Universitatea "Lucian Blaga" din Sibiu

Interdisciplinary PhD school

PhD domain: Industrial Engineering

PhD Thesis

DEVELOPMENT OF MULTIFUNCTIONAL EXTRACTS FROM HIBISCUS PETAL AND PEONY FOR USE IN FOOD AND TEXTILE TECHNOLOGIES

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Thank you,

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KEYWORDS: extraction, bioactive compounds, "green technology", technological applications.

PHD THESIS SUMMARY

1. INTRODUCTION

More and more scientific studies show that maintaining the health of the population requires a proper management of social resources, economic relations but more importantly, natural capital. Many of today's health problems are rooted in the same socio-economic inequalities and reckless consumption patterns that endanger both current and future human health (McMichael & Beaglehole, 2000).

In this regard, special interest is given to plants that have been used in traditional medicine due to the rich content of bioactive compounds in their composition. A multitude of bioactive compounds in plant composition are widely available for inclusion in human diets as alternative forms of current medicine (Cowan, 1999). In significant proportions, plants are used in the pharmaceutical industry and nutritional supplements as primary sources for the extraction of natural compounds with beneficial effects, especially for preventive purposes, but sometimes also therapeutic.

Among the fundamental needs that modern industry has as a result of overexploitation and globalization is sustainability and the so-called "green chemistry" in order to capitalize on and develop natural extracts, with many applications in various industries, such as: pharmaceutical, food, textile, cosmetics etc., to replace synthetic compounds with natural ones. Therefore, this doctoral thesis aims to use non-polluting, improved extraction technologies as alternatives to classical extraction technologies to obtain natural extracts rich in bioactive compounds, from hibiscus and the common garden peony.

The objectives of the thesis:

The main objective of this doctoral thesis is to capitalize on bioextracts obtained by classical and modern extractive technologies from hibiscus and garden peony in order to replace synthetic additives and dyes.

In order to achieve the main objective of the doctoral thesis, the following scientific objectives were proposed:

1. Identification and testing of conventional (maceration) and unconventional extraction technologies (ultrasound-assisted extraction) of anthocyanins from hibiscus extracts by establishing the optimal extraction parameters and their dosing from the extracts obtained.
2. Identification, establishment and testing of optimal extraction parameters of various polyphenolic-type bioactive compounds from garden peony petals by conventional (maceration) and unconventional extraction technologies (ultrasound-assisted extraction) and their dosing from the extracts obtained.
3. Development of enzyme-assisted extraction technologies based on the use of fungal cellulases in order to increase the efficiency of extraction of bioactive compounds from hibiscus and garden peony petals.
4. Testing the antioxidant potential of hibiscus extract on edible hemp oil.
5. Testing their dyeing capacity of hibiscus extract and better quality of cotton fabrics.

2. STATE-OF-THE-ART ANALYSIS OF THE BIOACTIVE POTENTIAL OF PIGMENT-RICH PLANTS

2.1. Hibiscus and garden peony flowers - sources of bioactive compounds

In the present study, the class of interest of the organic substances synthesized by the plants is that of bioactive compounds with polyphenolic structure, they have a very wide applicability and are spread throughout the plant, often being accumulated in different parts of the plant, such as roots, fruits, petals, bark and leaves (Oancea & Grosu, 2013).

The genus *Hibiscus* is a widespread genus that includes several perennial species, used in the form of tea / infusion due to its rich composition in bioactive compounds, especially those with polyphenolic structure (Sindi et al., 2014)..

Hibiscus flowers have been used in the past in traditional medicine and food industry in various forms, as a flavoring or natural dye for the production of various products (Wang et al., 2001; Oliver, 1960).

The garden peony is a perennial plant that belongs to the genus *Paeonia*, which includes several species, very few studied in terms of bioactivity. Among the research results is the fact that 256 bioactive compounds have been identified in different anatomical parts of the plant (Dienaitė et al., 2019).

For the analysis of the obtained polyphenols, the most important steps are the initial preparation of the plant material and the extraction method. The last one is an important step and depends mainly on the nature of the sample from which the extraction is performed, the chemical properties of the polyphenolic compounds concerned but also the number of aromatic rings and hydroxyl groups in the structure of the compounds, polarity and concentration, therefore it is difficult to choose a single method of preparation and extraction of compounds with polyphenolic structure for many plants. Choosing the method of extraction of polyphenols is an important step in the analysis of compounds with polyphenolic structure. In order to obtain a high extraction yield, the following parameters must be taken into account: extraction time, extraction temperature, type of solvent, solid / solvent ratio and number of extractions performed for each sample (Khoddami et al., 2013).

Lately, research involving solvent extractions of polyphenolic compounds has focused on the use of an ecofriendly solvent that has no negative implications for human health or the environment. Other studies illustrate that an acidic extraction medium favors the extraction of a higher content of polyphenolic compounds from plants (Davidov-Pardo et al., 2011; Sindi et al., 2014).

2.2. Beneficial effects on human health

The main biological function of bioactive compounds with polyphenolic structure is antioxidant (Oancea et al., 2011), therefore epidemiological studies indicate that a rich diet in foods containing large amounts of polyphenols reduces the risk of various types of cancer and cardiovascular disease. This function being a key factor in the treatment of many diseases, they have an anticancer, anti-inflammatory, antimicrobial, antiallergic, antiviral, antithrombotic, hepatoprotective and anti-diabetic role (Mori et al., 1999; Rocha et al., 2014; Ozcan et al., 2014; Działo et al., 2016; Khoddami et al., 2013).

2.3. Natural antioxidants - source of oxidative stabilization of edible oils

Considering that the food industry is in constant search of effective natural dyes with low toxicity, there are studies demonstrating the applications in the food industry of *Hibiscus sabdariffa* extract.

In addition to the consumption of vegetables and fruits recommended by health organizations, human needs an increased intake of polyunsaturated fatty acids that the human body can not produce, especially α-linoleic acid (Omega-3), linoleic acid (Omega-6) and oleic acid (Omega-9), which are mainly found in fish oils. Hemp seeds contain about 25% protein and 35% oil fat. Hemp oil usually contains 50–70% linoleic acid (Omega-6), 15–25% α-linolenic acid (Omega-3), which is approximately the ratio of 3: 1, being the optimal ratio to human nutritional needs (Da Porto et al., 2012).

Fats, oils and fat-based foods are generally very sensitive to oxidation after prolonged storage and exposure to heat, which limits the shelf life, affecting not only their sensory qualities but also their nutritional value. Consequently, they need antioxidant protection, and this can be achieved through various methods including the use of antioxidants as oxidation inhibitors (Oancea et al., 2013).

Although synthetic antioxidants have been preferred in the food industry and have been very successful, there has recently been growing concern about their toxicity. More and more scientists are testing the use of natural antioxidants to preserve various foods to replace synthetic antioxidants (Oancea et al.., 2013; Oancea et al., 2015).

2.4.Natural dyes - aspects regarding the use in ecological dyeing of textile substrates

Dyeing textiles with natural dyes was an expensive process, synthetic pigments being much cheaper, easier to produce and therefore their applications became accessible to all. However, in recent years specific tests have shown the allergenic and toxic effects of synthetic dyes, so that natural pigments are becoming more popular and replace synthetic ones (Abel, 2012).

Natural dyes have no carcinogenic effects and produce soothing and warm colors. They also have a much higher biodegradability and environmental compatibility compared to synthetic ones. Plants are potential sources for the extraction of natural dyes due to their wide availability in nature (Chandravanshi și Upadhyay, 2013).

3. CONTRIBUTIONS REGARDING THE INFLUENCE OF EXTRACTION TECHNOLOGIES ON THE CONTENT OF BIOACTIVE COMPOUNDS IN HIBISCUS AND PEONY

3.1. INTRODUCTION

Polyphenolic bioactive compounds have in their structure more than one aromatic ring, with one or more hydroxyl groups, being classified into flavonoid and non-flavonoid compounds. The applicative potential of these biochemical compounds with polyphenolic structure, as well as their abundance in flowers are some of the reasons for their selection in order to meet the objectives of this doctoral thesis.

In addition to various medical applications, these bioactive compounds with polyphenolic structure are used in various industrial sectors, for example in the food, cosmetics, pharmaceutical and solar panel cell industries (Martínez et al., 2017; Wang et al., 2001).

One of the most important aspects regarding the performance of the extraction of bioactive compounds with polyphenolic structure from plant materials is the identification of the optimal extraction conditions, because these chemical compounds can become unstable and sensitive to degradation. Their stability is affected by several factors such as: pH, storage temperature, extraction temperature, chemical structure, concentration, light, oxygen and solvents used for their extraction (Moţa et al., 2016).

The objectives of this research chapter are to test conventional extraction technologies (maceration) and unconventional (ultrasound-assisted extractions) of compounds with polyphenolic structure from hibiscus and peony, establishing optimal extraction parameters and dosing them from the extracts obtained.

The bioactive compounds with polyphenolic structure targeted in this study are: anthocyanins, flavonoids, polyphenols and tannins.

To achieve these objectives, experimental sets on maceration at different parameters and ultrasound-assisted extraction were performed, varying the environmental, process and instrument parameters.

The dosing of bioactive compounds with polyphenolic structure from the extracts obtained following the application of extraction techniques, both conventional and non-conventional, was performed by UV-VIS spectrophotometric techniques.

3.2. Materials

Hibiscus and peony petals, dried and crushed, were used. And for carrying out the experiments of extraction and dosing of the chemical compounds from the extracts, specific solvents and chemical reagents were used which were of analytical purity and all the solutions used were made with distilled water.

3.3. Equipment

The experimental part of the current study was conducted using the equipment of the laboratories of the Research Center for Food Biotechnology of the Faculty of Agricultural Sciences, Food Industry and Environmental Protection at the University "Lucian Blaga" in Sibiu.

3.4. Experimental methodology

The extraction of anthocyanins from the dried petals of hibiscus and peony was performed by both unconventional and conventional methods, according to Figure 1. For both maceration and ultrasonic extraction, 2 environmentally friendly solvents were chosen for extraction.

To perform a comparative study of the extraction efficiency of hibiscus flowers between classical and unconventional methods, the solvent that proved to be most effective in the experiments where the extraction was performed by maceration was chosen. In addition, due to the increased efficiency of anthocyanin extraction in acidic environment (Nollet, 2000) the efficiency of the acidified solvent was also tested.

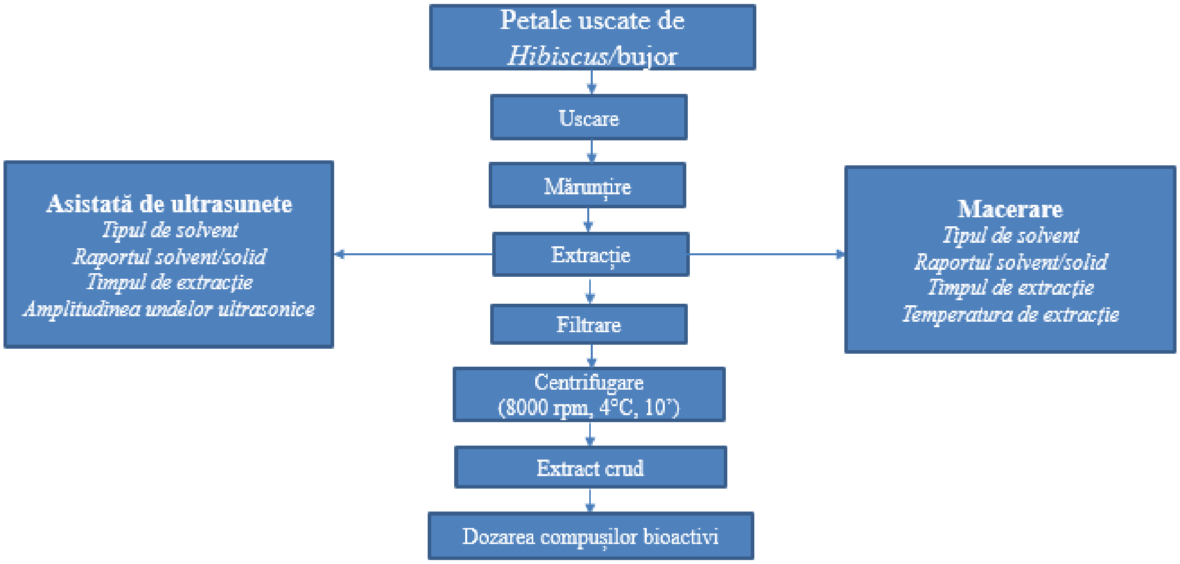

Figure 1. Stages of extraction of anthocyanins from the petals of hibiscus and peony by maceration and ultrasound-assisted.

3.4.1. Spectrophotometric determination of bioactive compounds content with polyphenolic structure

Quantitative determination of compounds with polyphenolic structure from the analyzed samples was performed each by specific spectrophotometric methods.

3.5. Mathematical modeling and statistical analysis of extraction parameters

Following the results of spectrophotometric analyzes, mathematical modeling was performed using multiple regressions by analyzing the influence of experimental factors, considered independent variables on the amounts of extracted compounds.

3.6. Statistical analysis

Statistical testing of the differences between the anthocyanin content obtained for both extraction methods was performed using the Kruskal-Wallis test (analysis of variance) (Kruskal & Wallis, 1952).

The t-student test and the F test (Fisher-Snedecor) were also applied.

3.7. Results and discussions

3.7.1. Influence of maceration and ultrasound-assisted extraction parameters on the hibiscus anthocyanin content

Extraction parameters such as extraction temperature, extraction time, type of solvent used, solvent / solid ratio, ultrasonic wave amplitude and extraction medium (pH) have a significant influence on the content of polyphenolic compounds extracted from hibiscus flower petals.

From the results obtained from the analyzes performed for the extraction of anthocyanins from hibiscus petals by maceration at a temperature of 40°C, the following optimal extraction parameters presented in Figure 2 were identified:

- Extraction solvent: ethanol 70% acidified 1% with acetic acid
- Solvent/solid ratio: 20/1
- Extraction time: 20 minutes

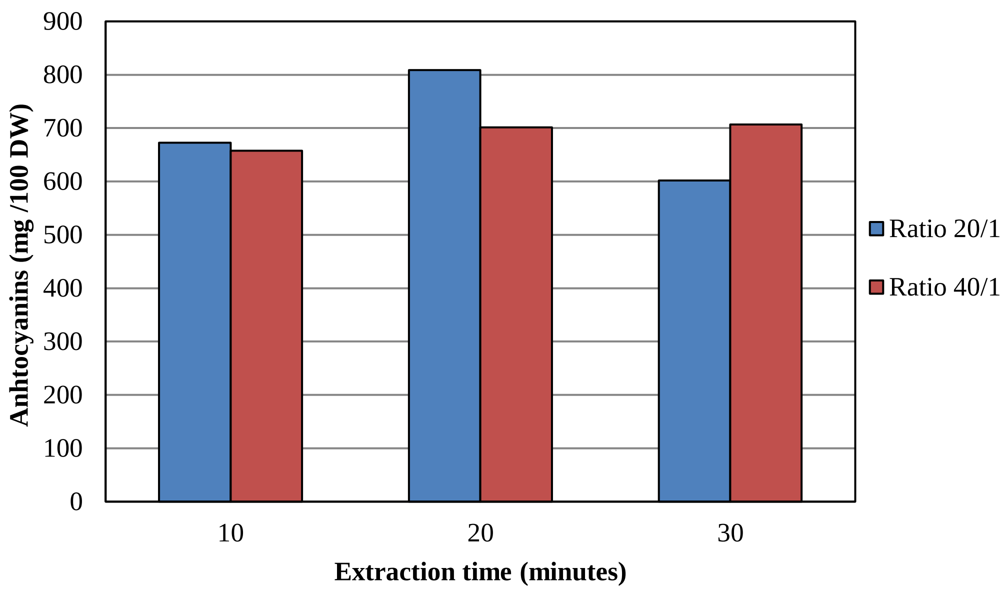

Figure 2. Anthocyanin content of hibiscus ethanolic extracts acidified with 1% acetic acid, depending on the extraction time, at 40°C (maceration extraction).

For the ultrasound-assisted extraction of anthocyanins from hibiscus petals, the following optimal extraction parameters presented in Figure 3 were identified:

- Extraction solvent: ethanol 70% acidified 1% with acetic acid

- • Solvent/solid ratio: 40/1
- • Extraction time: 30 minutes
- • Amplitude of ultrasonic waves: 70%

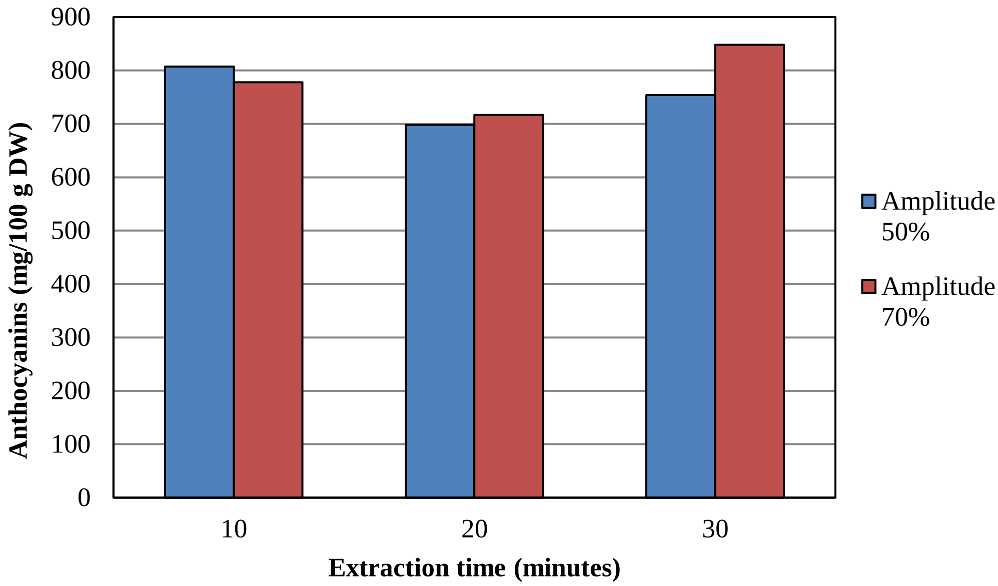

Figure 3. Anthocyanin content of ethanolic extracts 70% hibiscus acidified 1% with acetic acid, depending on extraction time, amplitude and solid/solvent ratio of 40/1 (ultrasound-assisted extraction).

Following the results obtained after the application of the two methods of extraction of bioactive compounds from hibiscus petals, we can say that the most efficient method of extraction was that assisted by ultrasound.

3.7.2. Influence of maceration and ultrasound-assisted extraction parameters on the polyphenolic content of the peony

Regarding the study of the influence of the parameters of maceration extraction of some bioactive compounds from the petals of peony (*Paeonia officinalis*), for the efficient extraction of anthocyanins, polyphenols and tannins, the following optimal conditions were identified:

- Extraction solvent: 70% ethanol

- Solvent/solid ratio: 48/1
- Extraction temperature: 40°C

and for the efficient extraction of flavonoids, the optimal conditions were:

- Extraction solvent: 70% ethanol
- Solvent/solid ratio: 24/1
- Extraction temperature: 40°C.

In the case of ultrasound-assisted extraction of anthocyanins and tannins from peony petals, the following optimal conditions have been established:

- Extraction solvent: 70% ethanol
- Solvent/solid ratio: 40/1
- Extraction time: 30 minutes
- Amplitude of ultrasonic waves: 50%.

For the ultrasonic assisted extraction of polyphenols from peony petals, the following optimal extraction parameters have been established:

- Extraction solvent: ethanol 70% acidified 1% with citric acid
- Solvent/solid ratio: 50/1
- Extraction time: 30 minutes
- Amplitude of ultrasonic waves: 50%.

For the ultrasonic assisted extraction of flavonoids from peony petals, the following optimal extraction parameters have been established:

- Extraction solvent: 70% ethanol
- Solvent/solid ratio: 50/1
- Extraction time: 20 minutes
- Amplitude of ultrasonic waves: 50%.

3.8. Mathematical modeling

The adequacy of different mathematical models was tested in order to identify the relationships between the anthocyanin content obtained and the independent variables.

The results obtained from the mathematical modeling confirm that a high content of anthocyanins in hibiscus extracts is influenced by independent variables depending on the parameters used and varied during the extractions.

Also, following the mathematical modeling of the results, confirms that a high content of polyphenolic compounds in peony extracts is also influenced by independent variables depending on the parameters used and varied during extractions.

It has been observed that along with the extraction time, the amplitude of the ultrasonic waves is an important factor for the ultrasonic assisted extraction. It can also be seen that a high amplitude at a higher temperature leads to increase the content of polyphenols during ultrasonic extraction for both types of solvent used.

3.9. Statistical analysis

The results of the two statistical tests applied indicate that there are no statistically significant differences in the average anthocyanin content of hibiscus extracts for the two extraction methods used.

According to the statistical analysis, the average content of total phenols and condensed tannins in the peony do not have a normal distribution for the extraction carried out by maceration. Thus, they cannot be tested with the t-student Test or the F-Fisher Test. Consequently, depending on the extraction solvent used, the content of total phenols and condensed tannins was tested using the Kruskal Wallis Test. For total phenols, the value of $p = 0.508$, the differences being statistically insignificant, and for condensed tannins the value of $p = 0.085$, the differences being statistically significant between these averages of the content for the solvent used.

For the average anthocyanin content obtained depending on the solvent used from the peony extracts there are statistically significant differences between the mean values ($p = 0.012$), these being represented in Figure 4.

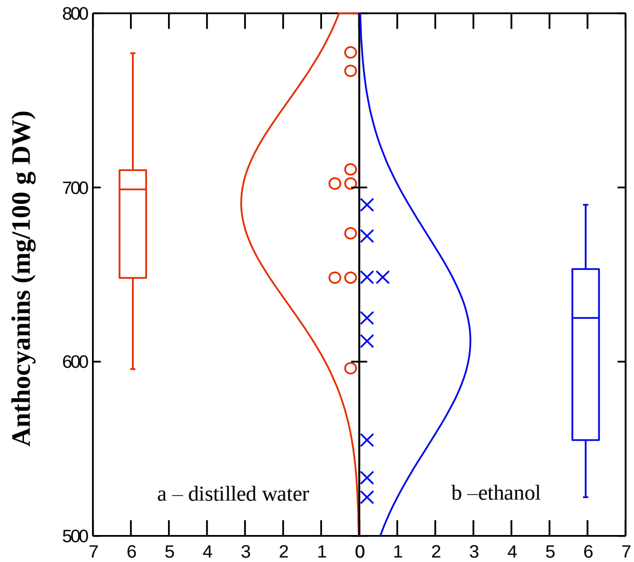

Figure 4. Distribution of experimental data as normal distribution for anthocyanins, extracted with distilled water (a) and 70% ethanol (b).

According to the statistical analysis, the polyphenols content does not have a normal distribution, being tested with the Kruskal Test and the value p = 0.773, being statistically insignificant differences depending on the solvent used for their extraction.

The rest of the analyzed bioactive compounds (anthocyanins, flavonoids and tannins) had a normal distribution, consequently they were statistically tested with the t-student test.

Depending on the solvent used, it is observed that there are statistically significant differences between the average values of anthocyanin content (p = 0.003), these being represented in Figure 5.

Statistical analysis shows that the average values of anthocyanin content obtained by ultrasonic assisted extraction from garden peony petals are statistically different depending on the extraction solvent used. Thus, for the ultrasound-assisted extraction performed with 70% ethanol, the average values of anthocyanins obtained are significantly higher, compared to those obtained with 70% ethanol acidified 1% with citric acid.

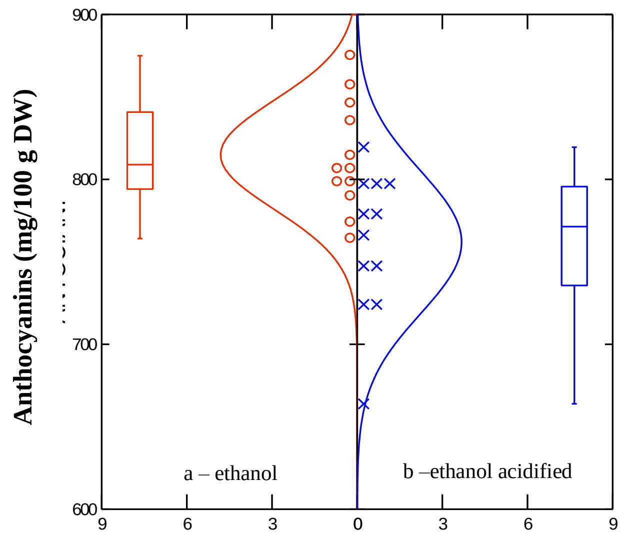

Figure 5. Distribution of experimental data as normal distribution for flavonoids, obtained with 70% ethanol (a) and 1% acidified 70% ethanol with citric acid (b).

For the content of condensed tannins obtained depending on the extraction solvent used, there are statistically significant differences between the averages of the values obtained ($p = 0.000$), these being represented in Figure 6.

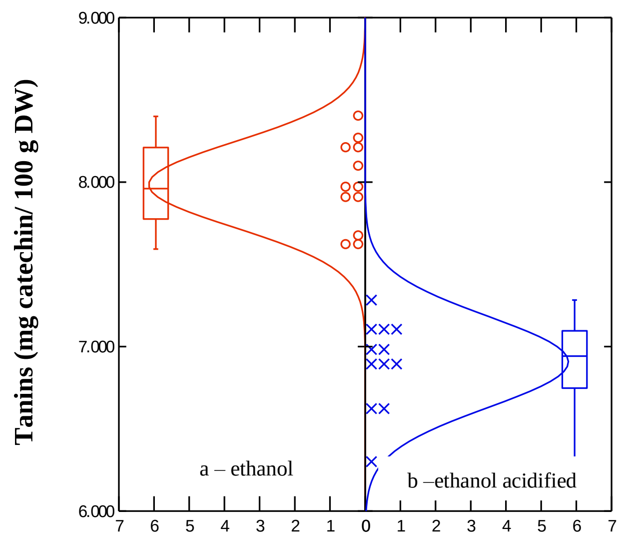

Figure 6. Distribution of experimental data as a normal distribution for condensed tannins, obtained with 70% ethanol (a) and 1% acidified 70% ethanol with citric acid (b).

4. USE OF ENZYME-BASED TECHNOLOGIES FOR THE SUPERIOR EXTRACTION OF HIOBUSCIOUS BIOACTIVE COMPOUNDS AND GARDEN PEONY

4.1. Introduction

Some of the polyphenolic compounds are attached to the cell wall, so, for their extraction, it is necessary to destroy the cell wall without affecting the compounds with polyphenolic structure. That is why it is necessary to develop an enzyme-based technology for the superior extraction of biologically active compounds from plants.

Therefore, **the main objective** of this study is to develop enzyme technologies based on the use of cellulase in order to increase the efficiency of extraction of bioactive compounds from hibiscus petals and garden peony. Enzyme-assisted extraction (cellulase) is applied for the first time to capitalize on compounds from the petals of the studied plants.

Thus, the influence of some parameters of enzyme-assisted extraction (incubation time) followed by ultrasound-assisted extraction on the content of bioactive compounds and the bioactive properties of the final extracts (total antioxidant capacity) are studied.

The research was conducted with a view to developing effective natural ingredients for use in natural food products or supplements and in dyeing fabrics.

4.2. Materials

In the present study, for the extractions of bioactive compounds with polyphenolic structure, hibiscus petals and garden peony petals were used.

Aspergillus niger cellulase enzyme was used to perform the enzymatic pre-treatment, making enzymatic solutions in acetate buffer pH 4.6.

All specific solvents and chemical reagents used for this study were of analytical purity.

4.3. Equipment

The experimental part was performed using the equipment of the Biochemistry Laboratory and the laboratories of the Research Center in Food Biotechnology of the Faculty of Agricultural Sciences, Food Industry and Environmental Protection, within the "Lucian Blaga" University of Sibiu.

4.4. Experimental methodology

After establishing the optimal parameters of ultrasound-assisted extraction from the experiments previously performed for hibiscus and peony, the increase of the extractive efficiency was tested by applying an enzymatic pre-treatment according to Figure 7.

The extraction was performed with optimal extraction solvents for each material, these being chosen after processing the results obtained after the previous experiments, maceration and ultrasonic assisted extraction.

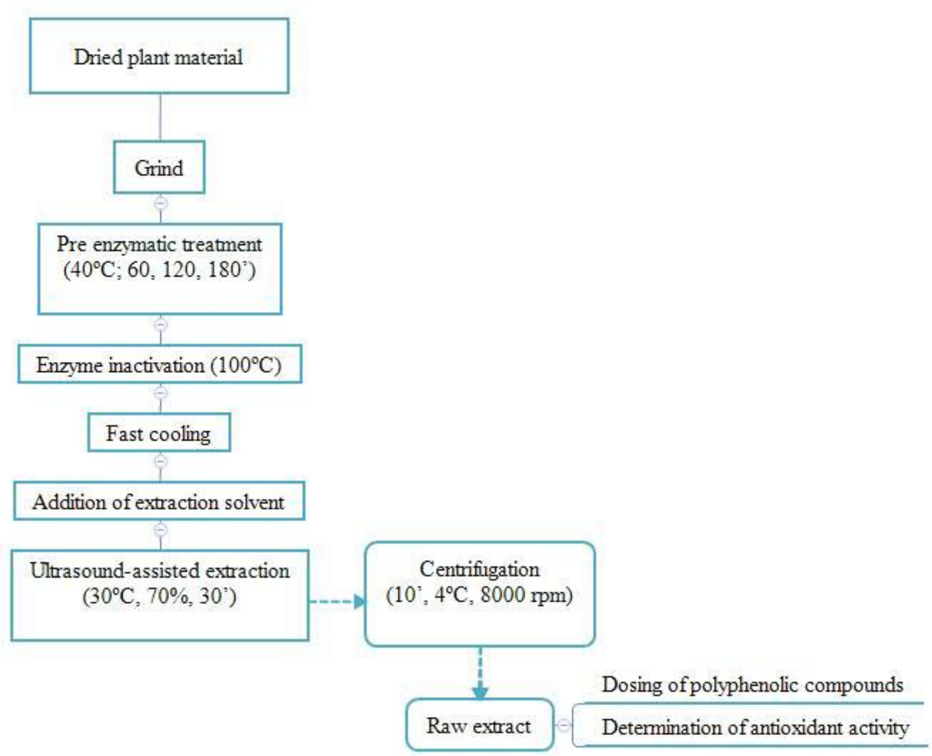

Figure 7. Experimental technical scheme of enzyme and ultrasound assisted extraction of bioactive compounds with polyphenolic structure from the petals of selected flowers.

The determination of the polyphenolic content from the obtained extracts was performed according to the specific spectrophotometric methods. Also, the determination of the antioxidant activity of the extracts was performed according to specific methods (FRAP, DPPH).

4.5. Results and discussions

Among the unconventional methods of extraction of bioactive compounds with polyphenolic structure, enzyme-assisted extraction has gained special importance, being successfully applied in recent years (Kalcheva-Karadzhova et al., 2014; Wang et al., 2010).

In the present study, enzyme-assisted extraction was applied for the first time to capitalize on bioactive compounds from hibiscus petals and garden peony.

The results of the analyzes show that the method of cellulase-assisted extraction is more efficient for obtaining high amounts of bioactive compounds from hibiscus (Table 1) and garden peony (Table 2).

Table 1. The content of bioactive compounds with polyphenolic structure from hibiscus samples, in the presence and absence of enzymatic pre-treatment (60 minutes).

Treatment type	Anthocyanins (mg cyanidin/ 100g)	Polifenoli (mg GAE/ 100 g)	Flavonoide (mg quercetin/100 g)	Taninuri (mg catechin/100 g)
Enzimatic	**668,115**	1336,743	**2715,513**	**6561,118**
Control	676,035	**1338,169**	2533,792	6271,147

Table 2. The content of bioactive compounds with polyphenolic structure from peony samples, in the presence and absence of enzymatic pre-treatment (60 minutes).

Treatment type	Anthocyanins (mg cyanidin/ 100g)	Polifenoli (mg GAE/ 100 g)	Flavonoide (mg quercetin/100 g)	Taninuri (mg catechin/100 g)
Enzimatic	**720,481**	**3985,507**	**2056,833**	**8917,8120**
Control	647,961	3428,178	1973,354	8605,0230

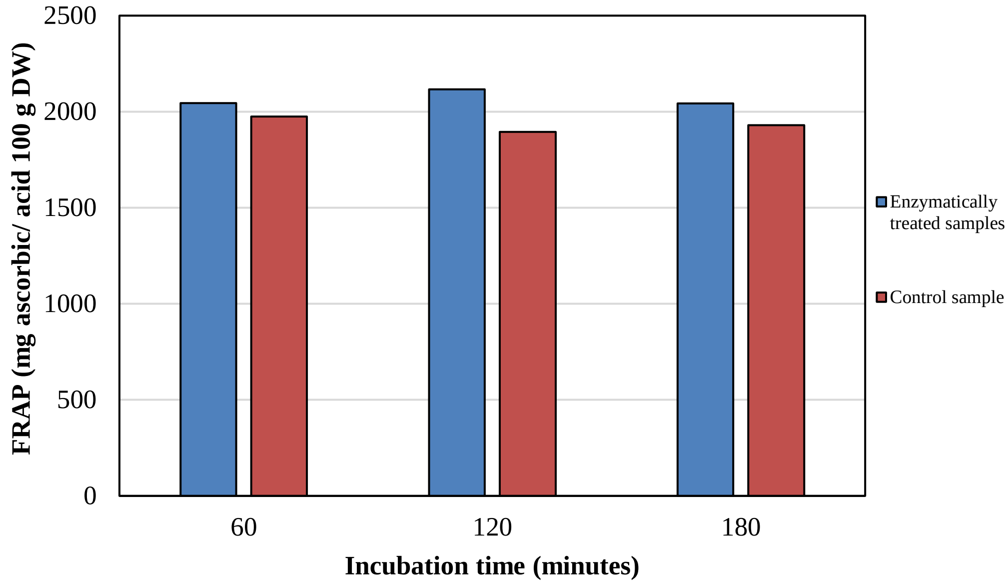

Figure 8. The influence of cellulase incubation time at 40 ° C on the antioxidant activity of FRAP of hibiscus extracts.

The results obtained in the case of samples where ultrasound-assisted extraction was preceded by enzyme treatment are presented in hibiscus (Fig. 8) indicate a high antioxidant activity, with average values higher than those without enzyme treatment.

For FRAP antioxidant activity, statistical analysis by Kruskal-Wallis test shows significant differences ($p = 0.050$) between pre-treated cellulase samples and control samples.

For the antioxidant activity of DPPH (Fig. 9), results were obtained that indicate that in the case of samples where ultrasound-assisted extraction was preceded by treatment there is a higher average antioxidant activity compared to those without enzymatic treatment.

The differences between the mean values of DPPH antioxidant activity for the two extraction methods are not statistically significant.

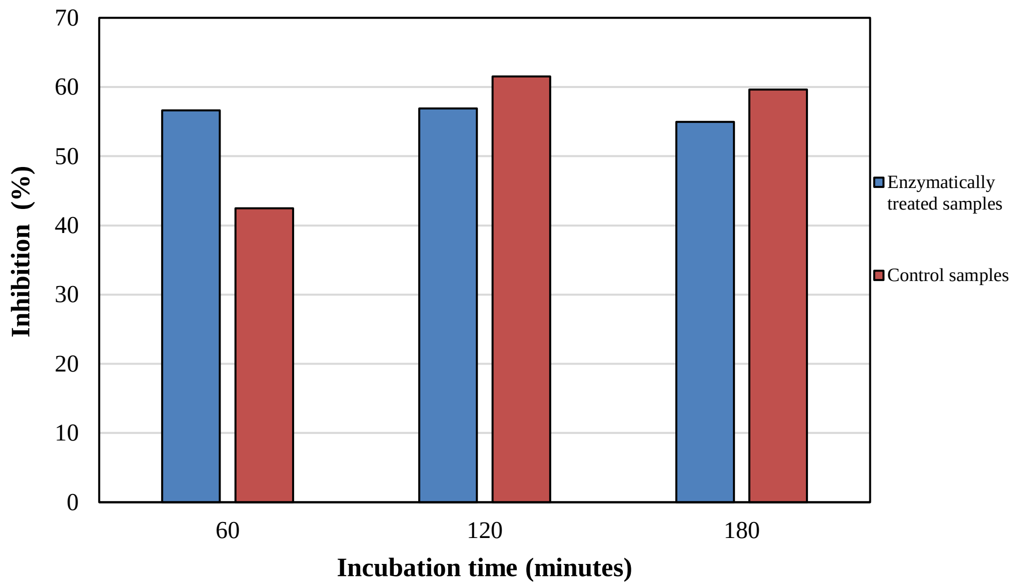

Figure 9. Influence of cellulase incubation time at 40 ° C on the antioxidant DPPH activity of hibiscus extracts.

The results obtained in the case of samples obtained from peony in which ultrasound-assisted extraction was preceded by enzyme treatment indicate an average value of antioxidant activity higher by 55.92% compared to that of samples without enzyme treatment (Fig. 10).

The obtained results indicate an antioxidant activity by the more efficient DPPH method in the control samples, in which the extraction was performed only with the help of ultrasound, without the combination with the cellulase-assisted extraction (Fig. 11).

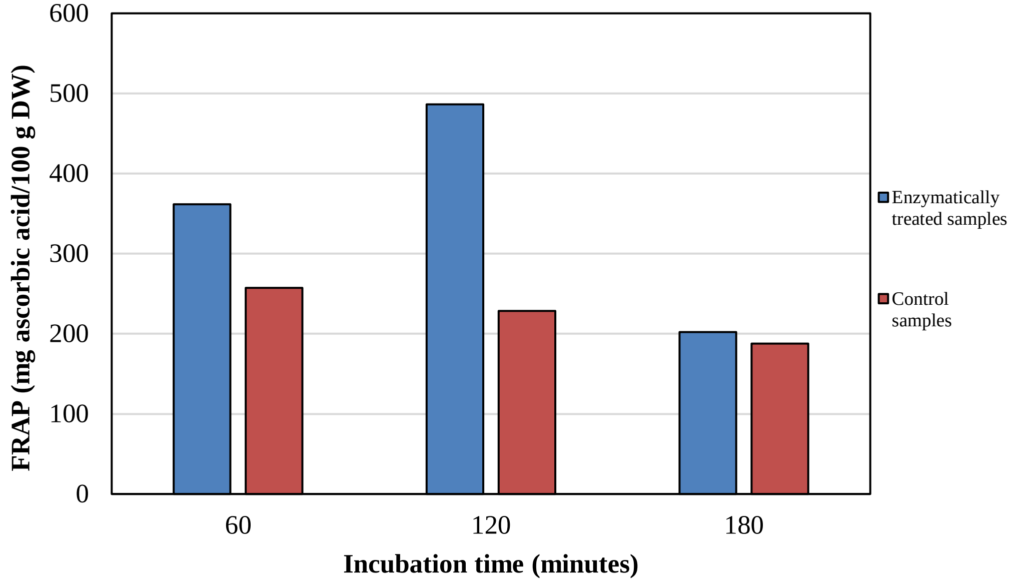

Figure 10. Influence of cellulase incubation time at 40 ° C on the antioxidant activity (FRAP) of peony extracts.

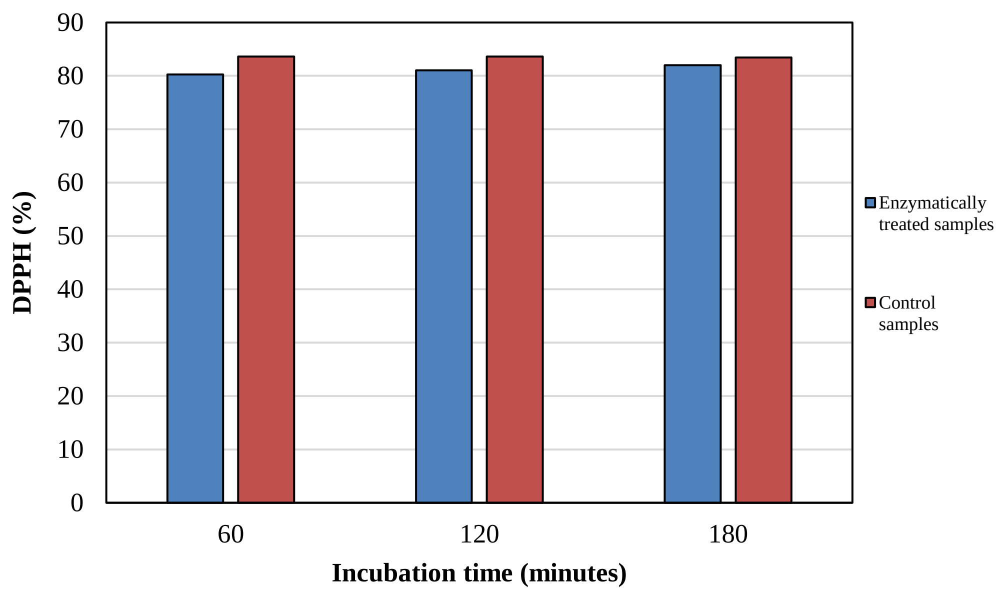

Figure 11. The influence of cellulase incubation time at 40 ° C on the antioxidant activity (DPPH) of peony extracts.

4.6. Statistical analysis

Statistical analysis of the data obtained from the extractions was performed by the Kruskal-Wallis test where significant differences ($p = 0.046$) were observed between the pre-treated cellulase samples and the control samples for the method for determining the antioxidant activity of DPPH.

For the analysis of the correlation coefficient of the content of different bioactive compounds, the Pearson test was performed for the obtained results and a significant positive correlation was observed between tannins and anthocyanins ($r = 0.877$) and marginally significant positive correlations between tannins and flavonoids ($r = 0.757$) , between anthocyanins and flavonoids ($r = 0.746$).

5. APPLICATION RESEARCH FOR HIBISCUS (*Hibiscus sabdariffa*) AND GARDEN PEONY EXACT (*Paeonia officinalis*)

5.1. Introduction

The need for healthy food-based consumption is one of the most important problems facing humanity and has led to a growing trend in the diversity of food and modern processing.

Cannabis sativa is a plant known as hemp, which has been used over the years to make various products, especially textile fibers. A new opportunity is given by the use of its seeds in obtaining an edible oil, rich in polyunsaturated fatty acids, recognized for its healing and beneficial effects (Da Porto et al., 2014).

Because the human body cannot produce alpha-linolenic (Omega 3), linoleic (Omega 6) and oleic (Omega 9) acids, hemp seeds can be a good alternative to animal oils. It contains polyunsaturated fatty acids, but also other compounds such as: minerals, vitamins (A, C and E), tocopherol, β-carotene. Due to the optimal ratio of polyunsaturated fatty acids Omega 3 and Omega 6 (1: 3), they are considered "healthy fats".

Due to concerns about the safety of synthetic additives, research has shown that artificial antioxidants used in the food industry should be replaced with natural ones due to potential negative effects on human health (Carocho et al., 2014). Hibiscus flowers are rich in bioactive compounds with polyphenolic structure, with antioxidant properties confirmed in the research in this doctoral thesis, which allow the research to be used in thermo-oxidative stabilization products of edible oils, namely hemp oil, which is the subject present research study.

Nowadays, the textile industry is oriented towards the replacement of synthetic pigments with natural ones, so that the good coloring capacity of hibiscus extracts makes them suitable for dyeing textiles such as cotton, linen, wool or silk, having a minimal negative impact on the environment and human health, compared to synthetic ones (Haddar et al., 2014).

The existence of a small number of studies aimed at the dyeing capacity and the high content of bioactive compounds with polyphenolic structure in the flowers of the peony obtained in previous experiments, contributed to a study that tested the dyeing capacity of these natural extracts.

The main objective of the study is to capitalize on extracts rich in bioactive compounds with polyphenolic structure, especially anthocyanins, in order to obtain new or improved products for food technologies and ecological dyes in textile technology.

5.2. Materials

In the present study, hibiscus and peony extracts were used, prepared using the optimal parameters described above (Chapter 3).

To perform the experiment in order to capitalize on the antioxidant potential of hibiscus extracts, hemp oil (*Cannabis sativa*) was used.

In order to make ecological paints with the extract obtained from hibiscus petals and peony, cellulose (cotton) textile specimens were used, washed and bleached, with a mass of 180 g/m2, with a composition of 100% cotton.

5.3. Equipment

The experimental part was performed using the equipment of the Biochemistry Laboratory and the laboratories of the Research Center in Food Biotechnology of the Faculty of Agricultural Sciences, Food Industry and Environmental Protection, within the "Lucian Blaga" University of Sibiu.

The fatty acid analysis was performed by the GC-MS method, using the Clarus 600 T spectrometer (Perkin ¬ Elmer) in the research laboratories at the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca.

5.4. Experimental methodology

Following the extractions performed from previous experiments for hibiscus and peony petals, those optimal parameters that were used to obtain a higher content of bioactive compounds with polyphenolic structure (especially anthocyanins), which was used, were identified and chosen. when making industrial applications.

5.4.1. Working methods for evaluating the potential of hibiscus bioextract to protect hemp oil against oxidation

In order to make a new product for the food industry, hibiscus oil stabilized with hibiscus extract, the antioxidant capacity of hibiscus extract was initially determined by the FRAP spectrophotometric method (Benzie & Strain, 1996), and the results were expressed in mg of acid equivalents ascorbic/100 g DW.

To evaluate the antioxidant potential using hemp oil, a lipid system of 0.2% anthocyanins (from hibiscus extracts) and 0.5% lecithin was added, added as an emulsifier. In parallel, a control sample (without hibiscus extract) and an oil sample with α-tocopherol (0.1%) were performed.

The samples applied in 2 cm layers in Berzelius glasses were stored in an oven for 10 days at a temperature of 60°C to accelerate the oxidation process. Periodically, at regular intervals of 2 days, the samples were analyzed to determine the peroxide index, the fatty acid composition, the FTIR analysis and the thermal stability by the DSC method.

5.4.2. Working methods on ecological dyeing of cotton fabrics with hibiscus and peony extracts

Hibiscus and peony extracts, rich in anthocyanins, were made according to the optimal conditions identified in previous processes.

Two methods of dyeing cotton fabrics were applied: the classical dyeing method by depletion and the unconventional ultrasonic dyeing method. The dyeing conditions that varied are the following: for the fixation of natural pigments, 3 types of mordants were used through a simultaneous treatment, among which 2 environmentally friendly mordants and a classic mordant, 2 different concentrations of mordants used, at a ratio of 50: 1 fleet.

Following the results obtained, analyzes of chromatic parameters, FT-IR and wash resistance analyzes were performed.

5.5. Results and discussions

5.5.1. Recovery of hibiscus bioextracts for the purpose of oxidative stabilization of hemp oil

In vitro antioxidant activity performed according to the FRAP method was 5146.24 mg ascorbic acid/100 g DW. The results obtained from hibiscus extract indicate that it is a good antioxidant, also proven by the inhibition of free radicals (DPPH method) of 87.77%.

Analytical results indicate that the type of hemp oil studied contains a large amount of unsaturated fatty acids (> 90%), mostly consisting of linoleic acid (86.53% of the total fatty acids in the oil).

In the first 7 days, major changes were observed in the composition of most fatty acids in oil samples:

- The level of saturated fatty acids increased by 31% in the controls, 92% for oil samples treated with α-tocopherol, and for the samples treated with the extract of hibiscus it decreased by 16.5%.
- The content of monounsaturated fatty acids decreased by 50.66% after 7 days of storage at 60°C for samples treated with hibiscus extract, compared to samples treated with α-tocopherol.
- The amount of polyunsaturated fatty acids decreased in the control samples and in those treated with α-tocopherol, correlated with a concomitant increase of monounsaturated fatty acids, after 7 days of storage at a temperature of 60°C; the amount of polyunsaturated fatty acids decreased by 10% in oil samples treated with α-tocopherol. In comparison, the samples treated with hibiscus extract showed an increase in the content of polyunsaturated fatty acids by 2.7% after 7 days of storage.

The percentage of total unsaturated fatty acids in the control samples and in the oil treated with α-tocopherol decreased after 7 days at 60°C, while it remained similar in the samples of hemp oil with hibiscus extract, demonstrating the protective effect of hibiscus extract against oxidative degradation of hemp oil.

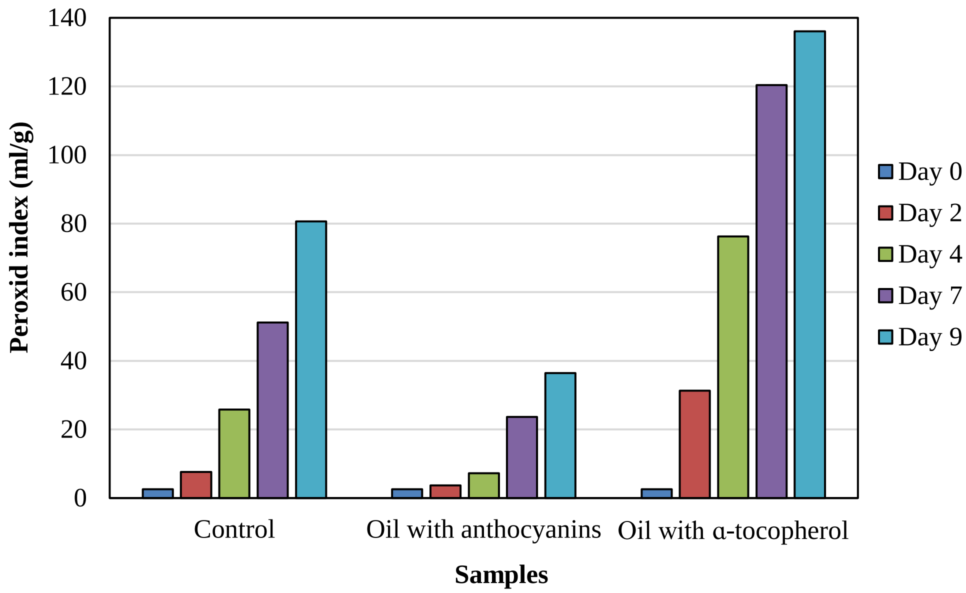

Figure 12. The time variation of the peroxide index for the hemp oil samples investigated.

Throughout the storage period, hemp oil samples with hibiscus extract indicate a slow increase in Ip compared to the other samples investigated, which indicates the efficiency in protecting the oil against oxidation (Fig. 12).

For the samples of hemp oil with hibiscus extract and α-tocopherol, respectively, the ATR-FT-IR analysis was performed to spectrally monitor the changes in the functional groups resulting from the accelerated oxidative degradation.

The results showed that after 9 days of oxidation at a temperature of 60°C, the differences in hydroperoxide absorbances compared to the control sample decrease as follows: α-tocopherol oil>Control>Anthocyanin oil. The low hydroperoxix content identified in the ATR-FTIR spectrum confirms the previous results from the Ip analysis of increased stability of hemp oil in the presence of hibiscus extract.

In this paper we also studied the kinetics of the process of thermo-oxidative degradation of hemp oil samples before and after storage at 60°C, monitored by the DSC method, calculating the kinetic parameters using the Ozawa-Flynn-Wall method.

The results obtained confirm that the hibiscus extract added to the hemp oil samples increases the thermo-oxidative stability at 60°C, consistent with the results on Ip and the evolution of the fatty acid composition by GC-MS (Fig. 13).

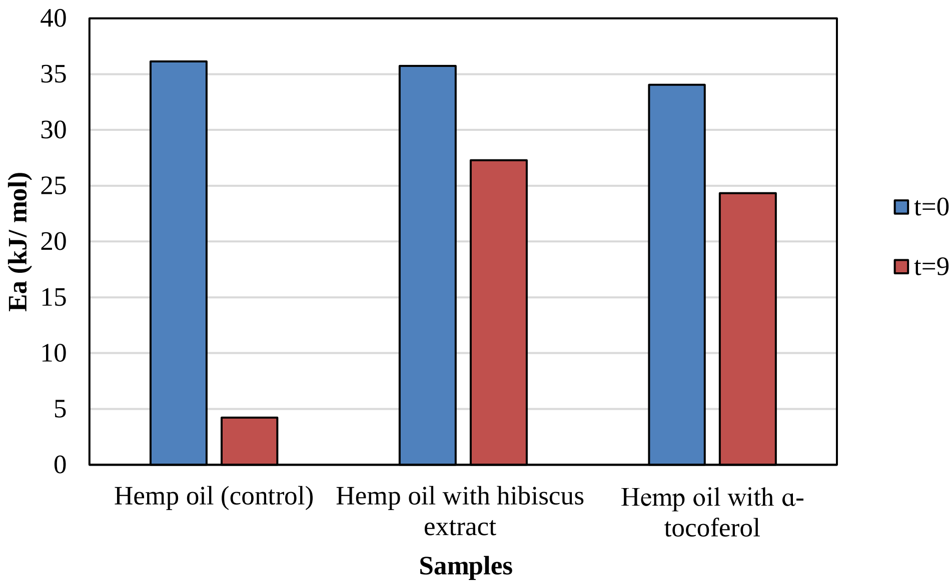

Figure 13. Variation of activation energy calculated for the samples of hemp oil, before and after thermo-oxidation at 60°C.

5.5.2. Recovery of bioextracts of hibiscus and peony by ecological dyeing of cotton fabrics

This chapter presents the results on the ability to dye cotton fabric with extracts of hibiscus and common red peony, respectively, with anthocyanins as the main species of dye.

After dyeing with natural hibiscus extract, by classical and modern dyeing techniques, cotton samples were analyzed by FT-IR spectroscopy to identify the presence of characteristic functional groups in cellulose substrates and hibiscus extracts where different absorption bands were identified.

Following the chromatic measurements of the fabrics dyed with hibiscus extract, it was observed that the brightness values and the color differences change in relation to the mordants used in the dyeing process.

Optical microscopy images of cotton samples dyed with hibiscus extract are shown in Figure 14 completing the results of the color analyzes performed.

The resistance to washing with soap solution of cotton samples dyed in different conditions with hibiscus extract was evaluated by comparison with the control samples, dyed by the two methods but not treated with mordant.

In general, the resistance to washing with soap solution has low values. The highest strengths of the cotton samples dyed with hibiscus extract were obtained in the case of sample 12, but also in the case of samples 4, 6, 8, dyed with hibiscus extract and simultaneously treated with tannic acid, citric acid 3 or sulfate of copper in a concentration of 3%, both by ultrasound and by depletion.

Also, after painting with natural garden peony extract, FT-IR spectroscopic analyzes were performed to identify the presence of characteristic functional groups in cellulosic substrates and peony extract, where different absorption bands were identified.

The results of the color changes measured by the CIELAB system for the differences in the chromatic coordinates of the specimens painted with peony extract showed that the brightness values (L *) and the color differences (ΔE *) changed according to the type of mordant used. in the process of dyeing cotton samples.

Optical microscopy images of cotton samples dyed with peony extracts, by various methods, in the presence or absence of mordants (citric acid 1 and 3%, tannic acid 1 and 3% and copper sulphate 1 and 3%) are presented in Figure 15.

The washing resistance of cotton samples dyed with peony extract obtained by different processes was evaluated by comparison with control samples, dyed and untreated with mordant. In general, the resistance to washing with soap solution (5g/l) of the samples in the present study shows average values, between 2 and 4.

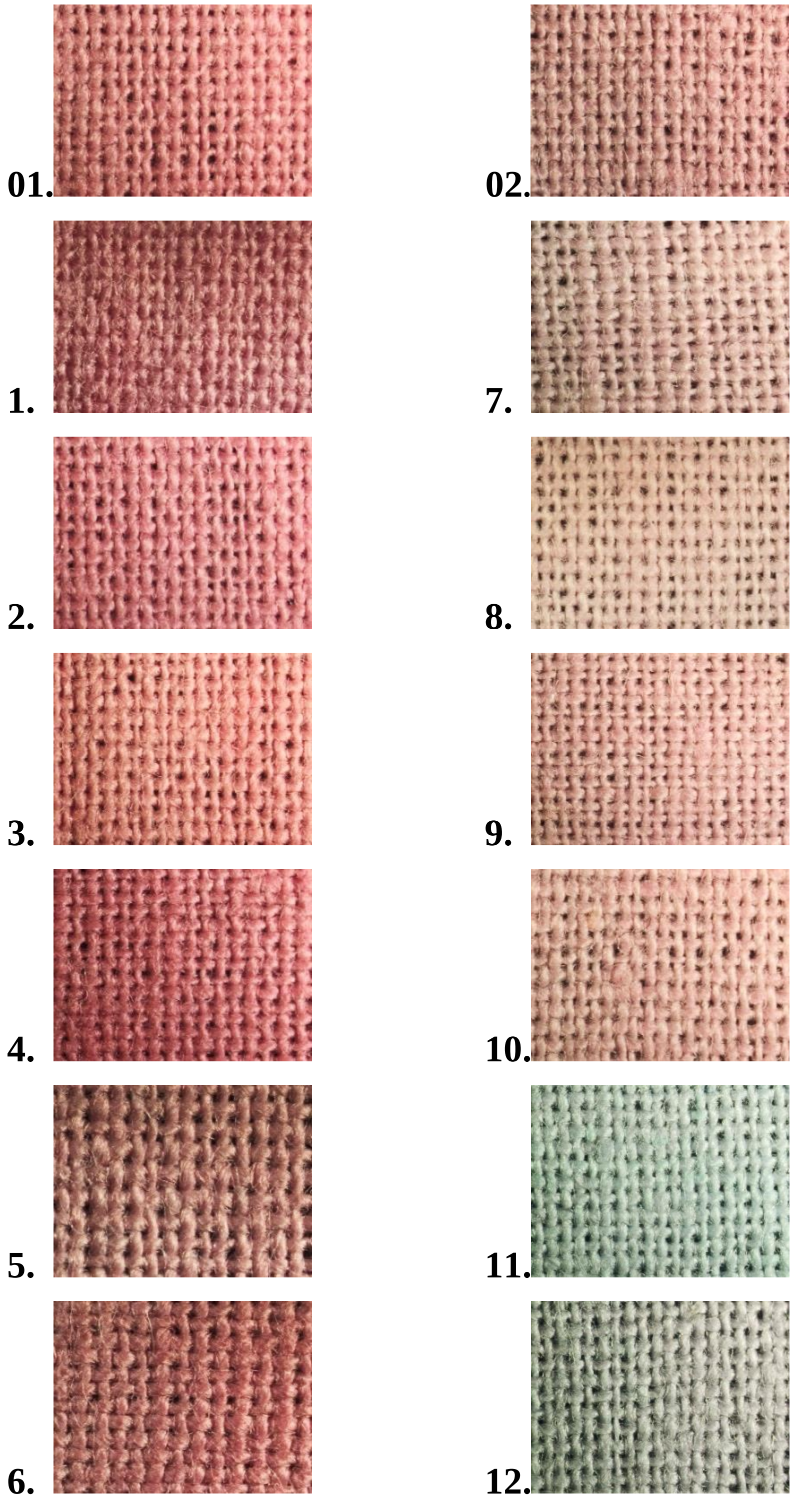

Figure 14. Optical microscopy images of textile substrates dyed with hibiscus extract.

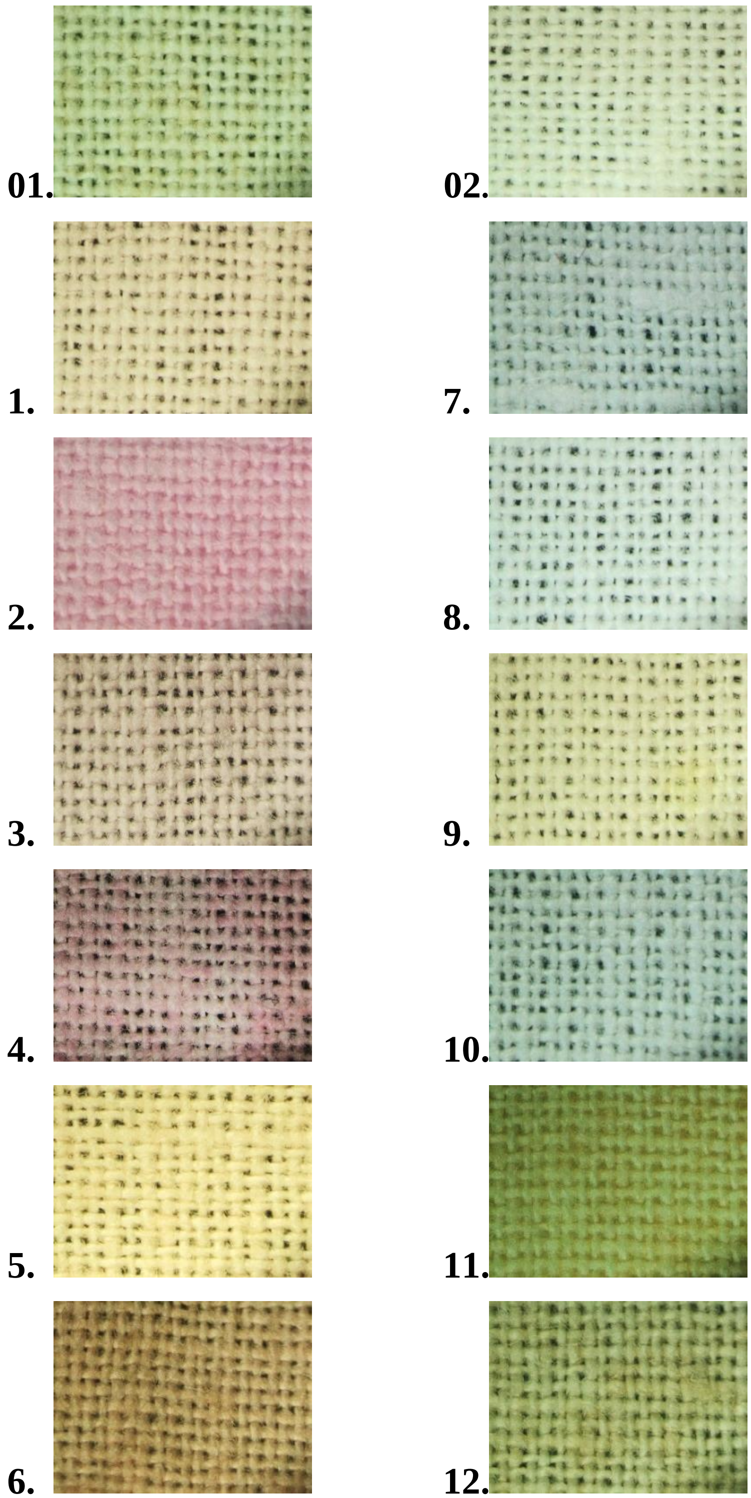

Figure 15. Optical microscopy images of textile substrates dyed with peony extract.

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7. GENERAL CONCLUSIONS, ORIGINAL CONTRIBUTIONS, RECOMMENDATIONS AND FUTURE RESEARCH DIRECTIONS

CONCLUZII GENERALE

The present research study involved the realization of some hypotheses, their testing by performing some experimental series, the improvement of the extraction processes by modifying the process parameters and the practical application. The obtained results allowed the realization of some mathematical interpretations and modelings through which the following conclusions can be formulated:

- Identifying the optimal conditions for the extraction of bioactive compounds by testing different experimental parameters. The extraction was performed by using "green technologies" based on the use of "environmentally friendly" solvents (water, ethanolic solutions), given the application purpose of the final extracts obtained.
- The initial processing of the samples has a positive effect on the content of bioactive compounds with polyphenolic structure obtained from extractions from peony petals, by facilitating extraction. The crushing of the dry plant material facilitates the extraction by increasing the contact surface between the plant material and the extraction solvent, thus the extraction efficiency is higher.
- Following the comparative study of the extractive efficiency of anthocyanins in *Hibiscus sabdariffa* petals using the two techniques, ultrasound-assisted extraction and maceration extraction, the latter proved to be less efficient. There were statistically significant differences between the two methods applied. The ultrasound-assisted extraction method can have economic advantages.
- For the series of maceration extraction experiments using two types of solvents, the content of anthocyanins extracted with ethanol 70% acidified 1% with acetic acid, the average value obtained was 19.21% higher compared to the samples in which its extraction made with 1% acidified water with acetic acid.
- For the series of ultrasound-assisted extraction experiments using the two types of solvents, the mean value of the anthocyanin content extracted with 1% acidified

water with acetic acid was 2.33% higher compared to samples where 70% acidified ethanol was used. 1% with acetic acid.

- Following the results obtained on hibiscus on the study of the influence of the extraction method, the type of solvent, the solvent/solid ratio, the extraction time and the amplitude of ultrasonic waves on the content of anthocyanin pigments, the optimal extraction conditions were identified.
- Although the use of acidified ethanol obtained higher values of anthocyanin content compared to those resulting from the use of acidified water, the statistical interpretation of the data shows that there are no statistically significant differences between the two types of solvents used.
- Regarding the study of the influence of maceration extraction parameters of some bioactive compounds from common peony petals (*Paeonia officinalis*), namely the type of solvent, solvent/solid ratio, extraction temperature and extraction time, for efficient extraction of compounds polyphenols, the optimal extraction conditions were identified.
- In the case of ultrasound-assisted extraction of bioactive compounds from peony petals, by testing the type of solvent, solvent/solid ratio, extraction time and amplitude of ultrasonic waves, the optimal extraction conditions were established.
- Development of an efficient modern extractive method that combines ultrasound-assisted extraction with enzyme-based technology to obtain large amounts of bioactive compounds with antioxidant activity from plant sources of interest in the present study.
- The results obtained from hibiscus petals and peony by applying this combined method show an average content of bioactive compounds with higher polyphenolic structure obtained for both types of biological material analyzed, highlighting the efficiency of the ultrasound-assisted extraction method preceded by with cellulase.
- For the extraction of biologically active compounds with polyphenolic structure from *Hibiscus sabdariffa* - hibiscus, the average values of the anthocyanin content obtained with enzymatic pre-treatment were higher compared to those of the samples. However, the differences are not statistically significant.

- For the extraction of biologically active compounds with polyphenolic structure from *Paeonia officinalis* - the common garden peony, the average values of bioactive compounds with polyphenolic structure obtained were higher in the case of assisted extraction of enzymes combined with ultrasound, compared to control samples.
- The study also aimed at in vitro testing of the antioxidant activity of extracts obtained by the combined extraction method, assisted by enzymes + ultrasound. Thus, for the samples in which the extraction was preceded by the enzymatic treatment, the antioxidant activity by the FRAP method is significantly higher than that for the control samples, for both types of flowers studied.
- The investigation of the oxidative stability of an edible oil of interest (hemp oil) in the presence of anthocyanin extract of hibiscus and peony respectively was performed using classical and modern methods of analysis (CG-MS, Ip, FTIR, DSC).
- Hibiscus extract led to improved thermo-oxidative stability at 60°C for 9 days of hemp oil compared to control oil samples (untreated) and those treated with α-tocopherol.
- The results of the GC-MS analysis on the level of saturated, monounsaturated and polyunsaturated fatty acids in the hemp oil samples show differences between the samples treated with antioxidants and the untreated ones. There was an increase of saturated fatty acids by up to 92% for control samples and a decrease of 16.5% for samples treated with hibiscus extract, indicating the effectiveness of the addition of natural antioxidant from hibiscus petals. Increases in the level of monounsaturated fatty acids by 50.66% were recorded in the case of samples treated with hibiscus extract compared to samples of oil treated with α-tocopherol.
- The results of the peroxide index analysis indicate a high efficiency of the hibiscus extract added to the hemp oil compared to the control sample, for protection against primary oxidation of lipids; moreover, after 2 days of accelerated oxidation at 60°C, α-tocopherol showed a pro-oxidant effect.
- The results of the thermal analysis by DSC technique, of the kinetic parameters (Ea) of the thermal degradation process of the oil samples confirm the previous

results regarding the efficiency of hibiscus extract in protecting hemp oil against thermo-oxidation tested at 60°C.

- Testing of hibiscus extract in textile technologies for ecological dyeing of cotton fabrics, in the presence and absence of metallic mordants ($CuSO_4$) and biomordants (citric acid, tannic acid) led to obtaining samples dyed in different shades, being evaluated by measurements color and standard wash tests. The results indicate color differences of the samples painted with natural extract (ΔE *) depending on the different conditions of the painting process. The FT-IR spectra confirm the dyeing with natural extract, by identifying the characteristic bands of the different functional groups.
- Dyeing of cotton fabrics with red garden peony extract by two processes, conventional and unconventional, in the presence and absence of classical mordant and biomordant was confirmed by FT-IR spectra, by the presence of characteristic bands of different functional groups. The chromatic characteristics of the painted cellulosic substrates, determined in the CIELAB system, showed higher values of the red coordinate (a *) and large positive differences in color intensity (ΔC *) for the samples painted by depletion in the presence of a higher acid concentration. citrus (3%). Large color differences (ΔE *) were observed for ecologically painted samples in the presence of a high concentration of copper sulphate (3%), followed by samples painted in the presence of citric acid and tannic acid using both types of paint.

ORIGINAL CONTRIBUTIONS

Through this research paper, important theoretical, experimental and applied contributions have been made in the field of natural extracts from widely cultivated plant petals, with the potential to be valuable products for the development of enriched foods or pigments used in ecological textile dyes:

- Contributions to the enrichment of the theoretical knowledge base regarding the bioactive and extractive potential of the plants of interest of the research study by identifying the classes of bioactive compounds present in the petals of the studied species.

- Studies on the main extraction techniques, conventional and unconventional, of polyphenolic compounds, on the antioxidant potential and coloring of extracts from plants of interest in this doctoral research study.
- Research on the influence of the type of extraction (maceration, ultrasound-assisted extraction, technologies based on the use of enzymes) and the parameters of extraction of bioactive compounds of polyphenolic type from hibiscus petals and red garden peony.
- Studies on the optimal extraction conditions for each species, mathematical modeling of data from extraction experiments.
- Characterization of the antioxidant properties of bioextracts obtained, both by classical methods (FRAP, DPPH) and by introducing them into a lipid system based on hemp oil.
- Improving the oxidative stability of hemp oil by adding hibiscus extract; characterization of the degradative process with the help of modern analytical techniques (spectrophotometric, GC-MS, FT-IR).
- The use of peony extract in ecological technologies for dyeing cotton fabrics, by classical (depletion) and modern (ultrasonic) methods, in the presence and absence of metallic mordants and biomorders; characterization by color determinations and resistance to washing by standard tests.

FUTURE RESEARCH RECOMMENDATIONS AND DIRECTIONS

The main study directions for further research in the field are:

- extension of experimental research for other vegetable, food or medicinal raw materials, from sustainable sources;
- research on the mechanisms of action of natural extracts in the evaluated systems;
- extending the applied research of natural extracts on other types of food and other textile supports.

LIST OF PUBLICATIONS RESULTING FROM DOCTORAL RESEARCH, PUBLISHED OR ACCEPTED FOR PUBLICATION

Articles published/accepted for publication in ISI listed journals according to WoS:

1. Oancea, S., **Perju, M.**, Olosutean, H., Influence of enzyme-aided extraction and ultrasonication on phenolics content and antioxidant activity of *Paeonia officinalis* L. petals, *Journal of the Serbian Chemical Society*, Vol. 85, pp. 1-12, 2020, DOI: https://doi.org/10.2298/JSC190807120O
2. Oancea, S., Drăghici, O., **Perju, M**., Dulf, Francisc V., Effect of roselle extract on the oxidative stability of hempseed oil, *Journal of Food and Nutrition Research*, Vol. 59, nr. 2, pp. 98–107, 2020.
3. Oancea, S., **Perju, M.**, Coman, D., Olosutean, H., Optimization of conventional and ultrasound-assisted extraction of *Paeonia officinalis* anthocyanins, as natural alternative for a green technology of cotton dyeing, *Romanian Biotechnological Letters*, acceptat 2020, https://www.rombio.org/

Articles published in ISI Proceedings (WoS indexed):

1. **Perju, M.**, Coman, D., Oancea, S., An experimental study on conventional and ultrasound-assisted extraction of *Hibiscus* anthocyanins for eco-dyeing of cotton substrates, Proceedings of the 19th International Multidisciplinary Scientific Geoconference SGEM 2019, Conference Proceedings, Vol. 19, issue 6.1, pp. 523-533, section Advances in Biotechnology, 2019, Albena, Bulgaria, https://www.sgem.org/index.php/elibrary?view=publication&task=show&id=6418
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Articles published in BDI/SCOPUS indexed journals:

1. Oancea, S., **Perju, M.**, Influence of enzymatic and ultrasonic extraction on phenolics content and antioxidant activity of *Hibiscus sabdariffa* L. flowers", *Bulgarian Chemical Communications*, Vol. 52, issue D, 2020, http://www.bcc.bas.bg/

Research papers published in the volumes of national/international conferences:

1. **Perju, M.,** Oancea, S., Valorisation of some indigenous plant extracts for industrial applications, Proceedings of the International Conference "Agri-Food Sciences, Processes and Technologies" AGRI-FOOD 2017 – Agriculture and Food for the XXI Century, May 11-13, 2017, Sibiu, Romania, pp. 7-15, 2017. ISSN 1843-0694 http://saiapm.ulbsibiu.ro/index.php/agri-food-2017
2. Radu, M., **Perju, M.**, Oancea, S., Valorificarea unor deșeuri horticole prinextracția de compuși biologic activi, Conferința națională studențească "Provocări și oportunități privindvalorificarea deșeurilor agro-alimentare", Sibiu, 17-18 mai 2018. http://saiapm.ulbsibiu.ro/index.php/2018/03/13/conferinta-nationala-studenteasca-provocari-si-oportunitati-privind-valorificarea-deseurilor-agro-alimentare/

Patents (OSIM proposal):

1. Oancea, R., S., Drăghicil, O., **Perju, M.,** Procedeu de obținere a uleiului de cânepă îmbogățit cu extract de *Hibiscus*, rezistent la degradarea termo-oxidativă, propunere de brevet OSIM, Nr. Cerere de brevet invenție A/01008, din 29.11.2018; Premiat la *Salonul internațional al cercetării științifice, inovării și inventicii "PRO-INVENT" 2019*, ediția XVII, Cluj-Napoca.

Member in research and development project:

1. Membru în proiectul de cercetare-dezvoltare PN-III-P2-2.2-CI-2018-1401, "Hempseed oil enriched with *Hibiscus* extract, innovative product resistant to oxidative degradation", 2018-2019, director de proiect prof. univ. dr. habil. Simona Oancea.