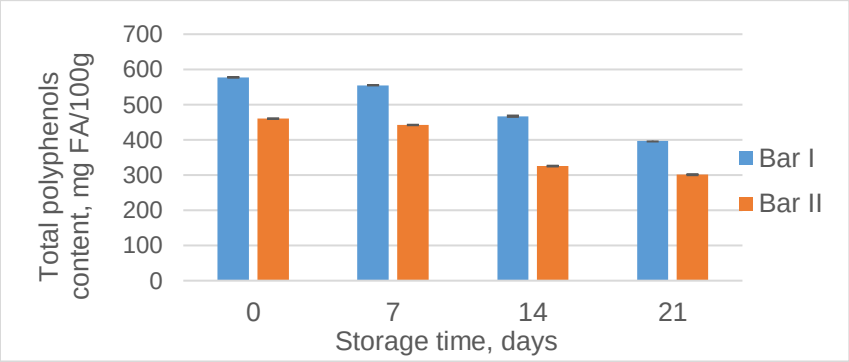


Figure 12.6. Evolution of total polyphenol content during storage at room temperature

The results presented in Figure 1.6. shows that both bars had a stable flavonoid content during storage. As in the case of polyphenols, bar I had a high flavonoids content.

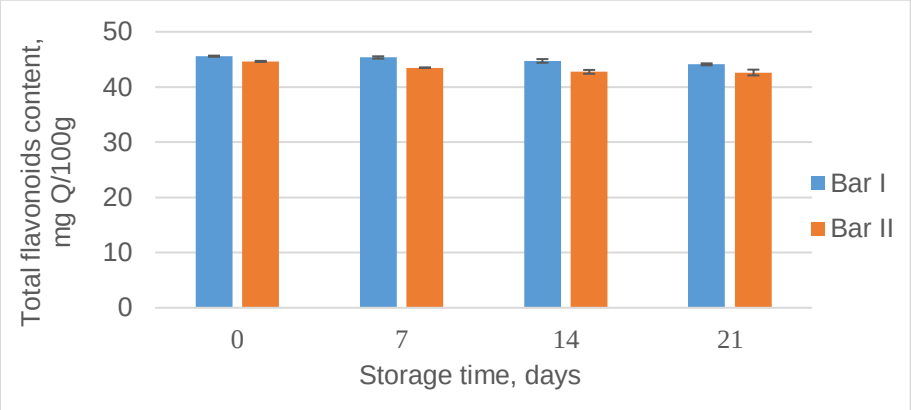


Figure 12.7. Evolution of total flavonoid content during storage at room temperature

From Figure 12.8. it is observed that both bars had high values of antioxidant activity. The antioxidant capacity of both bars decreases over time.

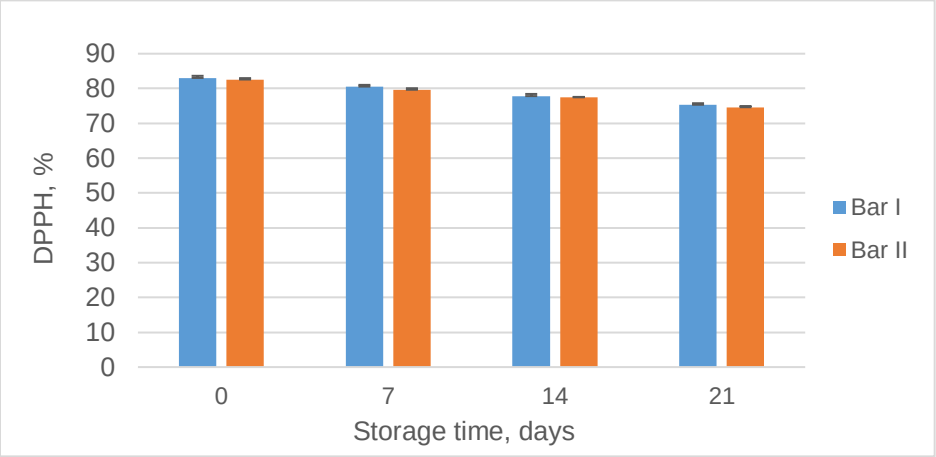


Figure 12.8. Evolution of antioxidant activity during storage at room temperature

Based on the analysis of IR absorption frequencies in the FTIR spectra of the samples we observe the same functional groups. Frequencies characteristic of sp3 C-H bonds, acyl (C-O) and phenolic (C-O) groups are present. These groups are registered in classes of compounds, polyphenols and flavonoids. Also, in both spectra we have intense bands characteristic of alkoxy (C-O-C) bonds present around 1200 cm^{-1}. Bindings belonging to the classes of compounds: polyphenols, flavonoids, glycosides. The frequency of carbonyl bonds is present around 1640 cm^{-1}. The region between 1500 and 1600 cm^{-1} is specific to the aromatic C = C groups, respectively N-H, groups that are found in peptides, amino acids and proteins.

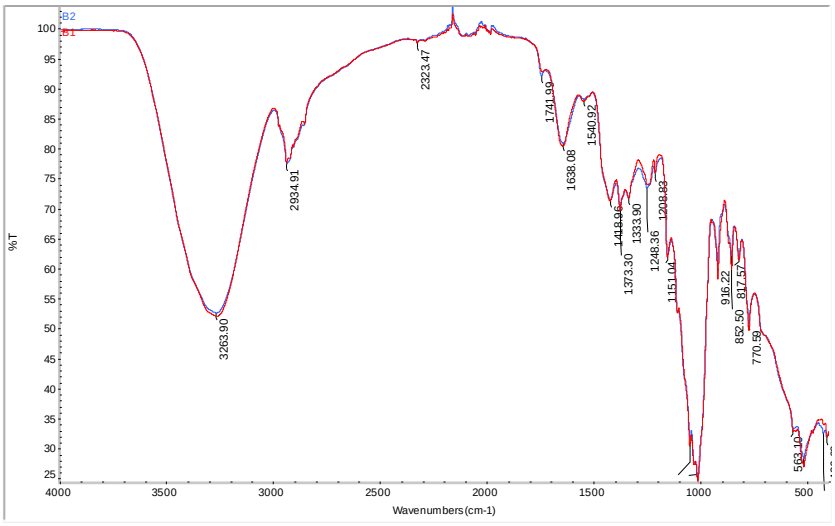


Figure 12.9. FTIR spectra of bars I and II

By comparing the IR data of gluten-free products (bar I-red; bar II-green) with the beebread (blue) it is observed in Figure 12.10., that in the bars are found most of the functional groups that we meet in the beebread. But due to the complex mixture of products, these functional groups are less obvious.

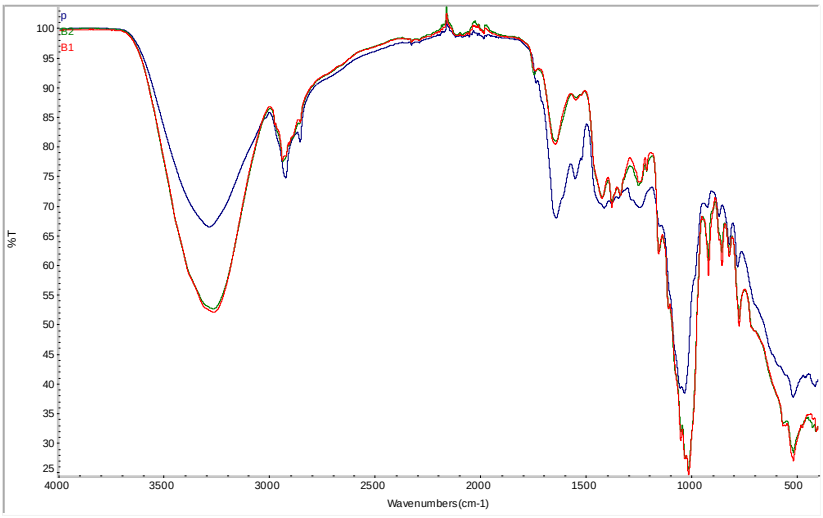


Figure 12.10. Overlapping FTIR spectra of the bars and beebread analyzed

The results of the ELISA test showed values below the limit of 20 mg/kg gluten, which indicates the absence of potentially allergenic proteins in bars. Since the ELISA method applied in this study has revealed the presence of gluten in products, the bars may be included in gluten-free products.

The results of the instrumental analysis of the texture are presented in Table 12.5. Instrumental analysis of the texture of the two samples revealed the same value for firmness, 3.62 N. Equal values are also noted for cohesiveness. Another parameter for which the values did not differ significantly is the gum: 0.34 N for bar I and 0.36 N for bar II. In the case of adhesion, a higher value can be observed in the case of bar II, 0.52 mJ, compared to 0.33 mJ in the case of bar I, which indicates that the addition of golden berries had the effect of decreasing the energy required to detach the sample from the penetration device. Regarding the elasticity of the samples, it can be seen that bar I had a higher deformation recovery capacity, 1.66 mm, compared to bar II, which recovered only 1.5 mm. The chewability was 0.52 mJ for bar I and 0.47 mJ for bar II, which shows that bar I requires more chewing time.

Table 12.5. The values of the textural parameters determined by the instrumental analysis

Nr. crt.	Textural parameter	UM	Bars I	Bars II
1	Firmness	N	3.62±0.68	3.62±0.28
2	Adhesion	mJ	0.33±0.08	0.52±0.14
3	Cohesion	-	0.10±0.01	0.10±0.02
4	Elasticity	mm	1.66±0.18	1.50±0.44
5	Gumminess	N	0.34±0.10	0.36±0.09
6	Chewability	mJ	0.52±0.02	0.47±0.08

The colorimetric parameters L*, a* and b* are presented in Tables 12.6. and 12.7. The values of the colorimetric indices did not vary much during the 21 days. The colorimetric indices a* and b* indicate that the bars have a shade of red-orange. Thus we can say that the bars keep their color throughout the shelf life.

Table 12.6. Colorimetric indicators of bar I

	Indicators	Initial	After 7 days	After 14 days	After 21 days
Bar I	L^*	35.94±0.57	35.03±0.04	34.05±0.03	33.89±0.02
	a^*	10.50±0.06	10.10±0.01	10.08±0.04	9.72±0.01
	b^*	12.68±0.06	11.87±0.03	11.50±0.01	11.11±0.02

Table 12.7. Colorimetric indicators of bar II

	Indicators	Initial	After 7 days	After 14 days	After 21 days
Bar II	L^*	33.81±0.02	33.70±0.06	33.68±0.09	32.95±0.03
	a^*	10.33±0.05	10.24±0.05	10.01±0.02	9.17±0.02
	b^*	10.92±0.13	10.60±0.11	10.46±0.16	10.07±0.04

The sensory attributes of food are important in the selection and purchase decision.

The results obtained from the sensory analysis on the nutrient bars are presented in Figure 12.10. According to the data obtained, it is observed that the appearance in the section, as a result of the breaking of the bars in two, had a high score of homogeneity, compactness, uniformity and firmness. Sensory attributes that easily differentiated the bars. In terms of crumbly (2.45; 2.55) and crunchy (2.45; 2.09) characteristics, they obtained approximately the same score on both bars. The texture had a score of 4.0 on both bars. The results showed that bar I was sweeter than bar II. The taste of beebread was felt in both bars, and the sour taste was easily noticed at the first bar. Following the sensory analysis, no significant differences were observed between the two bars.

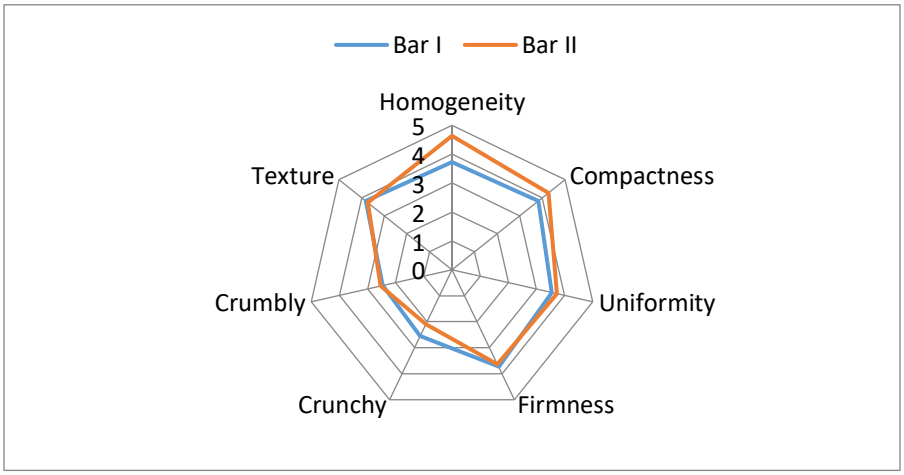


Figure 12.11. Sensory profile of bars

12.4. Partial conclusions

Following the research on the preparation and physical-chemical, biochemical and sensory characterization of functional products such as nutrient bar, the following conclusions can be drawn:

- recipes and technological scheme for making nutritional bars were established, based on germinated soryz flour, buckwheat honey, dehydrated fruits, beebread and almonds;
- the bars were analyzed from a physico-chemical, biochemical and sensory point of view;
- the functionality of the bars is given by the high content of biologically active compounds and by the high content of fibers;
- the analyzed bars do not have antigenic potential, thus being able to be successfully included in the category of gluten-free products;
- following the sensory analysis, no significant differences were observed between the two bars;
- the product is manufactured without the use of food additives, synthetic dyes, flavorings or genetically modified ingredients, being in harmony with nature;
- in conclusion, bars can be functional foods, a healthy alternative, with balanced nutritional and sensory characteristics.

13. General conclusions

The study of the doctoral thesis aimed at the advanced characterization of several honey samples, native and germinated soryz grains in order to obtain and characterize functional products for people with food intolerances. Based on the results of the experiments obtained and the partial conclusions presented at the end of each chapter of the experimental part, a series of general conclusions are highlighted, as follows:

- ✓ In the first stage of the research, several honey samples with different botanical and geographical origins were collected. Through physico-chemical, biochemical and rheological analyzes it was possible to determine the type of honey suitable for making bars.
- ✓ The presence of azole compounds and fungicide residues from 18 types of honey with different botanical and geographical origins was investigated. The selection of the compounds was made considering their appearance and potential use in Romanian agriculture. High-performance liquid chromatography coupled with Q-Exactive Orbitrap MS high-resolution mass spectrometer was used to identify residues. Since the SPE method shown best recoveries for the analytes selected, therefore was used in this study samples of honey. The results obtained showed that the honey samples are toxicologically safe for human consumption, as the concentrations of fungicide residues are low.
- ✓ The sorys grains were subjected to the germination process being monitored the physico-chemical and biochemical characteristics of the native and germinated grains.

- ✓ The results of the fundamental research obtained in the study were capitalized in order to make bars based on germinated soryz flour, buckwheat honey, dehydrated fruits, almonds and beebread. Thus, the recipe and the technology for obtaining gluten-free bars were established. The bars were analyzed from a physico-chemical, biochemical and sensory point of view. They can be considered functional products for people with gluten intolerance or those who want a healthy diet due to the absence of gluten, high fiber content and biologically active compounds.

14. Personal contributions and perspectives for further research

The researches of the doctoral thesis have a fundamental component and an applicative component and have been carried out in accordance with the scientific objectives of the doctoral thesis. The original contributions of the doctoral thesis can be summarized as follows:

- ✓ 18 samples of honey with different botanical and geographical origins were studied in detail.
- ✓ High performance liquid chromatography coupled with high-resolution Q-Exactive Orbitrap MS mass spectrometer was used to identify the presence of azole compounds and fungicide residues in the 18 honey samples.
- ✓ The obtained results were capitalized in order to develop functional products based on germinated soryz flour, buckwheat honey, dehydrated fruits, beebread and almonds. The bars made are rich in biologically active compounds and lack antigenic potential and can be successfully included in the category of gluten-free foods.
- ✓ The data obtained constitute reference elements for future research.

In the future, the application of the screening and quantification method developed and validated for the detection of residues of pharmaceuticals and fungicides is aimed for other matrices.

15. Dissemination of research results

The dissemination of the results of the research carried out during the entire duration of the doctoral studies were materialized in the following scientific papers published or communicated at national and international conferences as follows:

Articles published in journals indexed ISI

1. **Giorgiana-Valentina Blaga (Costea**), Carmen Lidia Chițescu, Elena Lăcrămioara Lisă, Caterina Dumitru, Camelia Vizireanu, Daniela Borda. 2020. Antifungal residues analysis in various Romanian honey samples analysis by high resolution mass spectrometry. Journal of Environmental Science and Health, Part B. https://doi.org/10.1080/03601234.2020.1724016 (**1,463**).
2. Roxana Elena Chiriac, Carmen Chitescu*, Daniela Borda, Mariana Lupoaie, Cerasela Elena Gird, Elisabeta-Irina Geană, **Giorgiana-Valentina Blaga**, Rica Boscencu. 2020. Comparison of the polyphenolic profile of Medicago sativa L. And Trifolium pratense L. sprouts in different germination stages using the UHPLC-Q Exactive hybrid quadrupole Orbitrap high-resolution mass spectrometry. Food and Drug Analysis, Molecules, 25, 2321 https://doi.org/10.3390/molecules25102321 (**4.411**).

3. Iuliana Aprodu, **Giorgiana Blaga**, Iuliana Banu. 2019. Comparative analyses of technological performance of multigrain milling with two experimental roller mills. Quality Assurance and Safety of Crops & Foods: 11 (2)- Pages: 117 – 125. https://doi.org/10.3920/QAS2018.1351

Articles published in journals indexed in Web of Science Core Collection

1. **Giorgiana Blaga**, Iuliana Aprodu, Iuliana Banu. 2018. Comparative analysis of multigrain and composite flours based on wheat, rye and hulled oat. Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1.

Articles published in journals indexed BDI

1. Marian Neculau, Vasilica Barbu, **Giorgiana-Valentina Costea**, Livia Pătrașcu, Camelia Vizireanu. 2016. Microbiologica analysis and the antioxidant capacity of edible biofilm enclosing *Bacillus subtilis*, Scientific papers, vol. 59/2016, nr.2, Agronomy series.

Publications in the volumes of international scientific events

1. **Giorgiana-Valentina Blaga (Costea)**, Camelia Vizireanu. 2018. Determination of antioxidant capacity in some types of honey from Romania, e-Book Proceedings Of The International Conference On Life Sciences, ISBN 978-88-85813-24-3, pp. 352-359.
2. **Giorgiana-Valentina Blaga (Costea)**, Elena Enachi, Andreea-Veronica Dediu, Rodica Mihaela Dinică, Vasilica Barbu, Camelia Vizireanu. 2020. Characterisation of Romanian honeys according to their physicochemical parameters and FTIR spectroscopy, International Conference ,,European Biotechnology Congress", Praga, Cehia.
3. **Giorgiana V. Blaga (Costea)**, Carmen L. Chițescu, Daniela Borda, Camelia Vizireanu. 2020. Evaluation of pesticide residues in honey from different geographic regions of Romania using U-HPLC-Q-EXACTIVE ORBITRAP HRMS. Nutritional Science&Food Chemistry, 16-17 September 2020.
4. **Giorgiana V. Blaga (Costea),** Roxana Elena Chiriac, Carmen Lidia Chițescu, Daniela Borda, Camelia Vizireanu. 2020. Comparative analysis of polyphenolic compounds in red clover (Trifolium pratense) and alfalfa (Medicago sativa) sprouts by UHPLC–ESI/ HRMS/MS. Nutritional Science&Food Chemistry, 16-17 September 2020.
5. **Giorgiana V. Blaga (Costea)**, Carmen L. Chițescu, Daniela Borda, Camelia Vizireanu. 2019. Evaluation of pesticide residues in honey using U-HPLC-Q-EXACTIVE ORBITRAP HRMS. International Symposium EuroAliment, 5-6 September 2019, Galați, România.
6. **Giorgiana Blaga**, Iuliana Aprodu, Iuliana Banu. 2018. Comparative analysis of multigrain and composite flours based on wheat, rye and hulled oat. International Conference "Agriculture for life, Life for agriculture", USAMV București 2018, 7-9 June 2018, București, România.
7. **Giorgiana V. Blaga (Costea)**, Camelia Vizireanu. 2018. Determination of antioxidant capacity in some types of honey from Romania. International Conference ,,Life Science", USAMV 2018, 24-25 May 2018, Timișoara, România.
8. **Giorgiana-Valentina Blaga (Costea)**, Iuliana Aprodu, Vasilica Barbu, Rodica Mihaela Dinică, Camelia Vizireanu. 2018. Influence of germination on chemical composition of

Sorghum oryzoidum. International Conference European Biotechnology Congress 2018, 26-28 April 2018, Atena, Grecia.

9. **Giorgiana V. Blaga (Costea)**, Andreea- V. Dediu (Botezatu), Rodica Dinică, Camelia Vizireanu. 2017. Physicochemical characteristics of germinated soryz. International Symposium EuroAliment, 7-8 September 2017, Galați, România.
10. **Giorgiana-Valentina Blaga (Costea)**, Marian Neculau, Livia Patrașcu, Camelia Vizireanu. 2017. A comparision between low amplitude oscillatory shear and forced flow in deteremining honey rheological behavior. International Conference European Biotechnology Congress 2017, 24-27 May 2017, Dubrovnik, Croația.
11. **Giorgiana-Valentina Blaga (Costea)**, Carmen Lidia Chițescu, Camelia Vizireanu. 2017. Application of U-HPLC -Q -Exactive Orbitrap HRMS in antifungal residues detection in honey. International Conference Agri- Food 2017, 11-13 May 2017, Sibiu, România.

Papers communicated at national scientific events

1. Mișu Cosmin, Istrati Daniela, Costantin Oana, Buruiană Cristian- Teodor, **Blaga Georgiana**, Furdui Bianca, Vizireanu Camelia. 2020. Influence of germination on pshysicochemical characteristics of soghum grain, Doctoral Schools Conference from UDJG, CSSD-UDJG, 2020.
2. **Giorgiana- Valentina Blaga (Costea),** Carmen Lidia Chițescu, Daniela Borda, Camelia Vizireanu. 2019. Identification of Antifungals Residues in Honey Samples by U-HPLC -Q -Exactive Orbitrap HRMS. Doctoral Schools Conference from UDJ – Galaţi CSSD-UDJG 2019, 13-14 June 2019, Galați, România.
3. **Giorgiana- Valentina Blaga (Costea)**, Iuliana Aprodu, Rodica- Mihaela Dinică, Camelia Vizireanu. 2018. Effect of Germination on the Composition of Soryz. Doctoral Schools Conference from UDJ – Galaţi CSSD-UDJG 2018, 7-8 June 2018, Galați, România.
4. **Giorgiana-Valentina Blaga**, Camelia Vizireanu. 2017. The antioxidant activity and physical properties of different floral origin honeys from Romania. Doctoral Schools Conference from UDJ – Galaţi CSSD-UDJG 2017, 8-9 June 2017, Galați, România.
5. **Giorgiana- Valentina Blaga**, Livia Pătrașcu, Camelia Vizireanu. 2016. Some rheological aspects of honey. Doctoral Schools Conference from UDJ – Galaţi CSSD-UDJG 2014, 2-3 June 2014, Galați, România.

Abstracts published in journals indexed ISI

1. **Giorgiana-Valentina Blaga (Costea)**, Iuliana Aprodu, Vasilica Barbu, Rodica Mihaela Dinică, Camelia Vizireanu. 2018. Influence of germination on chemical composition of Sorghum oryzoidum. International Conference European Biotechnology Congress 2018, 26-28 April 2018, Atena, Grecia.
2. **Giorgiana-Valentina Blaga (Costea)**, Marian Neculau, Livia Patrașcu, Camelia Vizireanu. 2017. A comparision between low amplitude oscillatory shear and forced flow in deteremining honey rheological behavior. International Conference European Biotechnology Congress 2017, 24-27 May 2017, Dubrovnik, Croația.
1. Marian Neculau, Mihaela Alina Ceoromilă, **Valentina Giorgiana Blaga (Costea)**, Gabriel Mustățea, Camelia Vizireanu, Structural and physicochemical properties of emulsified alginate-based film coatings for food products, International Conference ,,European

Biotechnology Congress", Dubrovnik, Croația, Journal of Biotechnology, 256S (2017) S44–S116.

Participation in activities organized by the University „Dunărea de Jos" from Galați

1. Training seminar for teachers "Rédaction médicale scientifique" CIDMEF-Sciences (Conférence, Formation, Réunion SaIN, PhD student, 2018
2. Student Scientific Communications Session 2018, From Farm to plate – Healthy for Sustainable Foods, guiding students
3. Student Scientific Communications Session 2017, From Farm to plate – Healthy for Sustainable Foods, guiding students
4. Student Scientific Communications Session, From Farm to plate – Healthy for Sustainable Foods, guiding students

Participation in national and international competitions

1. Dragomir Bălănică Carmelia Mariana, Munteniţă Cristian, **Blaga (Costea) Giorgiana Valentina**, Simionescu Aurel Gabriel. 2019. Agricultural use of sludge from treatment plants of municipal wastewater, UGAL INVENT Research and Innovation Salon organized by the University „Dunărea de Jos" from Galați.
2. Istrati Daniela-Ionela, Vizireanu Camelia, Furdui Bianca, Constantin Oana Emilia, Buruiană Cristian-Teodor, Mișu Dan Cosmin, **Blaga (Costea) Giorgiana-Valentina**, Dinică Rodica-Mihaela. 2021. Non-alcoholic fermented probiotic beverage from germinated sorghum and manufacturing process, INVENTICA 2021, Iași, România.

Member of national and international project teams

1. Project POSCCE-ID 1815, cod SMIS 48745, MORAS, engineer II, 2015-2020.
2. Organisation de seminaires doctoraux-appel a projets, PhD student, 2016-2017
3. PN-III-P2-2.1-BG-2016-0143 entitled „Solutions for multi-grain grinding"
4. Project nr. 9PCCDI/2018 Complex system of integral capitalization of some agricultural species with energy and food potential (VALINTEGR), 2018-2020

Patent

1. **Blaga (Costea) Giorgiana- Valentina**, Vizireanu Camelia, Istrati Daniela-Ionela, Aprodu Iuliana, Borda Daniela. Functional bar based on germinated soryz, buckwheat honey, dehydrated fruits and beebread and the obtaining process. 2021. A100435.
2. Istrati Daniela-Ionela, Vizireanu Camelia, Furdui Bianca, Constantina Oana Emilia, Buruiană Cristian-Teodor, Mișu Dan Cosmin, **Blaga (Costea) Giorgiana- Valentina**, Dinică Rodica- Mihaela. Probiotic non-alcoholic beverage fermented from germinated sorghum and process for obtaining it. 2020.A00765.