„Dunărea de Jos" University of Galaţi

Doctoral school of Fundamental Sciences and Engineering

PhD thesis

Characterization in bioactive compounds from some plants from the perspectives of using in foods

(PhD thesis summary)

PhD student,

RADU (LUPOAE) DANIELA SIMONA

Scientific Coordinator,

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Co-superviser scientific Coordinator

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Serie I 7: Food Engineering no. 12

GALAŢI

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Seria M: **Medicină**

Content

Introduction

The special emphasis on increasing the functionality of food has taken a significant **turn** in the design of food, from providing essential nutrients to support life and growth to preventing or even curing various forms of disease. Moreover, recent technological progress, changing consumer lifestyles and socio-economic trends around the world indicate the need to develop innovative technologies for obtaining **food with health benefits**. These elements are considered **key** and lead to the development of current production of **functional foods** on the global market.

The health benefits of functional foods derive from their content in biologically active compounds, such as polyphenolic compounds, carotenoids, vitamins, polyunsaturated fatty acids and peptides, which are naturally found in agri-food products, are formed during processing or extracted from other sources and is added to food. The literature already recognizes the **quality of functional ingredients** for a number of compounds, such as: fiber, protein, vitamins and antioxidants, which through various extraction techniques can be used in **re-modeling food**, in order to improve their functions. Bioactive components such as sterols, tocopherols, carotenes, terpenes and polyphenols can be used to formulate functional foods with improved antioxidant properties.

Our daily diet involves consuming a large amount of pigments such as carotenoids, anthocyanins and chlorophylls (Downham and Collins, 2000). In the human body, these compounds have important biological functions, which allow the fight against diseases such as cancer, cardiovascular disease, cataracts, arteriosclerosis, macular degeneration and others (Özkan and Bilek, 2014). The benefits to human health from the consumption of chlorophylls include antioxidant, anti-inflammatory, antibacterial, anticancer, odorizing and wound healing (Hosikian *et al*., 2010). Carotenoids provide beneficial effects in preventing certain cancers, cardiovascular and degenerative diseases (Nishino, 1998).

The choice of the doctoral topic " **Characterization in bioactive compounds from some plants from the perspectives of using in foods"** was based on the under-exploited functional potential of lavender in the food industry and on the general trend of increasing demand for functional foods on the world market. processing and production and consumer awareness of the role of food in health promotion. Therefore, the topic considered the possibility of identifying new sources of compounds from natural sources with antioxidant potential, which have the ability not only to delay or prevent the formation of reactive species in food, but also the development of technological variants of food, so that by increasing the consumption of these natural antioxidants to prevent the occurrence of oxidative processes in vivo, thus protecting the body against various diseases. It was also considered that, at industrial and commercial level, the applications of natural antioxidants are limited, due to their susceptibility to degradation by oxidation, isomerization, polymerization, copolymerization, etc., depending on environmental and processing conditions (oxygen, enzymes, light, high temperatures, acidic or alkaline pH), specific to the food industry (Schoefs, 2002; Marquez and Sinnecker, 2008; Kang *et al*., 2019).

The doctoral thesis entitled " ***Characterization in bioactive compounds from some plants from the perspectives of using in foods***" aimed at the extraction, identification and recovery of biologically active compounds from *Lavandula angustifolia* flowers in high functionality ingredients and the development of technologies for obtaining food with potential health benefits, by exploiting the phytochemical and biological potential of this plant. Lavender is known as a rich source of essential oils. Lavender (*L. angustifolia*) contains anthocyanins, phytosterols, sugars, minerals, coumaric acid, glycolic acid, valeric acid, ursolic acid, herniarin, coumarin and tannins (Prusinowska and Smigielski, 2014), with significant health effects such as: antioxidant, anti-inflammatory, anticancer, antifungal, antibacterial activity, etc.

The main **scientific objectives** of the doctoral thesis are:

- ✓ Establishing the phytochemical profile of extracts from lavender flowers (Lavandula angustifolia) obtained comparatively by different extraction techniques in correlation with antioxidant properties and processing stability, from the perspective of establishing optimal extraction and storage conditions.
- ✓ Microencapsulation of biologically active compounds from extracts into highly functional ingredients from the perspective of integration into functional foods, by improving the stability and controlled release characteristics.
- ✓ Development of two technologies for obtaining value-added products by adding microencapsulated functional ingredients.

The doctoral thesis is structured in two parts, as follows:

I. **THE DOCUMENTARY STUDY** is divided into 3 chapters that present synthetically different theoretical considerations from the literature on bioactive compounds in Lavandula angustifolia, focusing on health benefits. Theoretical and practical principles of extraction and microencapsulation techniques are also presented.

II. **OWN CONTRIBUTIONS** comprises 4 chapters in which the results of the research studies carried out during the doctoral internship are highlighted, briefly presented below:

Chapter 4, entitled **COMPARATIVE EVALUATION OF SOME EXTRACTION METHODS FROM THE PERSPECTIVE OF CONTENT IN BIOLOGICAL ACTIVE COMPOUNDS** presents the results obtained from the extraction, separation, identification and quantification of biologically active compounds from lavender flowers (carotene, carotene, clotene, flavonoids) (*Lavandula angustifolia*) using spectrophotometric methods and liquid chromatography (HPLC) and gas (GC-MS) techniques.

Chapter 5, entitled **DEVELOPMENT OF HIGH-FUNCTIONAL INGREDIENTS FOR POTENTIAL USES IN FOOD PRODUCTS**, presents the results obtained in the stages of microencapsulation and development of variants of functional ingredients and characterization of the resulting powders, from a biological, structural and phytochemical point of view.

Chapter 6, entitled **APPLICATIVE RESEARCH THROUGH THE DEVELOPMENT OF TECHNOLOGICAL VARIANTS FOR OBTAINING HIGH-FUNCTIONAL FOOD PRODUCTS**, presents the results obtained by developing two technologies for patenting two higher-functional products that exploit the potential of the plant, microencapsulated lavender, respectively a technology for obtaining an ice cream product based on whey protein isolate and lavender extracts and a pricomigdale product.

Each chapter of the experimental study includes the following subchapters: *Introduction*, *Objectives*; *Materials and methods*, *Results and discussion*, *Conclusions and References*.

Chapter 7, Final Conclusions, presents the main conclusions resulting from the experiments performed.

The doctoral thesis comprises **147** pages, which includes **50** figures and **23** tables. The documentary study represents 25%, and the experimental part 75%.

Finally, the original contributions of the doctoral thesis and the dissemination of the results obtained in the researched field are highlighted. Thus, the research results were capitalized by the elaboration of 3 scientific articles, published or in the process of publication, 1 article in journals listed Web of Science (accepted for publication in the International Food Research Journal) and 2 articles published in journals indexed Web of Science (The Annals of the University Dunarea de Jos of Galati, Fascicle VI - Food Technology). The results were also presented at 5 international and national conferences.

Carrying out the experiments within the doctoral thesis was possible due to the infrastructure of the Center for Integrated Research, Expertise and Technology Transfer (BioAliment-TehnIA) (www.bioaliment.ugal.ro), within the Faculty of Food Science and Engineering, “Dunărea de Jos” University ” from Galaţi.

The thesis was written under the scientific coordination of the steering committee:

- **Prof. dr. eng. Nicoleta STĂNCIUC** – PhD supervisor

- **Prof. dr. eng. Petru ALEXE** – PhD co-supervisor

- **Prof. dr. eng. Gabriela RÂPEANU** – coordinator of spectrophotometric analysis and degradation kinetics of polyphenolic compounds studies

- **Prof. dr. eng. Iuliana APRODU** – coordinator of phytochemicals and mciroencapusulation studies.

- **Conf. dr. eng. Liliana MIHALCEA** – coordinator of extraction studies.

CHAPTER 4.
COMPARATIVE EVALUATION OF SOME EXTRACTION METHODS FROM THE PERSPECTIVE OF CONTENT IN BIOLOGICAL ACTIVE COMPOUNDS FROM LAVENDER FLOWERS

4.1. General aspects

Research on the extraction and purification of biologically active compounds from various natural resources has grown exponentially over the last decade. Efforts have been directed towards identifying new sources of compounds with antimicrobial, antioxidants, anti-inflammatory, antidiabetic, properties, etc from different sources.

Lavender is intensively studied for its content in essential oils and less for its content in chlorophylls, carotenoids and polyphenolic compounds. Therefore, the present study aimed to test different extraction methods (conventional with solvents, combined and extraction with supercritical fluids) from the perspective of comparative evaluation of the content in biologically active compounds.

4.2. The objectives of the study

The main objective of the study was the comparative testing of three main categories of techniques for the extraction of biologically active compounds from lavender flowers: **solid-liquid extraction with different organic solvents** (conventional extraction), **combined assisted extraction**, using two solvent extraction variants, assisted by ultrasound and microwave, and **extraction with supercritical fluids**, using supercritical CO_2.

The extracts obtained were compared in terms of the content of biologically active compounds in lavender flowers, such as the main categories of chlorophylls, total carotenoids, fatty acids, volatile compounds, total polyphenols, total flavonoids and, antioxidant capacity, from the perspective of obtaining extracts rich in biologically active compounds, obtained in conditions of high yields, friendly, with potential for applicability in the food industry. A secondary objective of the study was to evaluate the thermal stability of some biologically active compounds targeted in the selected extracts, to assess the potential application of selected extracts as such in various production processes.

4.3. Materials and methods

4.3.1. Materials

Lavender (*Lavandula angustifolia*) was purchased from the local Romanian market and then planted. At an optimum degree of maturity, the flowers were transported to the laboratory and dried for processing. Lavender has been maintained in optimal conditions, regularly cleaned and irrigated occasionally, as it does not require a large amount of water. No pesticides or fertilizers were used on this lavender production, from which samples were collected for analysis in the doctoral thesis.

Making the powder: After drying in non-denaturing conditions (maximum 40°C), in the dark, the lavender was crushed using a crushing device.

4.3.2. Reagents used

1. HPLC purity methanol
2. 5% NaNO2 solution (m / v)
3. 10% AlCl3 solution (m / v)
4. 1M NaOH solution
5. Folin-Ciocâlteu reagent
6. 20% Na2CO3 solution (m / v)

7. 0.025 M KCl solution, pH = 1.0
8. 0.3 M CH3COONa · 3H2O solution, pH = 4.5
9. 1N HCl solution
10. Formic acid solution 3 - 5%
11. 70% ethanol solution
12. DPPH reagent (2,2-diphenyl-1-picrylhydrazyl)
13. TROLOX solution
14. Gallic acid solution

4.3.3. Equipment used

Julabo 5 water thermostat enclosure, Germany
High precision analytical balance, XS 403 SM, METTLER TOLEDO, Switzerland
Ultracentrifuge with cooling, HETTICH Universal 320 R, Germany
pH - meter S 20 K, METTLER TOLEDO, Switzerland
UV-VIS Spectrophotometer Biochrom Libra S22, 2017
Orbital shaker with analog stirring and thermostatic frequency control, LAB COMPANION COMECTA S.A.
Concentrator under vacuum AVC 2-18, CHRIST
Ultrasonic bath
Microwave oven
Natex Prozesstechnologie GembH supercritical fluid extraction plant, Ternitz, Austria, model 10-023 / 2011).

4.4. Methods

4.4.1. Solid-liquid extraction with different organic solvents of biologically active compounds from lavender flowers

The extract was obtained from dried and grounded lavender flowers. The test sample (2 g) was homogenized with 10 mL of various organic solvents, as follows: acetone, diethyl ether, hexane, methanol. It was homogenized using an orbital shaker at room temperature for 4 hours to extract the polyphenolic compounds. The resulting supernatant was removed and the extraction process was repeated. The two resulting supernatants were mixed and centrifuged at 8500xg at 4°C for 10 minutes.

Extraction of biologically active compounds by combined methods

In this case, solvents and two extraction methods used extensively in the literature were used in combination, namely ultrasound and microwave-assisted extraction.

4.4.2. Combined hexane:acetone extraction – ultrasound

In this case, 2 g of dry and grounded powder was homogenized with a mixture of n-hexane:acetone 3:1, followed by ultrasonication at 40°C for 30 and 40 minutes. After ultrasonication, the mixture was centrifuged at 8500xg at 4°C for 10 minutes. The supernatant was collected and the extraction was repeated 4 times. The resulting supernatant was mixed and concentrated at 40°C under reduced pressure to dryness (AVC 2-18, CHRIST).

4.4.3. Combined ethanol - microwave extraction

The combined solvent-microwave extraction was performed in ethanol in a microwave oven as follows: one gram of dried and grounded lavender powder was weighed and homogenized with 10 mL of ethanol (70%). This mixture was subjected to a microwave treatment using the following parameters for the extraction of biologically active compounds from lavender:

- power of 210 W, for 30 seconds, at a temperature of 64ºC;
- power of 310 W, for 20 seconds, at a temperature of 63.5ºC;
- power of 420 W, for 15 seconds, at a temperature of 64.5ºC.

4.4.4. Supercritical CO_2 extraction

Lavender harvested in 2017 and dried at a temperature of 25°C and finely grounded before extraction at the colloidal mill (model UMC 12 - 2012, manufacturer Stephan, Germany).

Supercritical CO_2 extraction plant (Natex Prozesstechnologie GembH, Ternitz, Austria, model 10-023 / 2011) consisting of extractor E (2 liters usable volume) - figure 1 and two separation stages (each with 1.5 liters usable volume) has integrated ABB software (ABB - Mannheim, Germany). The extractor *E* and the two separators *S1* and *S2* respectively are equipped with a heating system to ensure the optimal temperature conditions necessary for the extraction process (**Figure 4.1.**).

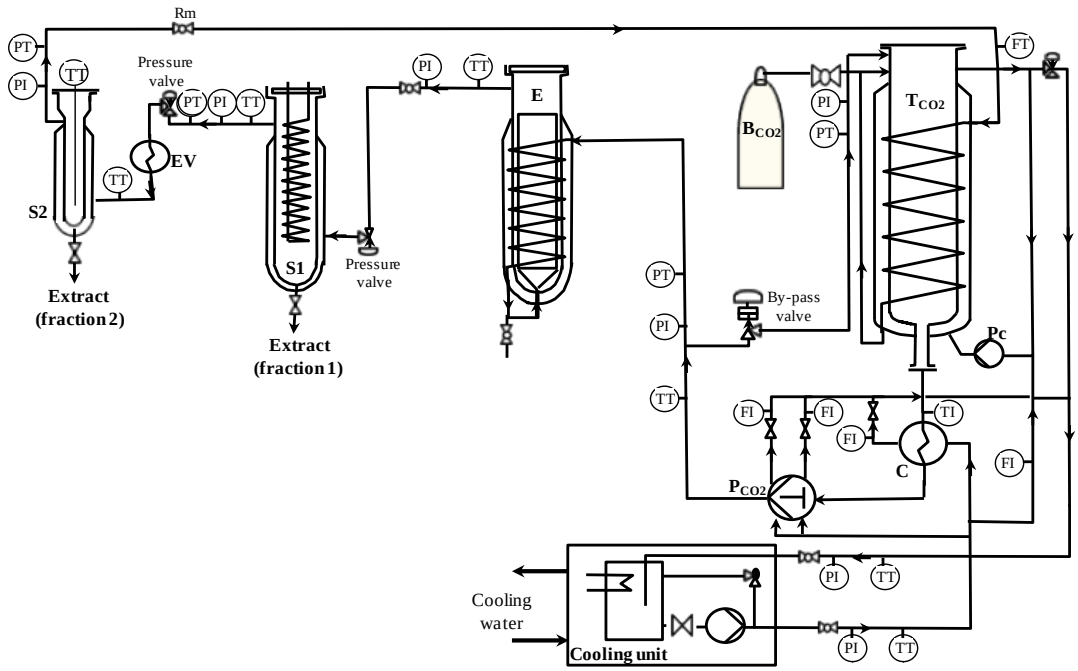


Figure 4.1. Outline principle of the supercritical fluid extraction plant

T_{CO2} - CO_2 tank; B_{CO2} - CO_2 cylinder; E - extractor; S1 - separator 1; S2 - separator 2; PCO_2 - process pump for CO2; Pc - circulation pump for CO_2: C - pre-cooler; EV - evaporator; Rm - manually operated valve; TT - temperature transmitter; TI - temperature indicator; PT - pressure transmitter; PI - pressure indicator; FI - flow indicator; FT - flow transmitter.

During the extraction process, CO_2 (99.99% purity provided by Messer S.A., Romania) circulates in the liquid state from the T_{CO2} tank in the pre-thinning system C to the absorption part of the P_{CO2} pump. The pre-cooling system C must achieve a temperature difference of 15°C relative to the "boiling" point of the CO_2, to avoid the cavitation phenomenon in the pump. Through the P_{CO2} pump, CO_2 coming from the working tank through the pre-cooling system is compressed to the extraction pressure (300 - 400 bar), thus exceeding the critical state (7.38 MPa). CO_2 circulates through the coil in the double jacket of the extractor E, then circulates from the bottom up through the plant material, being brought to the extraction temperature of 60°C, the temperature control being performed automatically through a temperature transmitter.

After leaving the extractor E, CO_2 reaches the pressure regulating valve being de-energized at a pressure of 150 bar, but above the critical state. Thus, it reaches the separator S1, provided with a heating coil to compensate for the temperature difference due to the isenthalpic expansion, respectively to maintain the temperature at 60°C to separate and accumulate the oil-resins in the liquid phase.

CO_2 passes from the separator S1 to the second pressure regulating valve, where the pressure is reduced to the values of the second separation stage 50 bar. In the second pressure

regulating valve, CO_2 passes from the phase of overcoming the critical state in two phases. Thus, after the second pressure relief valve, it is estimated that approximately 30% of CO_2 is in the liquid state. This liquid CO_2 is vaporized in the EV vaporizer. According to the vaporizer, CO_2 has only a gaseous state. Only low-viscosity oil-resins (essential oils, water, esters, etc.) reach the S2 separator.

CO_2 without extract passes through the filter to retain impurities, the mass flow is recorded with an automatically controlled flow meter, then circulates through the cooling coil in the double jacket of the working tank where CO_2 is liquefied and thus ensures the recirculation of the solvent.

Table 4.1. Discontinuous system extraction parameters (Nadalin et al., 2014)

C30 Extractor		Separator 1 – S1	Separator 2 – S2	CO_2 Flow, kg/h	Gravimetric yield,%
I	**300 bar/60°C/1 h**	**150 bar/60°C 1 h and 2 h**	**50 bar/25°C 1 h and 2 h**	**20,27**	**4,67**
	300 bar/60°C/2h			20,10	2,05
II	400 bar/60°C/1 h			17,78	1,94
	400 bar/60°C/2h			unfinished	unfinished
I+II	300 bar/60°C/3h	150 bar/60°C	50 bar/25°C	20,16	6,59
	400 bar/60°C/3h	150 bar/60°C	50 bar/25°C	unfinished	unfinished

4.4.5 Determination of carotenoid and chlorophyll contents from the lavender extracts

The resulting extracts are diluted in the extraction solvent and the absorbance is read at 470 nm for total carotenoids, 663 nm and 645 nm for chlorophylls. Arnon's (1949) 1-4 relations were used to calculate the concentration of carotenoids and chlorophylls:

$$\text{Chl } a = 0{,}0127 \times A_{663} - 0{,}00269 \times A_{645} \quad (1)$$

$$\text{Chl } b = 0{,}0229 \times A_{645} - 0{,}00468 \times A_{663} \quad (2)$$

$$\text{tot Chl} = 0{,}0202 \times A_{645} + 0{,}00802 \times A_{663} \quad (3)$$

$$\text{Total carotenoids} = (1000 \times A_{470} - 1{,}82 \times A_{663} - 85{,}02 \times A_{645})/198 \quad (4)$$

4.4.6. The total polyphenol content was determined using the Folin-Ciocâlteu colorimetric method (Gutfinger, 1981). 0.10 mL of sample was used, plus 1 mL of ultrapure water and 1 mL of Folin-Ciocâlteu reagent. The mixture was stirred vigorously and allowed to stand for 10 minutes, after which 0.8 mL of 6% sodium carbonate was added. The mixture is incubated for 60 minutes at room temperature. The absorbance of the supernatant is read on the spectrophotometer at a wavelength λ=765 nm. Gallic acid was used as a standard for the calibration curve, and the results obtained were expressed as mg gallic acid/mL extract (mg GA/mL) (**Figure 4.2**.).

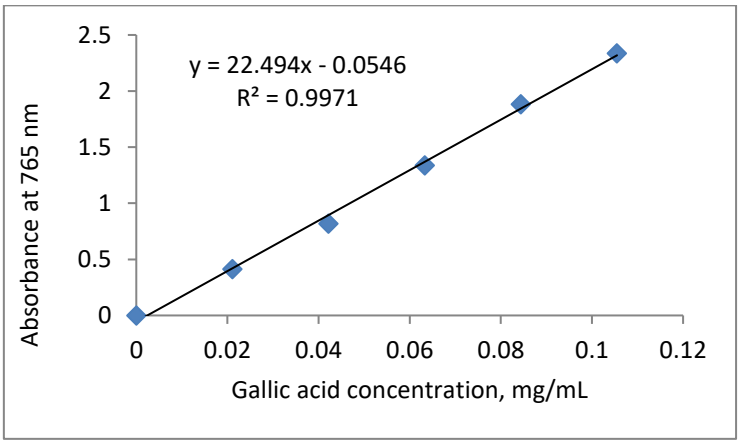


Figure 4.2. The calibration curve with gallic acid, mg GA/mL

4.4.7. The total flavonoid content was determined by using the method described above by Dewanto et al., (2002). A volume of 0.250 mL of diluted extract (1:10) is mixed with 1.25 mL of distilled water and 0.075 mL of 5% sodium nitrite. The mixture was allowed to react for 5 minutes, after which 0.15 mL of 10% aluminum chloride was added. The mixture is allowed to stand for 6 minutes to react. Then 0.5 mL of 1M sodium hydroxide is added. The absorbance of the resulting mixture is read immediately at a wavelength of 510 nm against a blank. The total flavonoid content is determined using the standard catechin curve and is expressed as mg catechin equivalents/g lyophilized sample ± SD (mg CE/g) (**Figure 4.3.**).

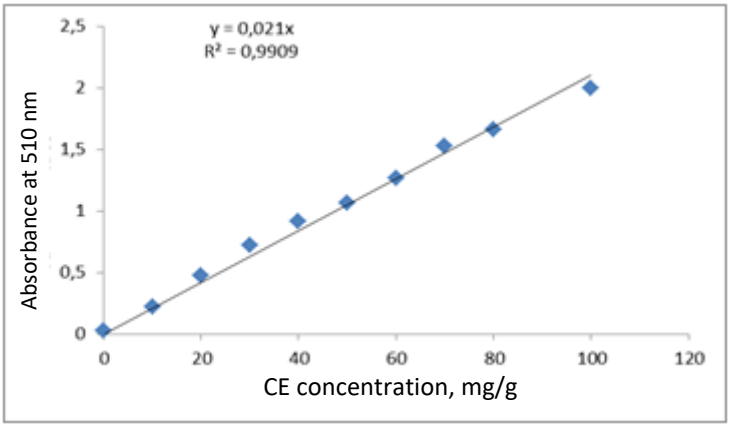


Figure 4.3. The calibration curve for the determination of the total flavonoid content, mg CE/g

4.4.8. Determination of antioxidant activity was performed using the DPPH method (2,2-diphenyl-1-picrylhydrazyl)

Preparation of DPPH stock solutions: Weigh 25 mg of DPPH and makeup to the mark with methanol in a 100 mL volumetric flask. The stock solution of DPPH is diluted 1:10 with methanol. (OBS: DPPH is insoluble in water so the DPPH solution must be freshly prepared before each experiment.)

Preparation of TROLOX stock solution: Weigh 25 mg of TROLOX and makeup to the mark with 10% ethanol in a 50 mL volumetric flask. The TROLOX concentrations used to achieve the standard curve are: 1.25; 2.5; 5; 10; 15; 20; 25 µM.

Reaction volumes in the test tube:

- more than 100 µL TROLOX of each concentration is added 3,9 mL of DPPH solution
- more than 100 µL / mL of plant extract, 3.9 mL of DPPH solution is added

- for control - over 100 µL 10% ethanol solution add 3,9 mL DPPH
- read the absorbance at 515 nm after one and a half hours of rest in the dark.

Determination of antioxidant activity: For the expression of antioxidant activity in mMol Trolox/ml the standard curve from **Figure 4.4** was used.

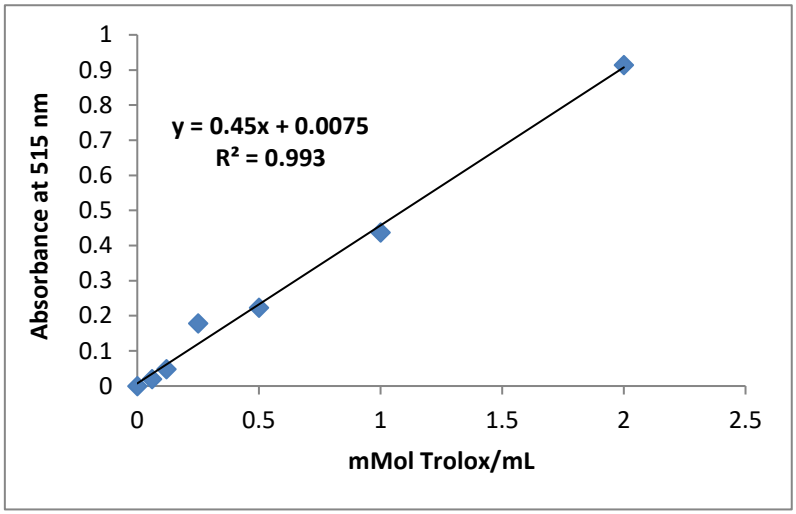


Figure 4.4. The calibration curve used to determine the antioxidant activity, mMol Trolox/mL

4.4.9. Absorption spectra

All extracts were tested by UV-Vis spectroscopy to verify the wavelengths at which the maximum absorption is recorded. The extracts were dissolved in solvents with which extraction was performed, a 1:3 dilution was performed, and the scan was performed in the spectral range 200-900 nm using the Cintra 202 spectrophotometer.

4.4.10. Determination of total fatty acids in lipids

To determine the fatty acids in the total lipids, 3 samples were selected, namely: the extract obtained by extraction with hexane and acetone followed by ultrasound for 20 and 40 minutes and the extract obtained by extraction with supercritical fluids, fraction S40.

Step 1. Trans-esterification of total lipids is a derivatization process, which consists of the cleavage of fatty acids from their bounded forms of triglycerides and other classes of lipids, subsequently binding to a methanol residue.

For the trans-esterification of fatty acids, the procedure described by Fărcaş et al., (2015) was applied. Thus, fatty acid methyl esters (FAME) from total lipids were derived by transesterification with catalyst acid using 1% sulfuric acid in methanol (Christie, 1989). Lipids (1 mg) were resuspended in 1 ml of toluene in a Pyrex tube fitted with a condenser. Two milliliters of methanolic H_2SO_4 (1% v / v) were added and the mixture was refluxed for 2 hours at 80°C. Water (5 ml) containing potassium chloride (5%) was added and the transmethylated fatty acids were extracted with hexane (2x5 ml) using Pasteur pipettes to separate the layers. The hexane layer was washed with water (4 ml) containing 2% potassium bicarbonate and dried over anhydrous sodium sulfate. Finally, the solution was filtered and the solvent was removed under reduced pressure in a rotary film evaporator.

Stage 2. Fatty acid analysis. Methyl esters (FAMEs) of fatty acids obtained from transesterification were analyzed with a gas chromatograph (GC) coupled with a mass spectrometer (MS), PerkinElmer Clarus 600 T GC-MS (PerkinElmer, Inc., Shelton, CT, USA) (Dulf et al., 2016). Column used: Supelcowax 10 (60 m × 0.25 mm i.d., 0.25 µm film thickness; Supelco Inc., Bellefonte, PA, USA). Temperature program: initial temperature, 140°C; then with

7°C/min it reaches 220°C, the temperature that finally remains constant for 23 min. Injector temperature: 210°C; split 1:24. Injected volume: 0.5 µL. Eluent: He, 0.8 mL/min. MS conditions: ionization energy 70 eV; trap current 100 µA; source temperature 150°C. Mass scanning interval m/z: 22–395, with 0.14 scan/s (0.02 s between scans). Identification of fatty acids: 1) based on retention times - comparison with retention times of fatty acids in a mixture of standards (37 components FAME Mix, SUPELCO # 47885-U); 2) comparison of mass spectra with those in the MS database (NIST MS Search 2.0).
Fatty acid concentrations were expressed in % of the area of total fatty acid areas.

4.4.11. Determination of volatile compounds in extracts by the ITEX / GC-MS method

To determine the fatty acids in the total lipids, 3 samples were selected, namely: the extract obtained by extraction with hexane and acetone followed by ultrasound for 20 and 40 minutes and the extract obtained by extraction with supercritical fluids, fraction S40.

The extraction of volatile compounds was performed by in-tube technique, according to a method described by Socaci et al. (2013), using 0.1g of S45 and S40 extract and 0.05 g of the extract obtained by ultrasound. The analysis of volatile compounds was performed using GC-MS QP-2010 gas chromatography equipment (Shimadzu Scientific Instruments, Kyoto, Japan) coupled with a mass spectrometer. The volatile compounds were separated using a Zebron ZB-5ms capillary column measuring 25 m × 0.25 mm and film thickness 0.25 µm. helium 1 mL/min and a ratio of 1:100 was used. The column oven temperature program was 50°C (maintained for 2 min) at 160°C at a rate of 4°C/min at 250°C with a growth rate of 15°C/min and maintained for 10 minutes. The injector, ion source, and interface temperature were set at 250°C. The MS detection used for this qualitative analysis was performed on a quadropolar mass spectrometer operating in full scan impact (40-500 m/z) at a ionization energy of 70 eV.

The volatile compounds were provisionally identified by first comparing the obtained mass spectra of each chromatographic peak with the NIST27 and NIST147 mass spectrum libraries (given a minimum similarity of 85%) and then whenever possible by comparison with indices of retention extracted from www.pherobase.com or www.flavornet.org (for columns with a stationary phase similar to the ZB-5ms column).

This technique provides a qualitative assessment of volatile compounds so that the relative percentage of each compound was estimated as a fraction of its integrated ionic area in the area of total ion chromatograms (ICT) (100%).

4.4.12. Heat treatment

Heat treatment was applied to verify the stability of the thermal treatment of the extracts. For thermal degradation kinetics experiments, the extract obtained by the combined extraction of hexane:acetone 3:1 and ultrasound for 30 minutes was selected. Thus, the extract with a concentration of 20 mg/ml was dissolved in ethanol of which 0.4 mL were subjected to heat treatment at different temperatures between 75 and 100°C, for 0-40 minutes. After the thermal treatment, the samples were placed in ice water to prevent the spread of degradation of biologically active compounds.

4.4.13. Kinetics of distortion reactions

The kinetic fractional conversion model described by equation 5 was used to describe the effect of the thermal treatment on biologically active compounds in the lavender extract. This kinetic model can be applied in the case of thermal degradation processes that take into account a positive value of the studied property after a prolonged duration of the heat treatment.

$$X_t = X_\infty + (X_\infty - X_I)\ exp\ (-k_I)$$

in which:

X_∞ - the equilibrium value for an infinite duration of the heat treatment;

X_i - the response value for native samples.

Equation 5 is valid in the temperature range in which the value X_∞ does not change with the change of the duration of exposure to different temperatures.

The kinetics of degradation of antioxidant activity was described using the first-order kinetic model, given by equation 6:

$$-\frac{dC}{dt} = k \cdot C$$

where *k* is the degradation rate constant (1/min). By integration, equation (6) becomes:

$$-\ln\left(\frac{C_0}{C}\right) = k \cdot t$$

According to the mathematical expression (7), ln C vs. time will be a linear function, the slope being *k* the degradation rate constant, according to **Figure 4.5**.

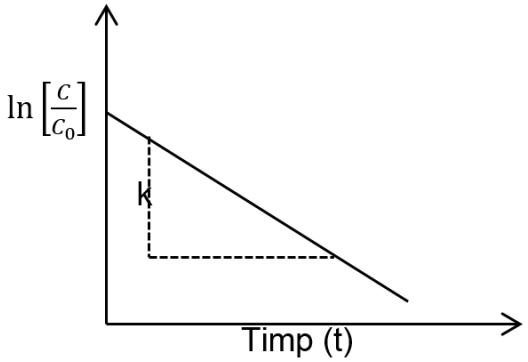


Figure 4.5. Determination of the first order reaction rate constant

The half-life ($t_{1/2}$) is given by the equations:

$$k \cdot t_{1/2} = -\ln(1/2) \quad (8)$$

$$t_{1/2} = \frac{\ln 2}{k}$$

The above mathematical expressions indicate that the half-life and constant rate of first-order reactions are independent of the initial concentration.

The transition from the native state to the degraded state requires a certain amount of energy to form an activated intermediate complex. This energy barrier has been called the reaction activation energy.

To calculate the activation energy it is necessary to determine the denaturation rate constants, which in this case were estimated using the kinetic model of fractional conversion, by linear regression using SAS Inc. software. (1999-2003).

The temperature dependence of the reaction rate findings, k (1/min) was described by the Arrhenius equation (equation 10.):

$$k = k_{ref} \exp\left(-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right)$$

in which:

T and T_{ref} represent the absolute temperature, respectively the reference temperature (K);

k_{ref} represents the constant of the reaction rate at the reference temperature;
It represents the activation energy (kJ/mol);
R represents the universal gas constant (8,314 J/(mol • K).
The activation energy was calculated using the linear regression of the line ln *k* as a function of 1/T (K).

4.5. Statistical analysis of data

All experiments were performed in triplicate. The results were expressed in terms of mean values. Statistical data analysis was performed using the Microsoft Excel software data analysis toolkit. The coefficient of determination (R^2) and the mean square error (*MSE*) was used as criteria for the fit of the model.

4.6. RESULTS AND DISCUSSIONS

In the studies performed, different extraction methods were tested and comparatively analyzed the content of chlorophylls, carotenoids, the content of total polyphenols (TPC), total flavonoids (TFC), fatty acids, volatile compounds, and antioxidant activity.

4.6.1. Screening of the global phytochemical profile of lavender flower extracts obtained by solid-liquid extraction with different solvents by spectrophotometric methods

For extraction with organic solvents, the following were used: acetone, diethyl ether, ethanol, n-hexane, and methanol. The results obtained by extraction with organic solvents are presented in **Table 4.2.**

Table 4.2. The content of biologically active compounds in the extracts obtained by solvent extraction

Compound	Acetone	Diethyl ether	Hexane	Methanol
TPC, mg AG/mL	0,06±0,01	0,05±0,01	0,008±0,001	0,05±0,01
TFC, mg EC/mL	84,74±2,31	52,71±3,45	10,61±0,89	121,85±2,54
Chlorophyll *a,* µg/mL	36,64±2,47	14,22±1,47	6,50±0,78	23,38±2,41
Chlorophyll *b,* µg/mL	36,08±2,74	4,93±1,75	2,51±0,96	18,27±1,07
Total carotenoids, µg/mL	14,43±1,24	4,90±1,45	2,05±0,71	11,18±0,45
Antioxidant activity, mMol Trolox/mL	0,68±0,17	0,46±0,12	0,26±0,07	1,28±0,23

From **Table 4.2**, a significantly different distribution of the extraction degree of the biologically active compounds can be observed depending on the type of solvent. Thus, none of the solvents used is distinguished by a high degree of extraction for total polyphenolic compounds (TPC). Total flavonoids were extracted in the highest concentration in methanol (121.85 ± 2.54 mg CE/mL), followed by acetone (84.74 ± 2.31 mg CE/mL) and diethyl ether (52.71 ± 3.45 mg CE/mL), with a lower hexane concentration of 10.61 ± 0.89 mg CE/mL.

The most effective solvent for the extraction of chlorophyll a, b and total carotenoids was acetone, with 36.64 ± 2.47 µg/mL, 36.08 ± 2.74 µg/mL and 14.43 ± 1.24 µg/mL, respectively followed by methanol with 23.38 ± 2.41 µg/mL chlorophyll *a*, 18.27 ± 1.07 µg/mL chlorophyll *b* and 11.18 ± 0.45 µg/mL.

The extract with the strongest antioxidant activity was the one obtained with methanol, with 1.28 ± 0.23 mMol Trolox/mL, which is probably given by the higher TFC content.

4.6.2. Comparative screening of the phytochemical profile of lavender flower extracts in ultrasound-assisted extraction

For ultrasound extraction, different types of solvent combinations were used, namely n-hexane-acetone in a ratio of 1:1, 3:1, and ethanol, for 30 minutes at 40°C.

The results obtained by combined extraction are presented in **Table 4.3.**

Table 4.3. Global phytochemical profile of lavender flower extracts in ultrasound-assisted extraction

Compound	Hexane:Acetone 1:1	Hexane:Acetone 3:1	Ethanol (70%)
TPC, mg GA/g SU	nd	5,41±0,08	0,08±0,007
TFC, mg CE/ g SU	nd	4,20±0,85	7,40±0,40
Chlorophyll *a,* µg/ g SU	0,53±0,02	4,50±1,16	1,04±0,02
Chlorophyll *b,* µg/ g SU	0,56±0,07	6,40±0,39	1,32±0,15
Total carotenoids, µg/g SU	0,28±0,06	2,71±0,28	0,84±0,06
Antioxidant activity, mMol Trolox/g SU	2,68±0,37	4,46±0,98	1,32±0,02

From **Table 4.3** it can be seen that the extraction with a mixture of hexane and acetone 3:1 allowed to obtain the highest content in chlorophyll, polyphenol compounds, flavonoids, and antioxidant activity.

4.6.3. Comparative screening of the phytochemical profile of lavender flower extracts in microwave-assisted extraction

The technique was also applied for the extraction of biologically active compounds from lavender flowers, at different parameters (**Table 4.4**.).

Table 4.4. The content of biologically active compounds in extracts obtained by microwave-assisted extraction

Compound	MW 15 sec (420 W, 64,5ºC)	MW 20 sec (310 W, 63,5ºC)	MW 30 sec (210 W, 64ºC)
TPC, mg GA/mL	3,56±0,01	0,26±0,01	0,26±0,01
TFC, mg CE/mL	1,10±0,12	0,11±0,04	0,10±0,02
Chlorophyll *a,* µg/mL	4,59±0,09	4,16±0,01	3,05±0,02
Chlorophyll *b,* µg/mL	2,39±0,02	2,22±0,04	1,34±0,04
Total carotenoids, µg/mL	2,48±0,06	2,95±0,26	1,82±0,01
Antioxidant activity, mMol Trolox/mL	1,13±0,04	0,52±0,01	0,32±0,01

A high degree of extraction can be observed for the polyphenolic compounds in the first variant (extraction time 15 sec, power 420 W, temperature 64.5ºC). For chlorophylls and carotenoid compounds, there are significant differences between the first two variants and variant three, the decrease in potency with the increase of the extraction time did not lead to the increase of the concentration of biologically active compounds in the extracts.

4.6.4. Comparative screening of the phytochemical profile of lavender flower extracts at extraction with supercritical fluids

Extraction with supercritical CO_2 (SCE) is performed at low temperatures, normally below 60°C, which protects the thermolabile compounds in the extract.

In **Table 4.5.** the values obtained for the biologically active compounds in the S45 fraction are obtained, obtained after 1 hour and 3 hours of extraction at 300 bar, respectively.

Table 4.5. Content of biologically active compounds in extracts obtained by extraction with supercritical CO_2, fraction S45

Compound	1 h of extraction 300 bar	3 h of extraction 300 bar
TPC, mg GA/g SU	67,56±1,42	80,95±0,67
TFC, mg CE/g SU	15,67±2,98	34,02±0,51
Chlorophyll *a,* mg/g extract SU	6,78±0,98	5,22±0,12
Chlorophyll *b,* mg/g extract SU	3,45±0,78	2,95±0,16
Total carotenoids, mg/g SU	18,24±0,04	15,67±1,45
Antioxidant activity, mMol Trolox/g SU	28,89±2,34	34,02±0,51

From **Table 5.5**., it can be seen that the extraction with supercritical CO_2 led to the production of oleoresins with different phytochemical profile, noting a high concentration of flavonoids after 3 hours of extraction, while the extraction for 1 hour resulted in the extraction of some higher concentrations of chlorophylls and total carotenoids.

4.6.5. Comparative analysis of the overall phytochemical profile of lavender flower extracts selected for further experiments

From the experiments performed, it was observed that the combined extraction n-hexane: acetone 3:1 ratio with ultrasound, under extraction conditions 30 min at 40°C (E1), extraction in ethanol 70% in combination with ultrasound (E2), under extraction for 30 min at 40°C, and the extraction with supercritical CO_2 (E40 and E45) allowed control of the phytochemical profile, with obtaining high concentrations of hydrophobic compounds in E1, hydrophilic in E2 and both categories in E40 and E45 (**Table 4.6**.).

Table 4.6. Phytochemical profile of lavender flower extracts

Phytochemical compounds	E1	E2	E40+E45
Total chlorophylls, mg/g SU	3,19±0,12	nd*	13,13±0,45
Chlorophyll *a,* mg/g SU	2,10±0.08	nd	8,22±0,12
Chlorophyll *b,* mg/g SU	0,69±0,02	nd	4,95±0,16
Total carotenoids, mg/g SU	0,42±0,01	nd	28,24±0,04
Total polyphenols, mg AG/g SU	19,45±0,45	180,80±2,91	89,95±0,67
Total flavonoids, mg EC/g SU	23,61±0,75	80,77±2,53	67,02±0,51
Antioxidant activity, mMol/g SU	17,54±3,98	108,33±0,86	102,36±3,56

In **table 4.6**. the global phytochemical profile of the three extracts obtained by different extraction techniques is presented. It can be seen that the ultrasound-assisted ethanol extraction (E2) allowed to obtain extracts with a high content of polyphenols and flavonoids, which led to a remarkable antioxidant activity of 108.33 ± 0.86 mMol Trolox/g S.U. Assisted extraction of supercritical fluids was superior to ultrasonic extraction in terms of extraction of hydrophobic compounds, allowing to obtain an extract with a significantly higher content of chlorophylls, total carotenoids, and flavonoids.

4.6.6. Comparative evaluation of the individual phytochemical profile of lavender flower extracts obtained by solvent extraction, ultrasonic-assisted extraction, and extraction with supercritical fluids by chromatographic methods

The chromatographic profile of the extract obtained from lavender flowers revealed the presence of eleven polyphenolic compounds, but the concentration of polyphenolic acids was predominant (**Figure 4.6**). The highest concentrations were recorded by vanillic acid and cinnamic acid. At the same time, ferulic acid and rosemary acid were found in high concentrations. Quercetin revealed a relatively low concentration in the lavender flower extract along with apigenin.

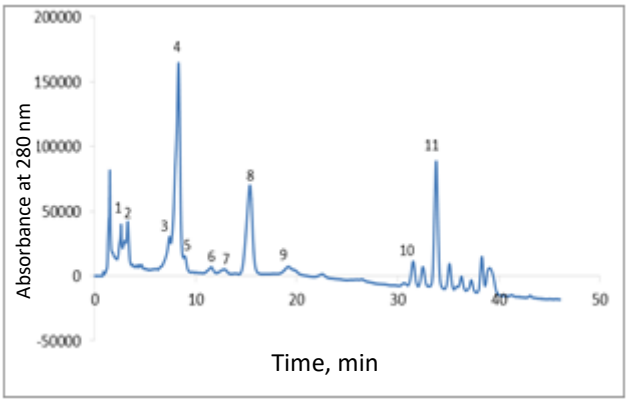


Figure 4.6. Chromatographic profile of lavender flower extract - Peak (1) - gallic acid; Peak (2) -3,4-hydroxybenzoic acid; Peak (3) - chlorogenic acid; Peak (4) - vanillic acid; Peak (5) - caffeic acid; Peak (6) - p-coumaric acid; Peak (7) - quercetină; Peak (8) - ferulic acid; Peak (9) - apigenină; Peak (10) - rosmarinic acid; Peak (11) - cinnamic acid.

For the extracts obtained, high-performance liquid chromatography and gas chromatography techniques were used to determine their phytochemical profile, as well as the antimicrobial character of the essential oil extracted from *L. angustifolia*. The chromatographic profile highlighted the presence of eleven different polyphenolic compounds, in the highest concentrations being found caffeic acid, rosmarinic acid, and 4-hydroxybenzoic acids.

The hydrophobic extracts obtained by assisted ultrasonic extraction (UAE) and the two extracts obtained by assisted extraction of supercritical fluids were analyzed comparatively in terms of the content of volatile compounds, the results being presented in **Table 4.5**.

Table 4.5. Profile of flavor compounds in lavender extracts

Compound	% of the total peak area		
	SCE (S40)	SCE (S45)	E1
2-Pentanone, 4-hydroxy-4-methyl-	-	-	9,24±0,89
2(5H)-Furanone, 5.5-dimethyl-	-	-	0,37±0,10
1-Hexanol	0,05±0,01	0,08±0,01	-
Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	0,03±0,01	0,13±0,02	-
Origanene	0,06±0,01	0,15±0,02	-
α-Pinene	0,30±0,02	0,77±0,04	-
3-Heptanone, 6-methyl-	0,11±0,05	0,11±0,01	-
Heptane, 2.5.5-trimethyl-			0,24±0,07
Camphene	0,77±0,09	1,97±0,11	-
n.i	0,17±0,02	0,86±0,11	-
2-Thujene	0,04±0,01	0,08±0,01	-
4(10)-Thujene (Sabinen)	0,10±0,01	0,47±0,02	-
β-Pinene	0,25±0,05	0,41±0,03	-
1-Octen-3-ol	0,20±0,05	0,17±0,01	-
3-Octanone	1,30±0,07	1,09±0,05	0,30±0,10
β-Myrcene	1,34±0,10	1,32±0,11	2,83±0,45
Butanoic acid, butyl ester	0,15±0,01	0,18±0,01	-
n.i	0,09±0,01	0,10±0,01	-
Acetic acid, hexyl ester (1-Hexyl acetate)	0,85±0,08	0,80±0,11	-
α.-Terpinene	0,08±0,01	0,11±0,01	-
p-Cymene	0,83±0,10	1,16±0,04	0,23±0,01

Limonene	3,48±0,54	4,87±0,58	0,97±0,15
Eucalyptol	16,72±0,98	17,62±1,20	2,10±0,58
β-trans-Ocimene	4,02±0,45	5,13±0,74	1,56±0,62
β-cis-Ocimene	1,58±0,23	1,60±0,12	1,48±0,23
γ-Terpinene	0,16±0,01	0,36±0,01	-
6-Methyl-2-(2-oxiranyl)-5-hepten-2-ol	3,98±0,23	3,45±0,41	6,74±1,02
Linalool oxide (fr.1)	3,04±0,25	2,66±0,52	5,76±0,28
β-Linalool	19,94±1,01	20,44±1,20	15,18±1,20
Octen-1-ol, acetate	1,01±0,05	0,97±0,11	1,85±0,57
2,4,6-Octatriene, 2,6-dimethyl-, (E, Z)-	0,71±0,11	0,58±0,15	0,18±0,02
Camphor	10,3±0,74	7,82±1,21	4,39±0,23
Lavandulol	0,35±0,01	0,33±0,08	-
Borneol	1,76±0,05	2,38±0,04	3,16±0,78
1-Terpinen-4-ol	1,41±0,47	1,42±0,09	1,46±0,06
n.i			1,30±0,24
Butanoic acid, hexyl ester	-	0,37	-
n-Hexyl butanoate	0,44±0,04	-	-
α-Terpineol	-	0,20±0,01	-
Isobornyl formate	-	0,11±0,01	0,22±0,21
Linalool acetate	18,08±0,87	15,28±1,25	25,86±1,24
Lavandulyl acetate	2,63±0,56	2,13±0,52	5,62±0,78
n.i	0,15±0,08	0,08±0,01	1,06±0,06
8-Hydroxylinalool	-	-	2,54±0,24
Nerol	0,14±0,09	0,11±0,01	0,53±0,08
u.i.	0,38±0,11	0,24±0,05	1,17±0,09
Caryophyllene	1,73±0,12	1,20±0,02	1,86±0,08
α-Bergamotene	0,08±0,01	0,05±0,01	-
β-Farnesene	0,62±0,02	0,49±0,01	0,43±0,09
γ-Muurolene	0,09±0,01	-	-
n.i	0,15±0,04	0,17±0,01	0,98±0,14
Acetic acid. hexyl ester	-	-	0,39±0,07

GC-MS analysis of the three types of extracts allowed the identification of 44 and 26 volatile compounds in CO_2 and E1 extracts, respectively.

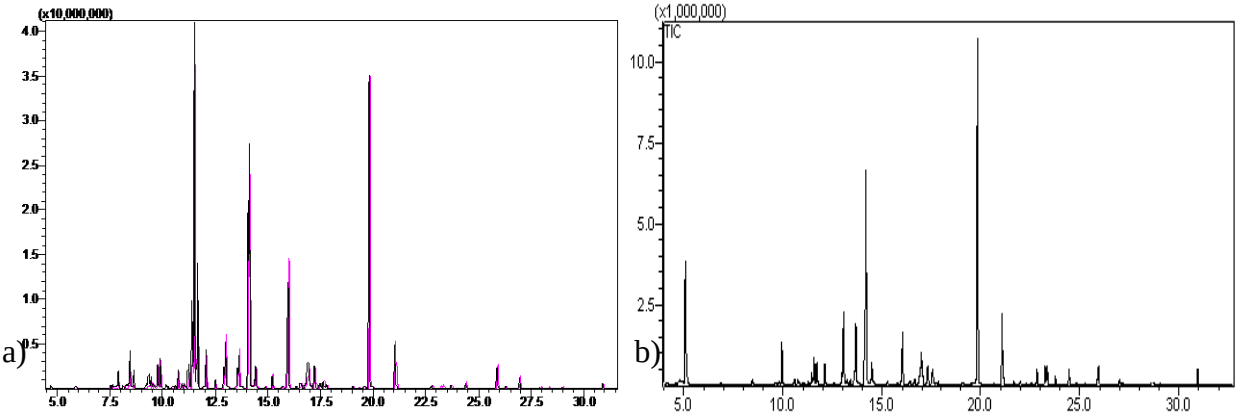


Figure 4.7. GC-MS chromatograms for the identification and quantification of volatile compounds in extracts obtained by extraction with supercritical fluids (a) (S45 - black, S40 - pink) and ultrasonic-assisted extraction (b)

In the CO_2 extract, 7 main compounds (*β*-linalool, eucalyptol, linalool acetate, *β*-trans-ocimene, and limonene) were identified (**Figure 4.7**), while in the E1 extract 7 main compounds were also identified, consisting of linalool acetate, *β*-linalool, 6-methyl-2- (2-oxirane) -5-heptane-2-oil, linalool oxide, lavandulyl acetate, and camphor.

The extract obtained by extraction with supercritical fluids is richer in volatile compounds. For example, fractions S40 and S45 contain *α*-pinene, 3-heptanone, 6-methyl-, camphene, sabinen, *β*-pinene, 1-octen-3-ol, etc., while extract E1 contains a double amount of *β*- myrcene, 6-Methyl-2- (2-oxiranyl) -5-hepten-2-ol, linalool oxide (fr.1) and octen-1-ol, acetate and a significantly lower amount of camphor. Significant differences were also identified in the content of limonene, eucalyptol, *β*-trans-ocimen, *β*-Linalool, with a higher proportion in the S45 fraction and lower in the E1 extract. Regarding the linalool acetate content, E1 extract showed the highest content (25.86 ± 1.24%), followed by the fraction S40 (18.08 ± 0.87%) and S45 (15.28 ± 1.25%).

In **Figure 4.8.** the chromatogram for identification and quantification of the fatty acid profile is presented, which shows 7 peaks in the CO_2 extract (**Figure 4.8. a**) and 8 peaks in **Figure 4.8. b**. The type and proportion of fatty acids are shown in **Table 4.7**.

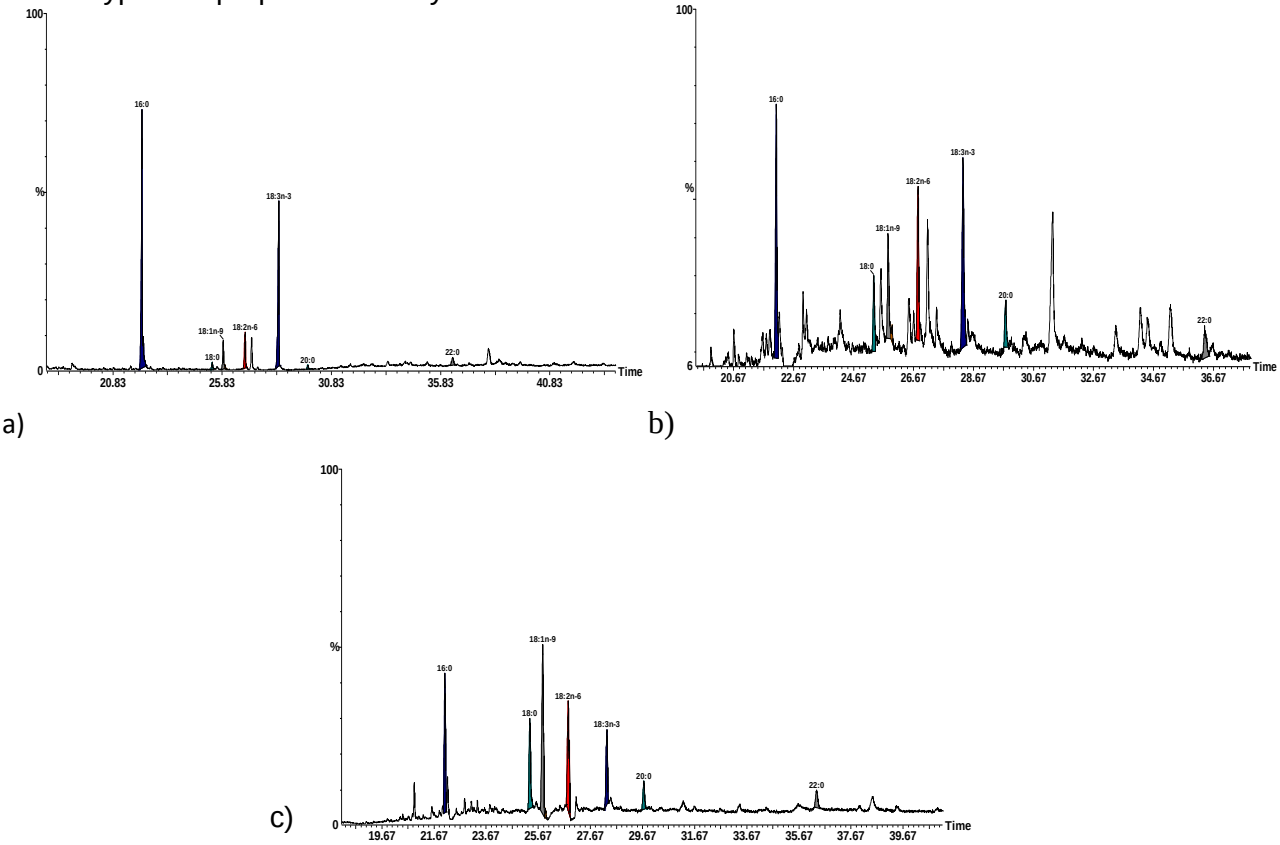


Figure 4.8. GC-MS chromatograms for identification and quantification of the total fatty acid profile of lavender extract CO_2 fractions S40 (a) and S45 (b) and extract E1 (c)

Table 4.7. Fatty acid composition (% of total fatty acids) in lavender flower extracts

Fatty acid	E40	E45	E1
α-linolenic acid (18:3n-3)	31,67±1,58	25,61±1,25	11,82±0,55
Palmitic acid (16:0)	49,80±2,31	25,10±0,87	18,71±0,80
Linoleic acid (18:2n-6)	7,66±1,02	16,81±1,05	19,66±0,85
Oleic acid (18:1n-9)	5,37±0,89	11,35±0,99	27,21±1,10
Stearic acid (18:0)	1,55±0,05	8,39±0,11	13,73±0,62
Behenic acid (22:0)	2,52±0,12	5,79±0,35	3,58±0,20
Arachidic acid (20:0)	0,86±0,16	5,63±0,52	4,62±0,25
Vaccenic acid (18:1n-7)	0,59±0,01	1,31±0,07	0,67±0,08

AGSs	54,73±1,25	44,91±1,53	40,64±1,35
AGMSs	5,93±1,20	12,66±1,28	27,88±1,20
AGPNs	39,33±1,48	42,43±1,87	31,48±1,35
n-3 AGPN	31,67±1,59	25,61±0,85	11,82±0,55
n-6 AGPNs	7,66±1,58	16,81±0,98	19,66±0,90
n-6/n-3	0,24	0,66	1,66
AGPN/AGM	0,72	0,94	0,77

The profile of fatty acids depends on the type of extraction. From **table 4.7**. it can be seen that the S40 fraction showed the highest proportion of α-linolenic acid (31.67 ± 1.58%) and palmitic acid (49.80 ± 2.31%), while the E1 extract showed a proportion higher than linoleic acid (19.66 ± 0.85%), oleic acid (27.21 ± 1.10%) and stearic acid (13.73 ± 0.62%).

It can be seen that the extracts showed a high concentration of polyunsaturated acids, with the highest value in the S45 fraction of 42.43% and a lower value of monounsaturated fatty acids, with the highest value recorded in extract E1 (27.88 ± 1.20%) and the lowest in the S40 fraction (5.93 ± 1.20%). N-3 acids had the highest contribution in the S40 fraction (31.67 ± 1.59%) compared to n-6 acids (7.66 ± 1.58%) and the lowest in E1, of 11.82 ± 0.55% and 19.66 ± 0.90%, respectively.

4.7. Evaluation of the stability of some biologically active compounds and the antioxidant activity at the heat treatment

4.7.1 Kinetics of chlorophyll degradation

The fractional conversion kinetic model given by equation 5 was used to describe the effect of heat treatment on biologically active compounds in the lavender extract.

The kinetics of thermal degradation was studied in the temperature range 75°-100°C. From **Figure 4.13**. it can be observed that the thermal degradation of chlorophyll followed a kinetic model of fractional conversion.

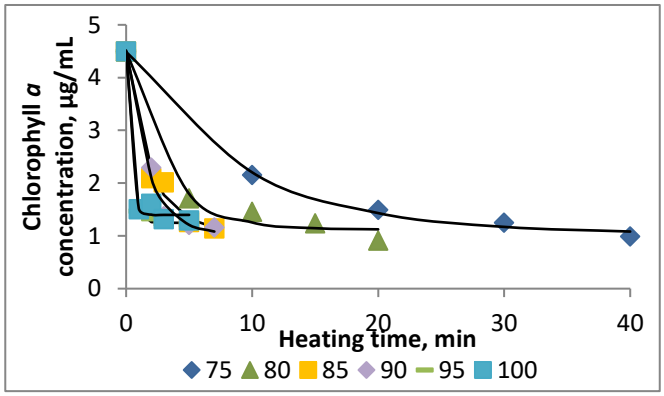


Figure 4.9. Fractional conversion kinetic model describing the thermal degradation of chlorophyll a in lavender extract

In **Figure 4.10**. the thermal degradation curves of chlorophyll *b* in the lavender extract are shown.

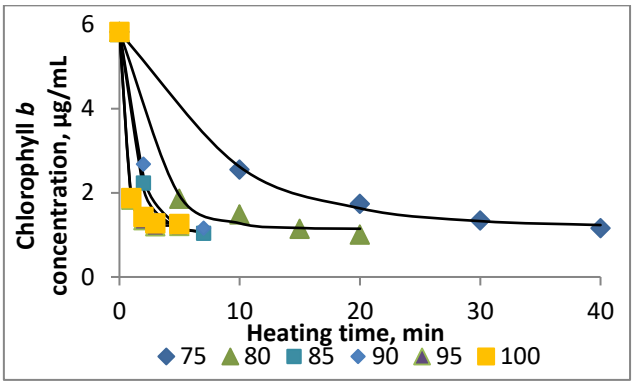


Figure 4.10. Fractional conversion kinetic model describing the thermal degradation of chlorophyll b in lavender extract

The degradation rate constants estimated by the fractional conversion kinetic model are shown in **Table 4.8**.

Table 4.8. Kinetic parameters estimated by the kinetic model of fractional conversion to thermal degradation of chlorophylls *a* and *b* in lavender extract

Compound	Temperature, °C	k (x10^{-2}), 1/min	C_∞	E_a, kJ/Mol
Chlorophyll *a*	75	10,83±1,15	1,03±0,09	**139,69±15,22**
	80	32,05±8,32	1,11±0,05	
	85	52,24±12,10	1,07±0,03	
	90	61,07±15,90	1,02±0,02	
	95	229,98±5,97	1,24±0,03	
	100	297,81±11,99	1,39±0,10	
Chlorophyll *b*	75	11,58±0,96	1,12±0,08	**123,49±18,24**
	80	34,92±6,18	1,14±0,12	
	85	61,82±5,86	1,04±0,07	
	90	70,90±6,49	0,98±0,03	
	95	208,71±9,72	1,22±0,02	
	100	220,82±16,23	1,28±0,03	

Based on the values of the degradation rate constants, it can be appreciated that the two bioactive components degrade similarly in the temperature range 75-80°C, while in the temperature range 85-90°C, chlorophyll *b* degrades at a faster rate. large and lower at higher temperatures.

4.7.2. Degradation kinetics of carotenoid compounds

The fractional conversion kinetic model given by equation 4 was used to describe the effect of the heat treatment on the total carotenoid compounds in the lavender extract.

In **Figure 4.11**. the thermal degradation curves of the total carotenoids in the lavender extract are shown.

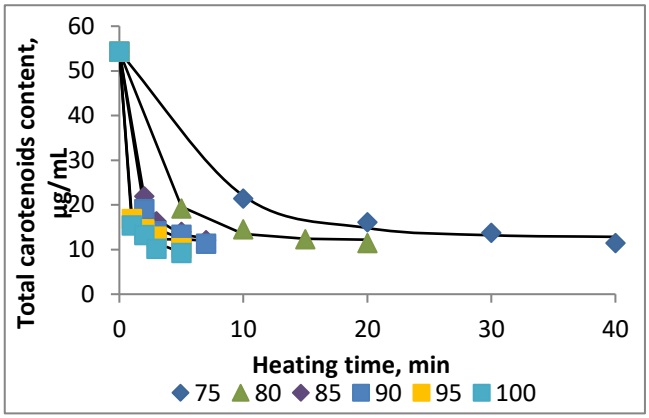


Figure 4.11. Fractional conversion kinetic model describing the thermal degradation of total carotenoids in lavender extract

The heat treatment had a significant effect on the total carotenoid content, causing a reduction of approx. 79% after 40 minutes at 75°C and 20 minutes at 80°C, respectively. At higher temperatures, total carotenoid losses were significant, ranging from 74% at 85°C to approx. 83% at 100°C after 5 minutes of holding (**Figure 4.11**). The degradation rate constants estimated by the fractional conversion kinetic model are shown in **Table 4.9**.

Table 4.9. Kinetic parameters estimated by the kinetic model of fractional conversion to thermal degradation of the total carotenoid content in lavender extract

Compound	Temperature, °C	k (x10^{-2}), 1/min	C_∞	E_a, kJ/Mol
Total carotenoids	75	15,05±2,01	12,73±1,07	**114,18±11,44**
	80	34,79±3,24	12,28±0,66	
	85	74,35±4,22	12,24±0,55	
	90	90,95±8,09	12,16±0,70	
	95	184,43±4,65	11,87±1,21	
	100	212,73±3,79	10,49±1,31	

From **Tables 4.8.-4.9**., a degradation with a higher velocity can be observed in the temperature range 75-90°C, followed by a similar degradation at temperatures of 95°C, while at 100°C, chlorophyll it degrades the fastest.

The thermostability of carotenoids is due to conjugated double bonds. When applying intense heat treatment, the structures are cleaved and molecular reactions occur in which the double bonds are involved.

4.7.3. Degradation kinetics of polyphenolic compounds

The fractional conversion kinetic model was used to describe the thermal degradation kinetics of the total polyphenolic compounds in the lavender extract.

The thermal degradation curves are shown in **Figure 4.12**.

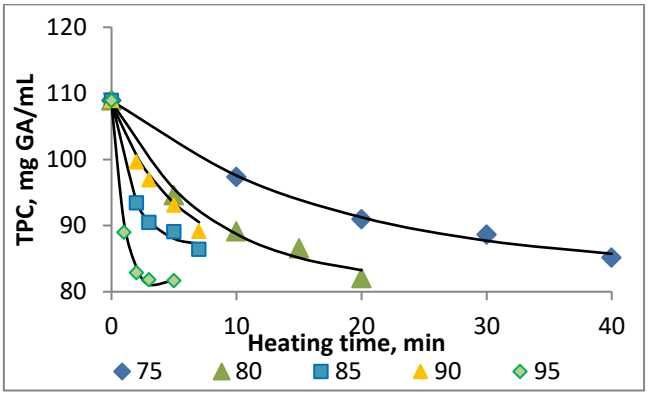


Figure 4.12. Fractional conversion kinetic model describing the thermal degradation of TPC in lavender extract

Degradation kinetics studies have shown the thermal stability of polyphenolic compounds in the lavender extract. From **Figure 4.13**. it can be seen that the heat treatment with a holding time of 5 min led to the preservation of the concentration of polyphenols in the proportion of ~ 87% at 80°C, ~ 82% at 85°C, ~ 79% at 90°C and ~ 75% at 95°C.

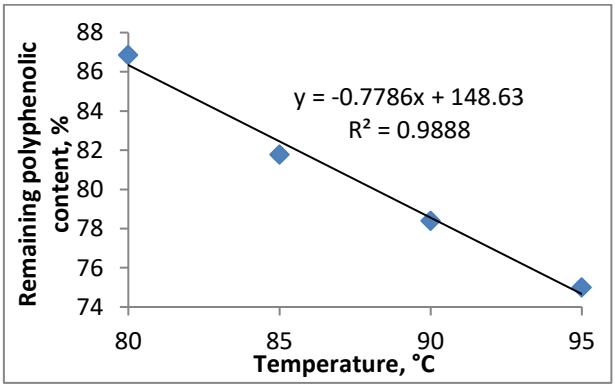


Figure 4.13. Percentage of TPC content in lavender extract after heat treatment in the range of 80-95°C for 5 min

The treatment at 100°C for 5 min led to an increase in the content of total polyphenols by approx. 9%, indicating an increase in the degree of extractability of polyphenolic compounds. In the present study, a decrease in TPC was observed in the first 3 minutes of the heat treatment, followed by a slight increase in concentration.

The degradation rate constants estimated by the fractional conversion kinetic model are shown in **Table 4.10**.

Table 4.10. Kinetic parameters estimated by the kinetic model of fractional conversion to thermal degradation of polyphenols in lavender extract

Compound	Temperature, °C	k (x10^{-2}), 1/min	C_∞	E_a, kJ/Mol
TPC	75	5,80±0,90	83,22±1,89	**94,65±8,19**
	80	12,94±3,30	81,18±2,58	
	85	20,35±8,91	86,85±0,93	
	90	30,82±0,19	84,62±1,56	
	95	38,79±1,50	79,50±1,08	
	100	58,69±1,54	77,57±1,89	

From **Table 4.10**., a slow degradation of polyphenols can be observed in the temperature range 75-80°C, followed by an accelerated degradation at temperatures between 85-95°C.

4.7.4. Flavonoid compounds

Following the heat treatment, high stability of flavonoid compounds was observed, without significant changes in content (**Table 4.11**.).

Table 4.11. Variation of the total flavonoid content at the heat treatment of lavender flower extract

Temperature, °C	Heating time, min	TFC, mg CE/mg extract
75	0	4,20±0,85
	10	3,93±0,42
	20	3,76±0,36
	30	3,83±0,74
	40	4,16±0,41
80	0	4,20±0,85
	5	3,94±0,74
	15	4,34±0,23
	20	3,78±0,58
	25	3,96±0,65
85	0	4,20±0,85
	2	5,01±0,45
	3	3,91±0,33
	5	4,04±0,41
	7	4,17±0,14
90	0	4,20±0,85
	1	4,00±0,47
	2	2,78±0,11
	3	4,18±0,25
	5	4,02±0,28
95	0	4,20±0,85
	1	2,11±0,28
	2	3,54±0,29
	3	3,79±0,47
	5	4,10±0,51
100	0	4,20±0,85
	1	3,42±0,11
	2	4,33±0,18
	3	3,58±0,41

The increase in the amount of flavonoids may be due to the formation of monomeric compounds resulting from the hydrolysis of the bonds between C-glycosides because in most plants flavonoids exist in the form of dimer or oligomer C-glycoside.

4.7.5. Kinetics of degradation of antioxidant activity

Heat treatment had a significant impact on antioxidant activity, causing a decrease of approximately 54% at 75°C after 40 minutes and 92% at 100°C after 3 minutes (**Figure 4.14**). The degradation kinetics of antioxidant activity followed a first-order model, which allowed the estimation of kinetic parameters, such as the constant of degradation rates and activation energy (**Table 4.11**.).

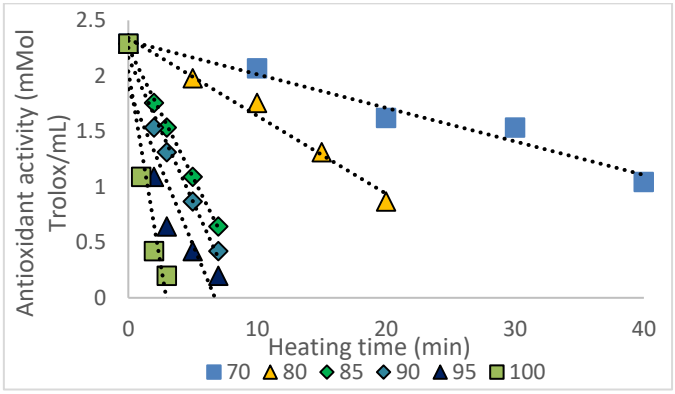


Figure 4.14. First-order kinetic model describing the thermal degradation of the antioxidant activity of lavender extract during heat treatment in the temperature range 75-100°C

Table 4.11. Kinetic parameters estimated by the first-order model for thermal degradation of antioxidant activity in lavender extract

Compound	Temperature, °C	k (x10^{-2}), 1/min	C_∞	E_a, Kj/Mol
Antioxidant activity	75	1,86±0,09	83,22±1,89	**158,69±29,11**
	80	4,69±0,87	81,18±2,58	
	85	17,87±1,28	86,85±0,93	
	90	23,49±2,37	84,62±1,56	
	95	34,01±1,27	79,50±1,08	
	100	77,35±3,78	77,39±1,21	

It can be seen from **Table 4.11**., that the antioxidant activity degrades at a slower rate throughout the temperature range, probably the thermal stability is due to the thermal stability of flavonoid compounds.

4.8. Partial conclusions

The main objective of the study was to test three different categories of extraction techniques and to analyze their global and individual phytochemical profile, from the perspective of selecting extracts with individualized profile, for particular uses. A secondary objective of the study was to test the thermal stability of some target compounds and to describe their thermal degradation based on kinetic models, from the perspective of using selected extracts as such in various industrial applications, to optimize thermal processes to preserve the biologically active potential.

The results obtained allowed the formulation of the following partial conclusions:

1. Three categories of extraction techniques were tested: a classical technique, solid-liquid extraction, two combined assisted techniques (solvents and ultrasound, solvents and microwaves), and extraction with supercritical fluids (supercritical CO_2). The study focused on several biologically active compounds, such as chlorophylls, carotenoids, fatty acids, volatile compounds, flavonoids, polyphenols, in correlation with antioxidant activity.
2. In the case of solid-liquid extraction, many organic solvents were used, such as acetone, diethyl ether, hexane, and methanol. Methanol made it possible to obtain an extract with the highest concentration of flavonoids (121.85 ± 2.54 mg EC/mL), and consequently the highest value for antioxidant activity (1.28 ± 0.23 mMol Trolox/mL). Highest concentrations of chlorophyll (36.64 ± 2.47 µg/mL for chlorophyll a and 36.08 ± 2.74 µg/mL for chlorophyll b) and total carotenoids (14.43 ± 1.24 µg/mL) were obtained by extraction with acetone.
3. In determining the absorption spectra, narrow bands were recorded in the different spectral ranges for chlorophyll a and b, in the blue range (408 nm for acetone and diethyl ether extract,

410 nm for hexane and 375 nm for methanol extract) and red (663 for acetone extract, 667 nm for diethyl ether extract, 668 nm for hexane extract and 665 nm for methanol extract).

4. For ultrasound-assisted extraction, the mixture of hexane:acetone in a ratio of 3:1 and ethanol was selected as a solvent for 30 min at 40°C, the results showing a significantly higher content of biologically active compounds in the extract obtained with the mixture. of hexane: acetone 3:1, except for the content of flavonoids, which were extracted with a higher yield in ethanol. For example, the extract obtained with a mixture of hexane: acetone in a ratio of 3: 1 showed a chlorophyll a and b content of 4.50 ± 1.16 and 6.40 ± 0.39 µg/mg extract, respectively, with a antioxidant activity of 4.46 ± 0.98 mMol Trolox/mg extract, high values were also recorded for the content of polyphenolic compounds, with 5.41 ± 0.08 mg GA/mg extract for total polyphenols and 4.20 ± 0.85 mg EC/mg extract for total flavonoids.

5. In microwave-assisted ethanol extraction, superior results were obtained under conditions of extraction time 15 sec, power 420 W, and temperature of 64.5ºC, the extract having a lower content of chlorophyll a and b compared to conventional extraction, 4.59 ± 0.09 µg/mL and 2.39 ± 0.02 µg/mL, respectively.

6. Extraction with supercritical fluids allowed to obtain two types of extracts, corresponding to the two separators, S40 and S45, with different global phytochemical profile, extraction at 300 bar, for one hour allowed to obtain a fraction S45 richer in chlorophyll and total carotenoids, compared to the extension of the extraction time to 3 hours, The extraction time influenced the concentration of flavonoids and polyphenols in the S45 fraction, with values of 80.95 ± 0.67 mg GA/g SU and 34.02 ± 0.51 mg CE/g SU, almost 20% higher for polyphenols and more than 2 times higher for flavonoids compared to extraction for 1 hour. The antioxidant activity was higher by about 18% in the case of the extract obtained after 3 hours of extraction.

7. For the comparative analysis, four extracts resulting from the combined extraction of n-hexane were selected: acetone 3: 1 ratio with ultrasound, under extraction conditions 30 min at 40°C (E1), extraction in 70% ethanol in combination with ultrasound (E2), in extraction conditions 30 min at 40°C, and the extraction with supercritical CO_2 (E40 and E45) allowed a control of the phytochemical profile, with obtaining high concentrations of hydrophobic compounds in E1, hydrophilic in E2 and both categories in E40 and E45, Thus, in the extract obtained by ultrasound with ethanol, the content of hydrophilic compounds (polyphenols and flavonoids) is significantly higher (180.80 ± 2.91 mg GA/g SU and 80.77 ± 9.53, respectively mg CE/g SU), while the supercritical extract has a remarkable content in both categories of compounds, with an antioxidant activity of 78.36 ± 89.56 mMol/g SU.

8. The extract obtained by combined ethanol extraction by ultrasound was analyzed for the individual chromatographic profile of the polyphenolic compounds, identifying the presence of eleven polyphenolic compounds, with a majority of polyphenolic acids. The highest concentrations were recorded by vanillic acid and cinnamic acid. At the same time, ferulic acid and rosemary acid were found in high concentrations. Quercetin revealed a relatively low concentration in the lavender flower extract along with apigenin.

9. The extracts obtained by combined solvent-assisted ultrasonic extraction (E1) and the two extracts obtained by assisted extraction of supercritical fluids (E40 and E45) were analyzed comparatively for the content of volatile compounds. Thus, the GC-MS analysis of the three types of extracts allowed the identification of 44 and 26 volatile compounds in CO_2 extracts and extracts obtained by ultrasound, respectively. In the CO_2 extract, 7 main compounds were identified (*β*-linalool, eucalyptol, linalool acetate, *β*-trans-ocimene, and limonene), while in the E1 extract 7 main compounds were also identified, consisting of linalool acetate, *β*-linalool, 6-methyl-2- (2-oxiranyl) -5-hepten-2-ol, linalool oxide, lavandulyl acetate and camphor.

10. In both extracts, the majority of the compounds accounted for more than 71% of the composition of the extracts. The extract obtained by extraction with supercritical fluids is richer in volatile compounds. For example, fractions E40 and E45 contain *α*-pinene, 3-heptanone, 6-methyl-, camphene, sabinen, *β*-pinene, 1-octen-3-ol, etc., while the extract obtained by ultrasound (E1) contains a double the amount of *β*-myrcene, 6-Methyl-2- (2-oxiranyl) -5-hepten-2-ol, linalool oxide (fr, 1) and octen-1-ol, acetate and a significantly lower amount of camphor .
11. Significant differences were also identified in the content of limonene, eucalyptol, *β*-trans-ocimene, *β*-Linalool, with a higher proportion in the E45 fraction and lower in E1. Regarding the content of linalool acetate, the extract obtained by ultrasound had the highest content (25.86 ± 1.24%), followed by the fraction E40 (18.08 ± 0.87%) and E45 (15, 28 ± 1.25%).
12. Fraction E40 showed the highest proportion of α-linolenic acid (31.67 ± 1.58%) and palmitic acid (49.80 ± 2.31%), while extract E2 showed a higher proportion of linoleic acid (19.66 ± 0.85%), oleic acid (27.21 ± 1.10%) and stearic acid (13.73 ± 0.62%),
13. All three extracts showed a high concentration of polyunsaturated acids, with the highest value in the E45 fraction of 42.43% and a lower value of monounsaturated fatty acids, with the highest value recorded in E2 extract (27,88 ± 1.20%) and the lowest in the E40 fraction (5.93 ± 1.20%). N-3 acids had the highest contribution in the E40 fraction (31.67 ± 1.59%), compared to n-6 acids (7.66 ± 1.58%) and the lowest in E2, of 11.82 ± 0.55% and 19.66 ± 0.90% respectively,
14. Compared to solvent extraction, a significant redistribution of the fatty acid profile can be observed, with an increase in the degree of extraction of palmitic acid and α-linolenic acid by 2.66 times, along with a decrease in oleic acid content. 5 times and linoleic acid with 2.56. Compared to the E45 fraction, the fatty acid profile in the E40 fraction reveals a decrease of approx. 2 times the concentration of palmitic acid, approx. 1.3 times the concentration of α-linolenic acid, but also an increase in the concentration of linoleic, arachidic and behenic acid.
15. Thermal degradation of biologically active compounds from lavender flower extract, obtained by combined technique, solvent-ultrasound (n-hexane mixture: acetone ratio 3:1, extraction time 30 minutes, temperature 40°C) followed by a kinetic model of fractional conversion, described by equation 6, which allowed the estimation of the kinetic parameters of thermal degradation, namely the constants of the thermal degradation rate and the activation energy for chlorophylls, carotenoids and polyphenols. Degradation of antioxidant activity has been described by the first order kinetic model.
16. Based on the values of the degradation rate constants, it could be appreciated that the main chlorophylls degrade similarly in the temperature range 75-80°C, while in the temperature range 85-90°C, chlorophyll b degrades at a faster rate. large and lower at higher temperatures.
17. The activation energies for the thermal degradation of chlorophylls *a* and *b* in the lavender extract had values of 139.69 ± 15.22 kJ/mol and 123.49 ± 18.24 kJ/mol, respectively, which indicates a higher susceptibility higher chlorophyll *a* at thermal degradation compared to chlorophyll *b*, under the studied conditions.
18. The heat treatment had a significant effect on the total carotenoid content, causing a reduction of approx. 79% after 40 minutes at 75°C and 20 minutes at 80°C, respectively. At higher temperatures, total carotenoid losses were significant, ranging from 74% at 85°C to approx. 83% at 100°C after 5 minutes of maintenance.
19. In comparison, total carotenoids degrade at a higher rate in the temperature range of 75-90°C, followed by a similar degradation at temperatures of 95°C, while at 100°C, chlorophyll *a* degrades the fastest .
20. It has been observed that the values estimated by the fractional conversion model for the total carotenoid concentration at an infinite duration of heat treatment (C∞) vary with temperature,

which indicates that under these conditions the final carotenoid concentration is temperature dependent.

21. The activation energy for the thermal degradation of the total carotenoid content in the lavender extract had values of 114.18 ± 11.44 kJ/mol, being lower compared to chlorophylls, which denotes a lower dependence of the rate constants degradation from temperature, so higher stability.

22. The heat treatment with a holding time of 5 min led to the preservation of the concentration of polyphenols in the proportion of ~ 87% at 80°C, ~ 82% at 85°C, ~ 79% at 90°C and ~ 75% at 95°C.

23. Treatment at 100°C for 5 min increased the total polyphenol content by about 9%, indicating an increase in the extractability of the polyphenolic compounds,

24. In the case of the total polyphenol content, a slow degradation was observed in the temperature range 75-80°C, followed by an accelerated degradation at temperatures of 85-95°C.

25. Among the compounds studied, polyphenols showed the lowest temperature dependence, the activation energy for thermal degradation of polyphenols in lavender extract having values of 94.65 ± 8.19 kJ/mol.

26. Following the heat treatment, a constant concentration of total flavonoids was observed, a phenomenon explained by the formation of monomeric compounds resulting from the hydrolysis of C-glycoside bonds because in most plants flavonoids exist in the form of C-glycoside dimer or oligomer.

27. The results obtained allowed the selection of optimal extraction conditions to obtain and use compositionally valuable extracts in subsequent experiments.

28. Varieties of extracts with high antioxidant activity have been obtained, which can be used to combat endogenous free radicals (oxidative enzymes, respiratory chain) or exogenous (smoking, toxins, air pollution), radicals that can damage nucleic acids, thus contributing to the development atherosclerosis, cataracts, cancer, ischemia, (vasoconstriction, thrombosis), gout, aging, dementia, diabetes, pulmonary fibrosis and Alzheimer's and Parkinson's diseases.

29. The study is also innovative and applied because no studies have been identified in the literature focusing on the chlorophyll, carotenoid, polyphenolic and fatty acid profile, the results obtained contributing to the drawing of a complete picture of the phytochemical profile of flowers. of lavender.

CHAPTER 5.
DEVELOPMENT OF HIGH-FUNCTIONAL INGREDIENTS FOR POTENTIAL USE IN FOODS

5.1. Introduction

Microencapsulation allows the creation of a physical barrier between the core and the materials to be encapsulated, thus protecting the biologically active compound from environmental conditions such as light, temperature, humidity, oxygen, but also interactions with other substances. The key factors for efficient microencapsulation are the type of encapsulating material, as well as the characteristics of the encapsulating compounds, the microencapsulation techniques, and the process parameters.

5.2. Objectives of the study

The aim of this study was the selection of complex matrices for microencapsulation of biologically active compounds extracted from lavender, from the perspective of the development of functional composites for the food industry, as well as the characterization of the resulting

powders. The technique selected for encapsulating the bioactive compounds in lavender was coacervation and lyophilization.

5.3. Materials and methods

The materials used in the experiments were described in the subchapter. **4.3.1**.

5.4. Reagents used

- HPLC purity methanol
- 5% NaNO2 solution (m / v)
- 10% AlCl3 solution (m / v)
- 1M NaOH solution
- Folin-Ciocâlteu reagent
- 20% Na2CO3 solution (m / v)
- 1N HCl solution
- Formic acid solution 3 - 5%
- 70% ethanol solution
- DPPH reagent (2,2-diphenyl-1-picrylhydrazyl)
- TROLOX solution
- Gallic acid solution
- Catechin solution

5.5. Equipment used

- Julabo 5 water thermostat enclosure, Germany
- High precision analytical balance, XS 403 SM, METTLER TOLEDO, Switzerland
- Ultracentrifuge with cooling, HETTICH Universal 320 R, Germany
- pH - meter S 20 K, METTLER TOLEDO, Switzerland
- UV-VIS Spectrophotometer Biochrom Libra S22, 2017
- Orbital shaker with analog control of stirring frequency and thermostat, LAB COMPANION COMECTA S, A,
- Concentrator under vacuum stroke 2-18, CHRIST
- Ultrasonic bath
- Microwave oven
- Freeze dryer MARTIN CHRIST ALPHA 1-4

5.6. Methods of extraction of biologically active compounds

5.6.1. Extraction of hydrophobic compounds

For the extraction of hydrophobic compounds, the combined extraction technique was applied, using a mixture of hexane: acetone in a ratio of 3:1 and ultrasound for 15 minutes for each extraction cycle, under controlled temperature conditions (40°C). Thus, 20 g of dried and ground lavender flowers were mixed with 200 mL mixture of hexane:acetone in a ratio of 3:1 and subjected to ultrasonic extraction. After extraction, the mixture was centrifuged at 8500xg at 4°C for 10 minutes, then filtered. The extraction was repeated 5 times, and the supernatant collected. After the 5 extraction cycles, the supernatant was concentrated to dryness using the AVC 2-18 vacuum concentrator, CHRIST. The extract was coded E1.

5.6.2. Extraction of hydrophilic compounds

For the extraction of hydrophilic compounds, the combined extraction technique was applied, using 70% ethanol and ultrasound for 15 minutes for each extraction cycle, under temperature-controlled conditions (40°C). Thus, 20 g of dried and ground lavender flowers were mixed with 200 mL of 70% ethanol solution and subjected to ultrasonic extraction. After extraction, the mixture was centrifuged at 8500xg at 4°C for 10 minutes, then filtered. The extraction was repeated 5 times, and the collected supernatant, After the 5 extraction cycles, the supernatant

was concentrated to dryness using the AVC 2-18 vacuum concentrator, CHRIST. The extract was coded E2.

5.6.3. Extraction with supercritical fluids

The supercritical fluid extraction protocol has been described in subchapter **4.4.4**. the extracts used were obtained under the conditions presented in **Table 5.1** .:

Table 5.1. Discontinuous system extraction parameters (Nadalin et al., 2014)

C30 Extractor		Separator 1 S1	Separator 2 S2	CO_2 flow, kg/h	Gravimetric yield,%
I	300 bar/60°C/1 h	150 bar/60°C 1 h	50 bar/25°C 1 h	20,27	4,67

The extracts obtained were coded E40 and E45.

5.6.4. Extraction characterization methods

Determination of chlorophyll content. The extracts were diluted in n-hexane-acetone, 3:1 ratio, after which the absorbance at 663 nm and 645 nm wavelengths was read. Relationships 1-3 were used to calculate the chlorophyll concentration.

Determination of total carotenoid content. The extracts were diluted in n-hexane-acetone, 3:1 ratio, after which the absorbance at 470 nm wavelength was read. For the calculation of the total carotenoid concentration, the relation 11 was used:

Total carotenoid = (1000xA470 –2.13 Chl a - 97.63 Chl b) / 209 (11)

The total polyphenol content was determined using the Folin-Ciocâlteu colorimetric method (Gutfinger, 1981). The method for the determination of total polyphenolic compounds has been described in subchapter **5.2.**

The total flavonoid content was determined using the method described above by Dewanto et al., (2002). The method for the determination of total flavonoid compounds has been described in subchapter **5.2**.

Determination of antioxidant activity using the DPPH method (2,2-diphenyl-1-picrylhydrazyl). The method for determining antioxidant activity has been described in subchapter **5.3**.

6.5. Methods for characterizing microencapsulated powders

Determination of encapsulation efficiency

To evaluate the efficiency of encapsulation of biologically active compounds, the following was determined:

- the total content of total chlorophyll, chlorophyll a, chlorophyll b, carotenoids, polyphenols, and flavonoids;
- the content of total chlorophyll, chlorophyll a, chlorophyll b, carotenoids, polyphenols, and surface flavonoids of microparticles (Idham et al., 2010).

The encapsulation efficiency (% EE) is calculated by equation 12:

$$\%EE = \frac{(Total - Suprafata)}{Total} x100 \quad (12)$$

Antimicrobial activity

Testing of antimicrobial activity against indicator microorganisms (*Aspergillus niger* MIUG M5, *Penicillium expansum* MIUG M11, *Bacillus subtilis* MIUG B1 and *Salmonella agona* MIUG BP1) was performed using the method described by Cortes-Zavaleta et al., (2014).

The growth inhibition ratio was calculated using the following formula (13):

$$RI = \frac{D_{M-D_P}}{D_M} X\ 100,\ \% \quad (13)$$

Structure and morphology of microencapsulated powders

The purpose of the CLSM analyzes was to highlight the size and morphology of the biologically active compounds in lavender extract encapsulated in different matrices. At this stage, a Zeiss LSM 710 confocal laser scanning microscopic system was used, and 3D images were captured and analyzed using ZEN 2012 SP1 software. The technical specifications of the equipment are: laser diodes (405 nm), Ar laser (458, 488, 514 nm), DPSS laser (solid state diode pump (561 nm) and HeNe laser (633 nm), AxioObserver Z1 reversing microscope, 40x apochromatic lens (with 1.4 aperture) and FS49, FS38 and FS15 filters.

Electron scanning microscopy

Morphological examination of fine powders (V3 and V4) was performed using electron scanning microscopy (FEI Quanta 200) using a low vacuum 15 kV voltage. The powders were fixed on an aluminum tube using double carbon adhesive tape. To increase the conductivity, the samples were coated with a thin layer of 5 nm gold, using a SPI-Module Sputter Coater (SPI Supplies, USA), using a current of 18 mA. SEM images were collected at different magnitudes between 500 and 100,000X.

Particle size

The particle size distributions were further determined by measuring the light intensity scattered by the laser beam as it passed through the fully hydrated sample. Particle size measurements were performed using the PA-200G Wet Laser Particle Analyzer (MRC, Holon, Israel).

Evaluation of cytotoxicity of microencapsulated compounds

The cytotoxicity of microencapsulated powders was evaluated by *in vitro* assays on a culture of fibroblasts from the NCTC stabilized line clone L929 by the Neutral Red method.

5.7. Statistical analysis

For the evaluation of cytotoxicity, the results represent mean values for 3 determinations ± standard deviation (SD). Statistical analysis of the results was performed using the Student t-test on pairs of samples. The differences were considered statistically significant at $p < 0.05$.

5.8. RESULTS AND DISCUSSIONS

5.8.1. Experimental variants of microencapsulation of biologically active compounds from lavender flowers

4 encapsulation variants were selected, two for the hydrophobic extract, one for the hydrophilic extract, and one for the supercritical CO_2 extract.

Encapsulation variant 1

It involved the use of a combination of agar and carboxymethylcellulose in a ratio of 2:1. The technological scheme for obtaining the microencapsulation variant 1, by complex coacervation and lyophilization is presented in **Figure 5.1**.

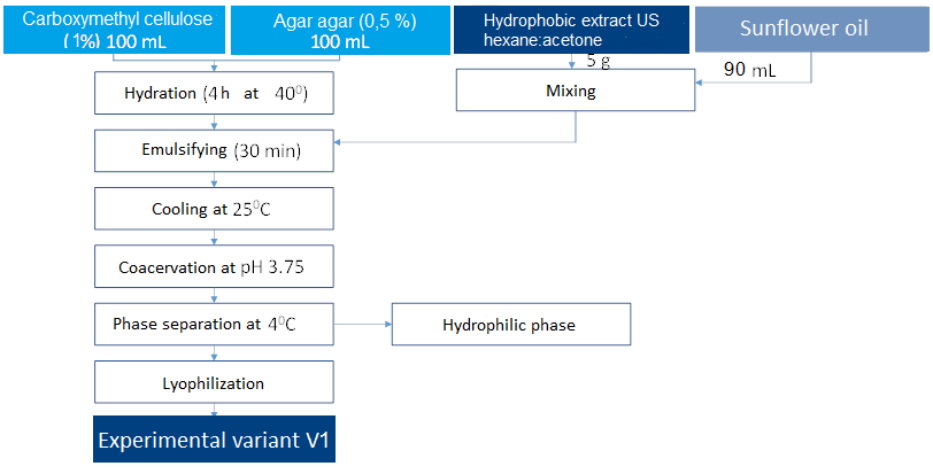


Figure 5.1. Technological scheme for obtaining microencapsulation variant 1, by complex coacervation and lyophilization

Encapsulation variant 2

It involved the use of a combination of agar and acacia gum in a ratio of 2:1. The technological scheme for obtaining microencapsulation variant 2, by complex coacervation and lyophilization is presented in **Figure 5.2**.

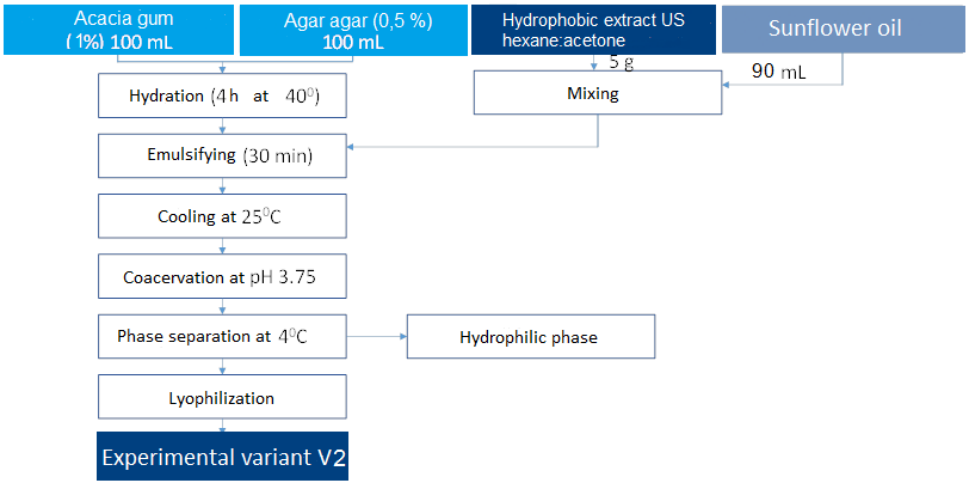


Figure 5.2. Technological scheme for obtaining microencapsulation variant 2, by complex coacervation and lyophilization

Encapsulation variant 3

It involves the use of a combination of whey protein isolate and carboxymethylcellulose in a 2:1 ratio. The technological scheme for obtaining the microencapsulation variant 3, by complex coacervation and lyophilization is presented in **Figure 5.3**.

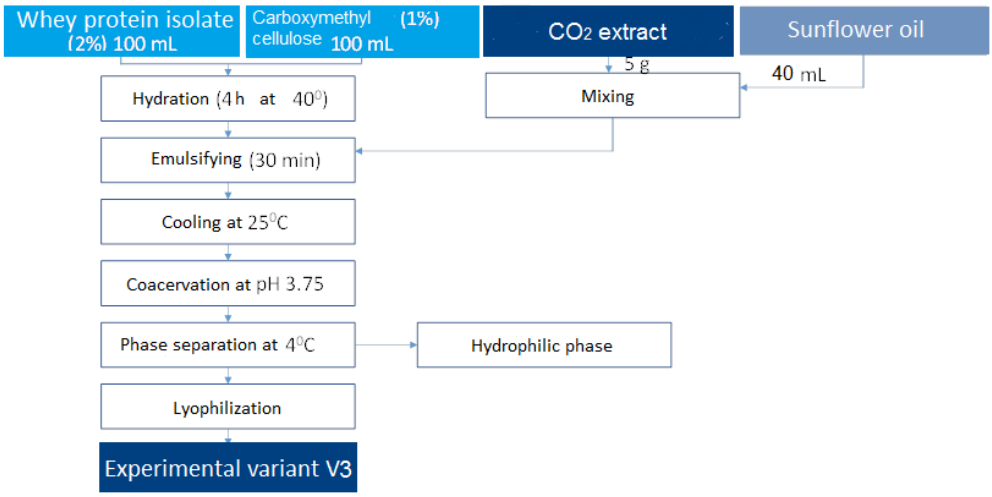


Figure 5.3. Technological scheme for obtaining microencapsulation variant 3, by complex coacervation and lyophilization

Encapsulation variant 4

It involves the use of a combination of whey protein isolate and acacia gum in a ratio of 2:1. The technological scheme for obtaining the microencapsulation variant 4, by complex coacervation and lyophilization is presented in **Figure 5.4**.

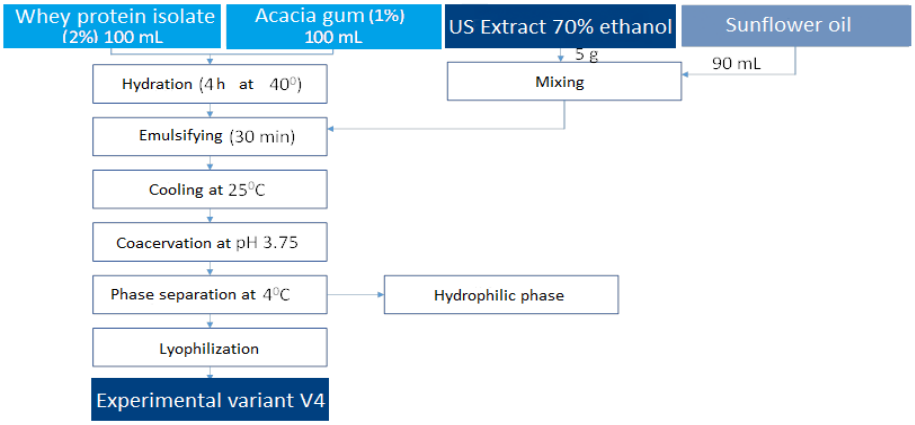


Figure 5.4. Technological scheme for obtaining microencapsulation variant 4, by complex coacervation and lyophilization

5.8.2. Comparative analysis of the global phytochemical profile of microencapsulated experimental variants

In our study, four encapsulation variants were tested. The efficiency of the microencapsulation was evaluated for each test compound, the values obtained is presented in **Table 5.2**.

Table 5.2. The encapsulation efficiency of microencapsulated variants

Phytochemical compounds	V1	V2	V3	V4
Total chlorophylls	41,97±1,70	49,02±1,33	95,22±1,85	94,65±1,44
Chlorophyll *a*	38,87±1,43	44,79±0,57	96,62±2,87	98,01±1,56
Chlorophyll *b*	43,90±3,56	51,79±2,55	90,61±1,20	90,61±1,36
Total carotenoids	49,32±3,16	50,14±2,97	94,79±1,29	97,04±1,20
Total polyphenols	61,79±1,98	61,92±1,54	93,88±2,01	93,93±2,01
Total flavonoids	73,36±2,87	69,82±1,47	90,22±1,20	90,72±2,47

From **table 5.2**. it can be seen that the use of proteins in the microencapsulation process has led to an increase in the encapsulation efficiency (EE) for each phytochemical compound. For example, for chlorophyll *a*, the EE was 38.87 ± 1.43% in variant 1, and 98.01 ± 1.56% in variant 4. Variants 3 and 4 showed the highest EE values for all analyzed compounds, which suggests that whey proteins are very effective encapsulating materials (**Figure 5.5**.).

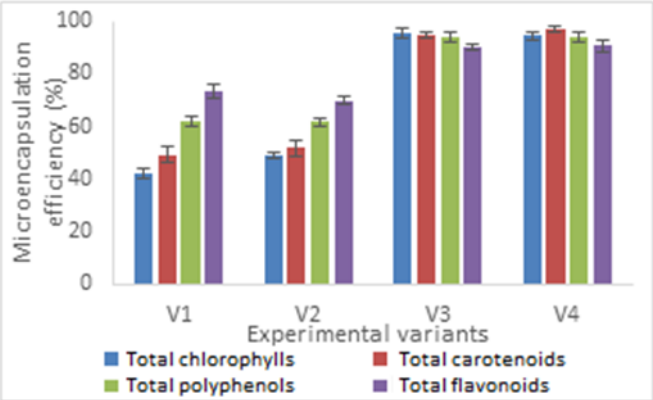


Figure 5.5. The microencapsulation efficiency of bioactive compounds in the 4 experimental variants

In **table 5.3**. is presented the phytochemical profile of the microencapsulated powder variants obtained.

Table 5.3. Phytochemical profile of experimental variants of microencapsulated powders

Phytochemical profile	V1	V2	V3	V4
Total chlorophylls, mg/g SU	1,54±0,11	0,88±0,03	0,43±0,05	0,27±0,04
Chlorophyll *a,* mg/g SU	0,61±0,01	0,68±0,02	0,17±0,07	0,10±0,06
Chlorophyll *b,* mg/g SU	0,93±0,10	1,05±0,10	0,27±0,02	0,16±0,03
Total carotenoids, mg/g SU	338,70±4,60	339,30±2,39	95,00±1,29	58,82±1,20
Total polyphenols, mg GA/g SU	5,17±0,42	5,47±1,54	9,71±2,01	10,78±0,10
Total flavonoids, mg CE/g SU	4,25±0,87	3,98±0,47	6,61±1,20	6,92±0,92
Antioxidant activity, mMol/g SU	2,85±0,12	3,44±0,32	4,77±0,26	4,80±0,15

A different phytochemical profile can be observed for the powder variants, with a higher content of chlorophylls and carotenoids in variants 1 and 2 (total carotenoid content of 338.70 ± 24.60 mg/g SU in V1 and 339.30 ± 2.39 mg/g SU in V2). Polyphenolic compounds and flavonoids were found in higher concentrations in variants 3 and 4, which led to a higher antioxidant activity.

5.8.3. Antimicrobial activity of the powders

In **Table 5.4**. the antimicrobial activity of the microencapsulated variants is presented. The highest antimicrobial activity, 100%, was identified in variants 1 and 2 against *Penicillium expansum* MIUG M11 and *Bacillus subtilis* MIUG B1 (**Figures 5.6-5.9**). From **Table 5.4**. it can be seen that the most resistant fungal strain proved to be *Aspergillus niger* MIUG M5 for all four variants, showing no or less antifungal activity (0 to 6.52%).

Table 5.4. Antifungal activity of experimental variants of microencapsulated powders

Microorganism	Inhibition ratio, %			
	V1	V2	V3	V4
Aspergillus niger MIUG M5	0±0,0	4,68±0,3	6,52±0,4	4,34±0,4
Penicillium expansum MIUG M11	**100±0,0**	**100±0,0**	29,03±0,5	58,83±0,5
Bacillus subtilis MIUG B1	22±0,5	**100±0,0**	50,0±0,5	18,51±0,8

Higher antimicrobial activity in variants 1 and 2 may be associated with higher carotenoid content and probably with free fatty acids.

However, our results showed a remarkable antibacterial effect mainly due to the presence of carotenoids and chlorophylls.

5.8.4. Morphological and structural analysis of microencapsulated variants by laser confocal microscopy

In the native samples obtained by microencapsulation (**Figure 5.10**.), Thin scales with irregular and asymmetrical shapes were observed, their emission being on the whole spectral range due to their complex composition. However, variants 1 and 2 appear to have a more uniform distribution of biologically active compounds within the microencapsulated biopolymer matrices.

Large formations were observed (244.57 - 306.93 µm as in **Figure 5.10. C**) with irregular shape and porous structure (such as microcavities or cracks with a diameter of 6-8 µm in **Figure 5.10. B**).

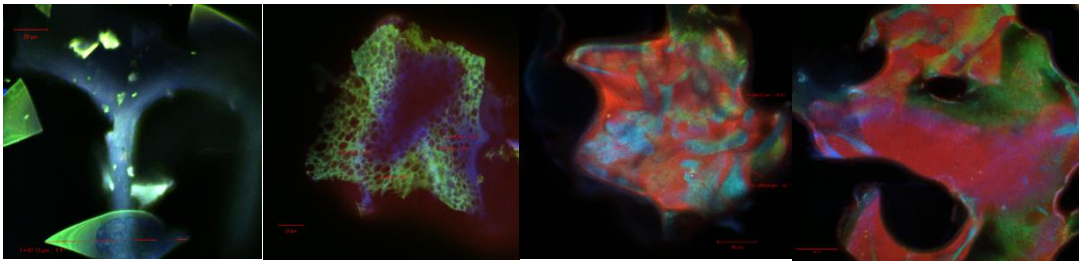


Figure 5.10. Images of native microencapsulated powders obtained by confocal microscopy: V1 (a), V2 (b), V4 (c), V3 (d)

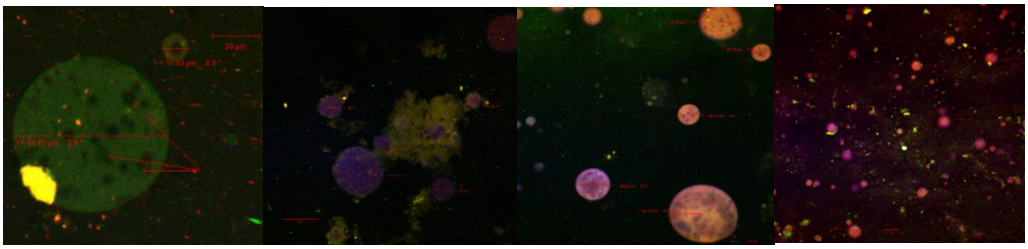


Figure 5.11. Images obtained by confocal microscopy of microencapsulated powders to which dyes have been applied: V1 (a), V2 (b), V4 (c), V3 (d)

In the samples of microencapsulated powders to which the dyes were applied, the presence of lipophilic compounds could be observed. They have a spherical shape with diameters between 10-30 microns (**Figure 5.11**.), In sample V3, the spheres have the smallest values, most having a diameter of less than 10 µm (**Figure 5.11. D**). In the other samples, 20% of the spheres have diameters greater than 60 µm (**Figure 5.11. A, b, c**). Moreover, among the giant spheres can be seen microvesicles (arrows in **Figure 5.11. A**), their presence suggesting a possible double encapsulation.

5.8.5. Morphological and structural analysis of microencapsulated variants by electron scanning microscopy (SEM)

Electron scanning microscopy was performed for V3 and V4 at different degrees of magnitude. In **Figure 5.12**. SEM images of the two variants at different magnitudes are presented, ranging from x100 to x25000.

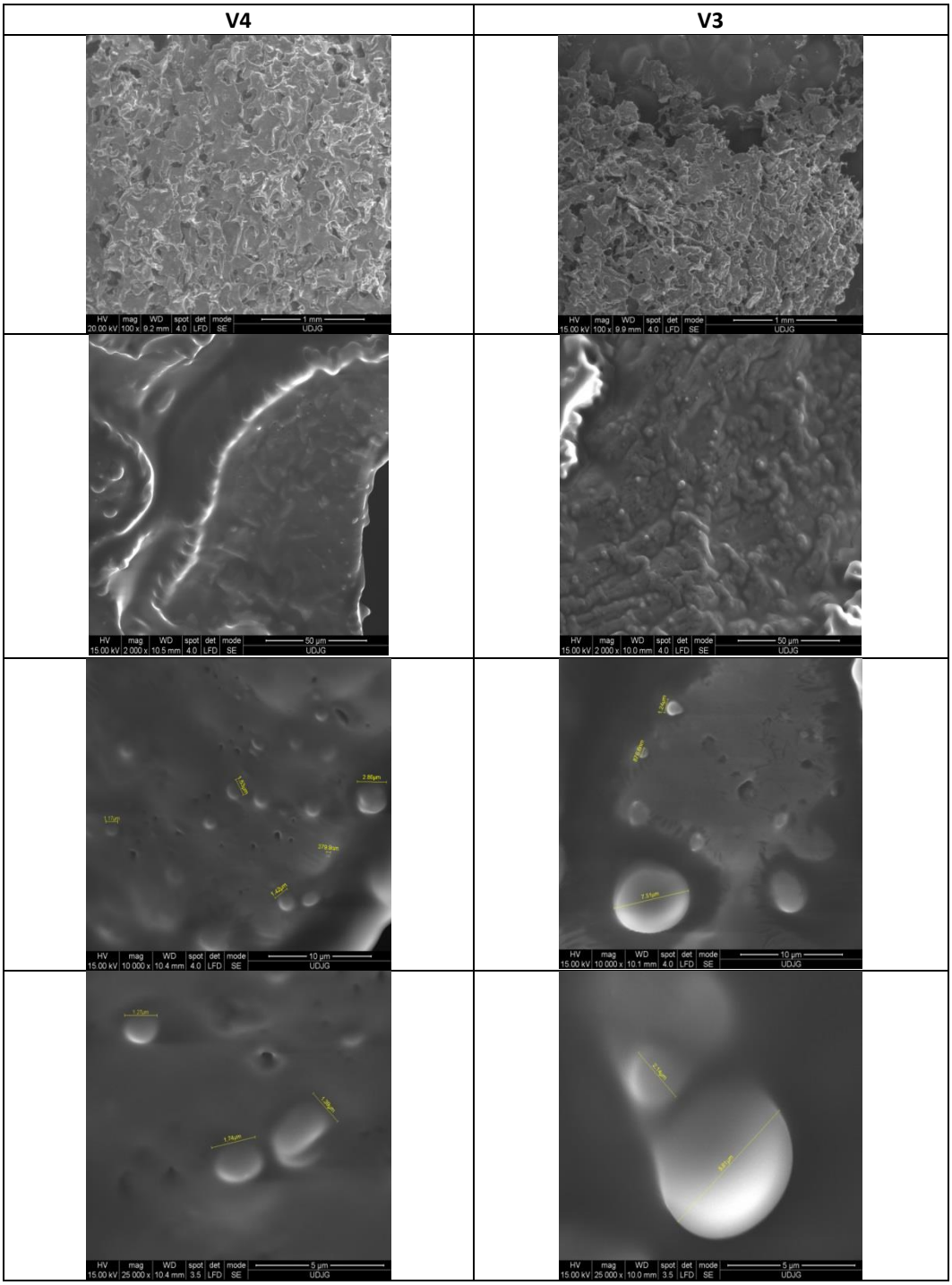


Figure 5.12. SEM images of V3 (a) and V4 (b) microencapsulated variants at magnitude X100

In **Figure 5.12**. it can be seen that both variants have spongy structures, with spherical or oval shapes, with dimensions between 582 nm and 7.51 µm in V3 and smaller in variant 4, between 380 nm and 2.86 µm.

5.8.6. Particle sizing

Analyzing the results presented in **Table 5.5**., it can be seen that for all microencapsulated powders, the coarser particle size is larger compared to the fine particles. The D values [3,2] decrease in the following order: variant 4> variant 2> variant 3> variant 1.

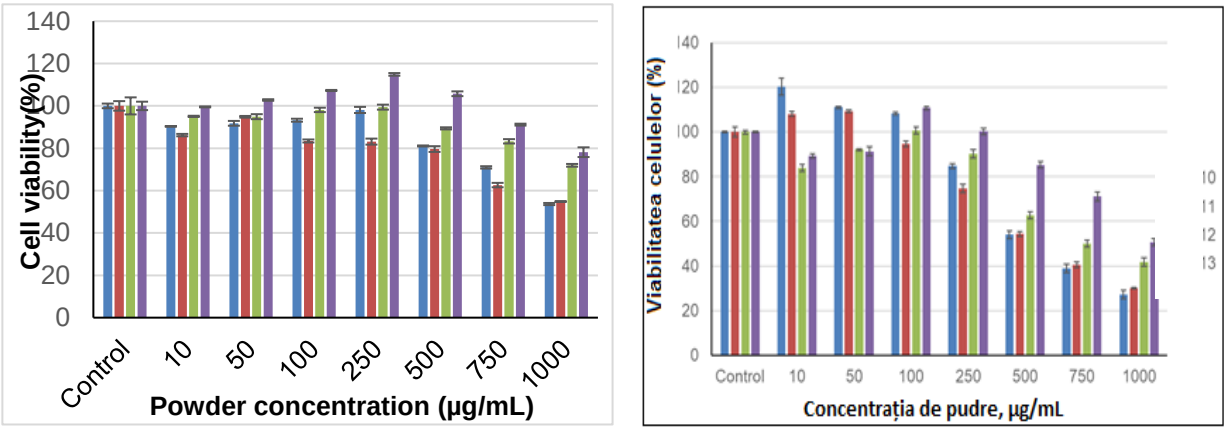
Table 5.5. Characterization of particle size in experimental variants

	Variant 1	Variant 2	Variant 3	Variant 4
D [3,2], µm	4,494	4,701	4,620	4,738
D [4,3], µm	4,684	5,048	4,909	5,108
D10, µm	3,613	3,648	3,635	3,654
D50, µm	4,497	4,608	4,563	4,629
D90, µm	5,698	7,612	6,665	7,860

The same trend was observed for the size of coarse particles present in the microencapsulated powders, the D values [4,3] varying from 4,684 µm recorded for variant 1 and 5,108 um for variant 4. The largest variation of the particle size was registered in the case of variant 4 when 80% of the sample volume had particle diameters between 3,654 and 7,860 µm. On the other hand, the most uniform microencapsulated powder in terms of particle size was variant 1, with the narrowest variation of dimensions D10-D90 from 3,613 to 5,698 µm.

5.8.7. Cytotoxicological analysis of microencapsulated variants

The viability of L929 fibroblast cells cultured in the presence of different concentrations from the experimental variants was determined, initially, by the Neutral Red test. The results obtained after 24 hours and 48 hours of cultivation are presented in **Figure 5.13**.



a) b)

Figure 5.13. Cell viability on L929 fibroblasts cultured in the presence of microencapsulated powders of lavender extracts

(Variant 1 blue, variant 2 red, variant 3 green and variant 4 purple) after 24 hours (A) and 48 hours (B), by the method Red Red, The results were expressed as a relative percentage compared to the untreated control sample, considered 100 % viable, The indicated values represent mean values ± SD (n = 3), * p <0.05 compared to the control sample

It can be seen that variants 1 and 2 had a similar behavior, after 24 hours of culture, variants 1 and 2 were cytocompatible in the concentration range 10-500 µg / mL, and the values for cell viability varied between 80-98 %. Values for cell viability were significantly higher than those for control (p <0.05), ranging from 108-120%. This behavior can be correlated with the release of biologically active compounds from encapsulation matrices.

Microencapsulated variant 3 was cytocompatible in the concentration range 10-750 µg/mL after 24 hours of culture (83-99% cell viability) and 10-250 µg/mL after 48 hours of culture (90-101% cell viability). At higher concentrations, cell viability decreased to 42% after 48 hours of culture.

After 24 hours of culture, variant 4 was cytocompatible throughout the tested concentration range of 10-1000 µg/mL, but after 48 hours of culture, the cytocompatibility interval decreased to 10-500 µg/mL.

Cell morphology was analyzed after 48 hours of culture (**Figure 5.14**.). It can be seen that the cells treated with the variants encapsulated in concentrations of 250-1000 µg/mL maintained their normal fusiform phenotype, characteristic of fibroblast cells, similar to untreated cells.

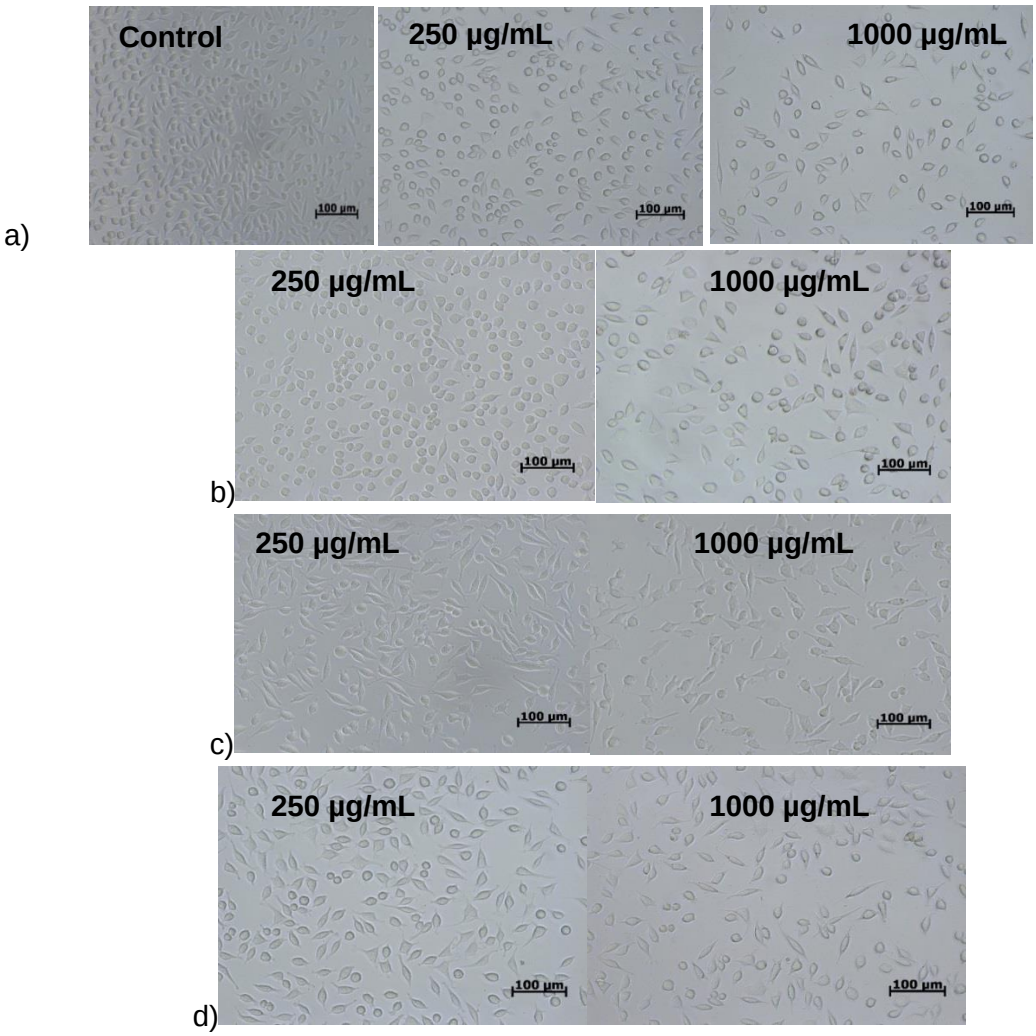


Figure 5.14. Optical microscopy images of L929 fibroblast cell culture cultured in the presence of variants 1-4 (ad), at concentrations of 250 µg/ml (left) and 1000 µg/ml (right), respectively, for 48 h, under conditions standard (37°C, atmosphere with 5% CO_2), Giemsa coloring.

5.9. Partial conclusions

Lavender is a rich source of valuable volatile compounds, carotenoids, chlorophylls, polyphenols, flavonoids, volatile compounds with significant biological activities, with different applicability. The main purpose of the present study was to test the possibility of using materials for encapsulation of biologically active compounds target from lavender extracts, from the perspective of developing composites with different applicability, which successfully meet the necessary conditions for use as ingredients. with high functionality.

The results obtained in this study allowed the formulation of the following partial conclusions:

1. In a first step, the extraction steps were repeated, except for the extraction with supercritical fluids, with a succession of 5 extraction steps, followed by the collection of supernatants, centrifugation and vacuum concentration;

2. The two ultrasound-assisted extractions varied as the solvent used, in the first variant using a mixture of n-hexane-acetone, a ratio of 3: 1, and in the second ethanol 70%, to control the predominant profile (hydrophobic or hydrophilic) of the extracts;

3. For co-encapsulation, complex coacervation and lyophilization were used as techniques, using as encapsulation materials polysaccharides (carboxymethylcellulose, agar agar and acacia gum) and whey protein isolate;

4. In the first encapsulation variant, which used the hydrophobic extract obtained by solvent and ultrasonic extraction in agar and carboxymethylcellulose combinations in a ratio of 2: 1, high values of encapsulation efficiency were obtained for the total flavonoid content, of approx. 74% and about 62% for polyphenols;

5. This variant showed a high total carotenoid concentration of 338.70 ± 24.60 mg/g SU, with a polyphenol concentration of 5.17 ± 0.42 mg AG/g SU and flavonoids of 4.25, respectively. ± 0.87 mg EC/g SU, which led to an antioxidant activity of 2.85 ± 0.12 mMol/g SU, with a 100% inhibitory antifungal activity against *Penicillium expansum* MIUG M11;

6. The second experimental variant, which used combinations of agar and acacia gum in a ratio of 2: 1 and hydrophobic extract, obtaining higher values for the encapsulation efficiency of chlorophylls and carotenoids compared to experimental variant 1, but lower for polyphenols and flavonoids;

7. Experimental variant 2 had a higher content of total chlorophylls, without significant differences in the profile of the other biologically active parameters, but showed a higher antioxidant activity of 3.44 ± 0.32 mMol/g SU and both antifungal activity (*Penicillium expansum* MIUG M11) and antibacterial (*Bacillus subtilis* MIUG B1);

8. Experimental variants 3 and 4 showed the highest values of microencapsulation efficiency for all compounds analyzed, which suggests that whey proteins are very effective encapsulating materials;

9. Thus, variant 3 showed an efficiency of the microencapsulation of chlorophyll a of 98% and almost 91% of chlorophyll b, while variant 4 showed similar values of 97% and 91% respectively;

10. The phytochemical profile of the two variants is different, with a content about 2 times lower of total chlorophylls in variant 4, which, however, had a relatively higher content of polyphenols;

11. Variants 3 and 4 showed different amtimicrobial activity, with inhibition ratios greater than 50% for *Penicillium expansum* MIUG in variant 4 and *Bacillus subtilis* MIUG B1 in variant 3;

12. The higher values of antimicrobial activities recorded for variants 1 and 2 may be a synergistic result of the fatty acid and carotenoid content, the significant antimicrobial effect being attributed to the dominant presence of carotenoids, in addition to the complexity of the fatty acid composition and characteristics of microorganisms;

13. Structural analysis revealed a more uniform distribution of biologically active compounds within microencapsulated biopolymer matrices for variants 1 and 2, confocal microscopy allowing the detection of large formations (244.57 - 306.93 µm) with irregular shape and porous structure (such as microcavities or cracks with a diameter of 6-8 µm);

14. In variant 3, spheres with a diameter of less than 10 µm were highlighted, while in the other samples, 20% of the spheres have diameters greater than 60 µm, which contain microvesicles, their presence may suggest a possible double encapsulation;

15. For all microencapsulated powders, the coarser particle size was larger compared to the fine particles;

16. Cytocompatibility studies showed that variants 1 and 2 had a similar behavior, after 24 hours of culture, being cytocompatible in the concentration range 10-500 µg/mL, and the values for cell viability varied between 80-98% ;

17. After 48 hours of culture, variants 1 and 2 stimulated cell proliferation in the concentration range of 10-100 µg/mL and 10-50 µg/mL, respectively, with an increase in cell viability between 108-120%, an associated phenomenon with the release of biologically active compounds from encapsulation matrices;

18. Microencapsulated variant 3 was cytocompatible in the concentration range 10-750 µg/mL after 24 hours of culture (83-99% cell viability) and 10-250 µg/mL after 48 hours of culture (90-101% cell viability)

19. Variant 4 was cytocompatible throughout the tested concentration range of 10-1000 µg/mL, but after 48 hours of culture, the cytocompatibility interval decreased to 10-500 µg/mL, with a stimulation of cell proliferation at concentrations. between 100-500 µg/mL;

20. Stimulation of cell proliferation is a consequence of the release of biologically active compounds from microcapsules, which have had a stimulating effect on cell metabolism,

21. In conclusion, the results obtained in this study offer new perspectives for the development of complex composites, with functionality demonstrated by selective, cytocompatible antioxidant and antimicrobial activity, for new formulations in food.

CHAPTER 6.
APPLICATION RESEARCH THROUGH THE DEVELOPMENT OF TECHNOLOGICAL VARIANTS FOR OBTAINING VALUE-ADDED FOOD PRODUCTS

6.1. Introduction

The development of the concept of functional foods or health foods among producers, which are foods that have a positive, proven health benefit, introduces the concept of functional ingredients, which can be perceived as components of foods with the role of improving well-being. short-term, but many health effects refer to the long-term mitigation of certain diseases.

6.2. Objectives of the study

The purpose of this study was to develop technologies for patenting value-added products, which exploit the functional potential of lavender and microencapsulated lavender extracts, respectively a technology for obtaining a product - ice cream with the addition of microencapsulated powders of lavender flower extracts in Whey protein isolate The main objectives of the study were:

- Selection of food matrices to develop value-added foods, especially in terms of antioxidant activity;
- Comparative analysis of the texture characteristics of products and analysis of sensory characteristics.

6.3. Materials and methods

6.3.1. Characterization of the antioxidant activity of products

All product variants were analyzed for antioxidant activity, which involved a preliminary stage of extraction from the food matrix.

6.3.2. Rheological analysis of ice cream mixes

The measurement of the rheological behavior of the ice cream samples performed at laboratory level was performed at least in duplicate using the controlled voltage rheometer (AR 2000, TA Instruments, New Castle, DE, **Figure 6.1**.). The specific software Rheology Advantage Data Analysis Program (TA, New Castle, DE) was used to analyze the results obtained. The temperature was monitored and kept constant during the measurements by means of a Peltier type temperature control system. All rheological measurements were made using a gap of 2000 μm.

Figure 6.1. AR 2000 Rheometer (TA Instruments, New Castle, DE)

The rheological characteristics of the ice cream samples were assessed by monitoring the following rheological parameters: storage modulus (G'), relaxation modulus (G"), deformation angle (δ), shear stress, and apparent viscosity. performed two types of tests: shear (rotational) and oscillators.

6.3.3. Sensory analysis

A panel of 14 tasters of different ages participated in the sensory analysis of the experimental ice cream variants, using the scoring method from 1-7. Tasters evaluated the following attributes: color, phase separation, aroma and taste attributes, such as sweetness, bitterness, flavor, rancidity, textural attributes evaluated when the spoon was placed in the ice cream box, such as: hardness, roughness, crumbly, gumminess, aeration, textural attributes evaluated during consumption, such as: ice cream, hardness (mouthfeel), roughness, watery, creaminess, smoothness and general impression (classification of samples in order of preference, 1 least appreciated sample - 7 most appreciated test).

6.4. RESULTS AND DISCUSSIONS

6.4.1. Technological variants for obtaining food with added value

The ice cream was made according to a classic recipe, in which variants 1 and 2 were added to milk as an ingredient. 6 product variants were made (varying the amount of microencapsulated lavender powder of 1%, 1.5%, and 2%).

The added value was tested in terms of antioxidant activity, rheological properties and sensory analysis.

Microencapsulated variants 3 and 4 were added in a classic macaroons recipe, with the addition of the experimental variants in a proportion of 1% to the total mixture mass obtained.

6.4.2. Evaluation of added value as antioxidant activity

The antioxidant activity of the experimental variants of ice cream is presented in **Figure 6.2**.

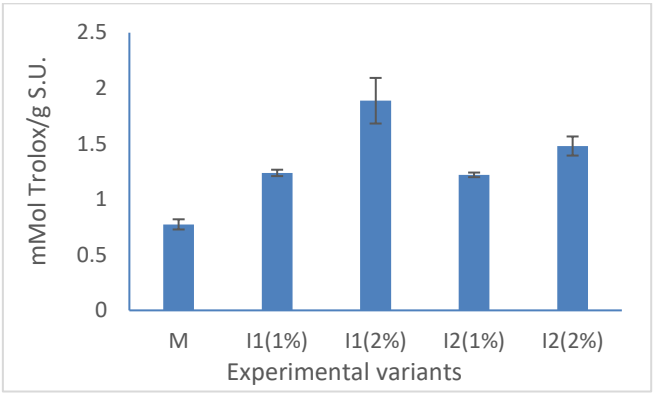


Figure 6.2. Antioxidant activity of ice cream variants with the addition of microencapsulated powder

It can be seen that the antioxidant activity was about 1.6 times higher in the experimental ice cream variant which used a percentage of 1% of the microencapsulated variant 1 and 2.5 times higher in the 2% variant. In the case of experimental variants that used microencapsulated variant 2, an increase of antioxidant activity was obtained 1.6 times at an addition of 1% and 1.9 times at an addition of 2%.

6.4.3. Testing of microencapsulated powders in other food matrices

Experimental variants of almonds showed significantly higher values of antioxidant activity, 9.40 ± 0.49 mMol Trolox/g SU in control, 9.73 ± 0.04 mMol Trolox/g SU for the variant that used microencapsulated powder V3 and 11 .28 ± 1.08 mMol Trolox/g SU for the variant that used V4 (**Figure 6.3**.).

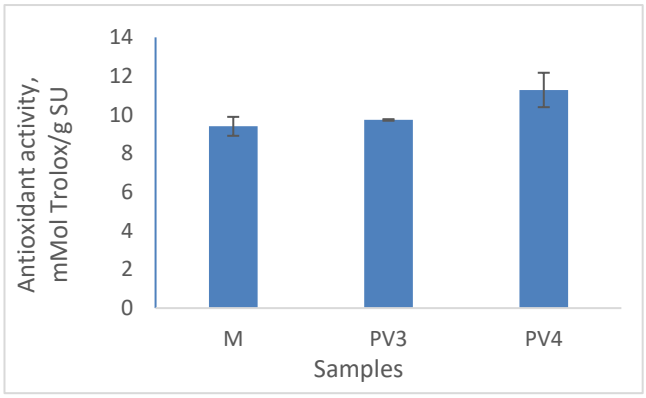


Figure 6.3. Antioxidant activity of almond variants with the addition of microencapsulated powder

Unlike ice cream, in this case, the increase in antioxidant value was only 3.5% in the first variant and 20% in variant 2.

6.4.4. Rheological analysis of value-added foods

Analyzing the results presented in **Table 6.1**. it can be seen that the G' values varied significantly depending on the type of sample under analysis. There are samples with the addition of 1 and 2% of variant 1, which showed values of modulus G' approximately 25 times higher than the control sample at 1 Hz.

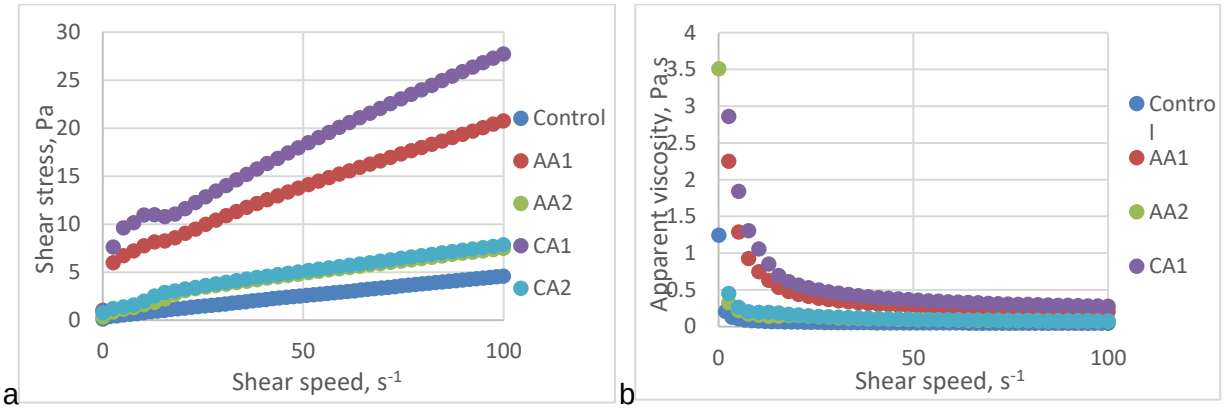
The intersection point of the G' and G'' curves for the analyzed samples varied depending on the type of ingredient used for the supplement (**Table 6.1.**).

Table 6.1. Rheological parameters recorded for ice cream samples at 4°C

Rheological parameters	Control	I.2.1	I.2.2	I.1.1.	I.1.2
Frequency scanning					
G' at 1Hz frequency, Pa	2,42	43,66	13,78	60,77	59,29
The relation between G' and G'' at a low frequency	G'>G''	G'>G''	G'<G''	G'>G''	G'>G''
Module reversal frequency G' and G'', Hz	2,51	10	-	15,85	39,81
Shear test					
Apparent viscosity at 50 s^{-1}, Pa·s,	0,051	0,282	0,098	0,368	0,103
T, %	0	20,92	1,02	19,84	2,91

I.1.1 - experimental version of ice cream with the addition of 1% of the encapsulated version 1,
I. 1.2. - experimental version of ice cream with the addition of 2% of the encapsulated version 1,
I. 2.1. - experimental ice cream variant with the addition of 1% of the encapsulated variant 2,
I.2.2. - experimental ice cream variant with the addition of 2% of the encapsulated variant 2,

The shear stress increased and the apparent viscosity decreased with increasing shear rate in the range of 0.1 - 100 s^{-1} (**Figure 6.4.**).



a b

Figure 6.4. Evolution of shear stress and apparent viscosity as a function of shear rate (AA1 - I 2.1. AA2 - I 2.2. AC 1 - I 1.1. CA2 - I.1.2.)

Regardless of the value of the shear rate, the increase of the shear stress and also of the apparent viscosity was noticed with the addition of the functional ingredient based on biologically active compounds from lavender.

6.4.5. Sensory analysis of value-added foods

In **Table 6.2.** the average scores obtained in the sensory analysis of ice cream are presented with the addition of different experimental variants of microencapsulated powder and for control.

Table 6.2. Mean values obtained from the sensory analysis of ice cream samples with the addition of microencapsulated powders

Sensory attribute	Sample				
	M	I.1.1.	I.1.2.	I.2.1.	I.2.2.
Color	5,28±0,72^{a}	4,14±0,86^{a}	5,35±1,15^{a}	1,85±1,65	5,28±1,20^{a}
Phase separation	Nu	Nu	Nu	Nu	Nu
Sweet	5,57±0,75^{a}	5,71±1,06^{a}	5,28±1,26^{a}	5,35±1,39^{a}	5,35±1,15^{a}
Bitter	1,14±0,36^{a}	1,28±1,61^{a}	1,35±1,08^{a}	1,28±0,72^{a}	1,50±1,16^{a}
Flavor	1,21±1,18^{a}	1,21±0,57^{ab}	4,35±2,20^{c}	3,50±1,74^{d}	5,42±1,45^{d}
Rancidity	1,14±0,36^{a}	1,14±0,53^{a}	1,14±0,36^{a}	1,14±0,36^{a}	1,07±0,26^{a}
Hardness	5,00±1,24^{a}	4,14±1,79^{a}	4,50±1,34^{a}	4,00±1,46^{a}	4,35±1,69^{a}
Roughness	4,14±1,70^{a}	2,85±1,91^{ab}	4,71±1,43^{ab}	2,78±1,47^{b}	3,67±1,78^{b}

Crumbness	3,71±1,59[a]	2,71±1,63[a]	3,35±1,82[a]	2,85±1,79[a]	3,35±1,86[a]
Gummy	1,85±1,16[a]	2,42±1,74[a]	2,78±1,88[a]	2,28±1,68[a]	5,57±1,82[a]
Aeration	3,57±1,74[a]	4,00±1,88[ab]	3,28±1,43[ab]	5,00±0,96[ab]	4,21±1,42[a]
Frozen	5,07±1,59[a]	5,79±1,05[a]	5,14±1,51[a]	4,78±1,84[a]	4,71±1,81[a]
Hardness (mouthfeel)	3,92±1,85[a]	3,78±1,04[a]	3,92±1,59[a]	4,07±2,12[a]	3,57±1,91[a]
Roughness (mouthfeel)	4,14±1,83[a]	4,28±1,63[a]	3,07±1,97[a]	3,57±1,55[a]	3,78±1,92[a]
Aqueous	3,21±1,57[a]	3,28±1,77[a]	2,78±1,52[a]	3,07±1,59[a]	3,14±1,40[a]
Creaminess	3,35±1,78[a]	4,85±0,94[a]	4,28±1,54[a]	4,78±1,36[a]	4,28±1,54[a]
Unctuosity	4,42±1,34[a]	3,92±1,63[a]	4,64±1,27[a]	4,57±1,15[a]	4,42±1,47[a]
General impression	4,35±1,21[a]	5,50±1,09[a]	5,28±0,61[a]	5,71±0,72[ab]	5,50±1,02[b]

Tasters did not report significant color differences ($p>0.001$) between the experimental variants. Significant differences were reported by tasters in terms of flavor, where variants with the addition of encapsulated lavender extracts showed a more pronounced flavor, especially for samples with a 2% addition. Overall, the most appreciated sensory test ($p<0.001$) was frozen with the addition of 1% of experimental variant 2, at the opposite pole being the control sample.

6.4.6. Development of a technology for obtaining ice cream with the addition of microencapsulated powder with lavender flower extract

In this subchapter, an adapted technological scheme for obtaining ice cream with the addition of microencapsulated powder with lavender flower extract is proposed, in a variable proportion, but not more than 2%, compared to the ice cream mix. Ice cream can have a variable fat content of between 2 and 5%.

The adapted technological scheme for obtaining ice cream with the addition of microencapsulated powder with lavender flower extract, in variable proportion is presented in **Figure 6.5**.

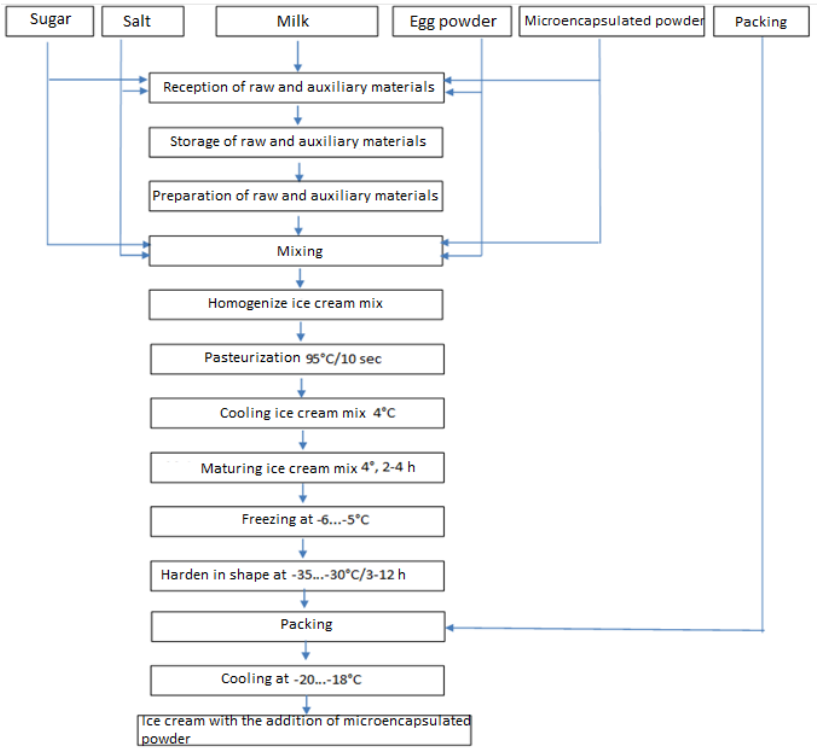


Figure 6.5. Adapted technological scheme for obtaining ice cream with the addition of microencapsulated powder with lavender flower extract

6.5. Partial conclusions

In this chapter, the possibility of capitalizing on the experimental variants obtained by microencapsulation of lavender flower extracts was tested, with the main purpose of contributing to the development of innovative technologies for obtaining food products with high functionality.

The results obtained in this chapter allowed the following partial conclusions to be drawn:

1. Taking into account the specific properties of the 4 experimental variants of microencapsulated powders, namely color, flavor, and content of biologically active compounds, the first step was to select optimal food matrices to highlight the specific flavor properties of volatile compounds such as and their release during consumption, to develop high-functional foods, especially in terms of antioxidant activity.
2. Thus, two matrices were selected: ice cream, because the sensation of cold accentuates the nuance of aroma specific to lavender and almonds. For both products, simple, easy-to-apply technologies were selected, in which experimental variants 1 and 2 were added in proportions of 1 and 2% in ice cream and variants 3 and 4 in the proportion of 1% in the almond mix.
3. In the ice cream samples, the high functionality was demonstrated by a value 1.6 times higher in the experimental version of ice cream which used a percentage of 1% of the microencapsulated version 1 and 2.5 times higher in the version with 2%.
4. In the case of experimental variants that used microencapsulated variant 2, an increase of antioxidant activity was obtained 1.6 times at an addition of 1% and 1.9 times at an addition of 2%.
5. Almond nuts showed significantly higher values of antioxidant activity, 9,40 ± 0,49 mMol Trolox/g SU in control, 9,73 ± 0,04 mMol Trolox/g SU for the variant which used microencapsulated powder V3 and 11, 28 ± 1.08 mMol Trolox/g SU for the variant that used V4, with an increase in antioxidant value was only 3.5% in the first variant and 20% in variant 2.
6. Ice cream mixes were tested for rheological properties, highlighting the fact that the samples with the addition of 1 and 2% of variant 1 showed values of modulus G 'approximately 25 times higher than the control sample at 1 Hz.
7. Irrespective of the analyzed sample, the G' values were higher than G'', suggesting that, at a temperature of 4°C, all ice cream samples retain their specific structure, showing specific behavior of solids at low-frequency values.
8. Also, for all the analyzed samples, regardless of the value of the shear rate, it was noticed the increase of the shear stress and implicitly of the apparent viscosity with the addition of the functional ingredient based on biologically active compounds from lavender.
9. Sensory analysis significantly differentiated the ice cream samples in terms of flavor, especially for the 2% addition samples.
10. Overall, the most sensory sample was frozen with the addition of 1% of the experimental microencapsulated powder variant 2.
11. An adapted technological scheme for obtaining ice cream with the addition of microencapsulated powder with lavender flower extract has been developed.

CHAPTER 7. FINAL CONCLUSIONS

In the current socio-economic context, there is a growing interest, both from the scientific, economic, and industrial environment, but also from consumers, oriented towards the link between food and health, with a significant impact on food demand. for which the potential to prevent undesirable effects has been demonstrated.

Numerous studies in the literature have highlighted, in vitro and in vivo the positive role of secondary metabolites in fruits, vegetables and various plants, with an impact on reducing the

death rate due to cardiovascular disease, common cancers, etc., the positive effects are due to the presence of various ranges of compounds in effective combinations.

Thus, the interest in compounds such as polyphenols, carotenoids, sterols, flavonoids, anthocyanins, fibers, peptides, polyunsaturated fatty acids, volatile compounds, etc. is mainly due to multiple biological effects, which include antioxidant, anti-inflammatory, anti-mutagenic, and anticancer activities. list only a few.

In this context, the approaches presented in the doctoral thesis entitled "**Characterization in bioactive compounds of some plants for use in the food industry**", which aimed to identify, quantify, and superior use of biologically active compounds in lavender flowers, for the development of potential functional ingredients, which can then be used in the development of foods with health benefits.

Lavender is intensively studied, mainly due to its content in essential oils, and less so for its content in polyphenols, chlorophylls, carotenoids, phenolic acids, flavonoids, or fatty acids. Lavender also has multiple applications in cosmetics and pharmaceuticals, in terms of essential oils, and is less known for applications in food.

Therefore, the objectives of the doctoral thesis were:

 Establishing the phytochemical profile of extracts from lavender flowers (*Lavandula angustifolia*) obtained comparatively by different extraction techniques in correlation with antioxidant properties and processing stability, from the perspective of establishing optimal extraction and storage conditions;

 Microencapsulation of biologically active compounds from extracts into ingredients with high functionality from the perspective of integration into functional foods, by improving the stability and characteristics of controlled release;

 Development of two technologies for obtaining value-added products by adding microencapsulated functional ingredients.

All three objectives were met, which allowed the formulation of partial conclusions presented at the end of each chapter of the experimental part, and some general conclusions, presented summarily, as follows:

A. From the analysis of the literature, a comprehensive study resulted in highlighting the importance of biologically active compounds for health. Lavender is an important source of essential oils, especially applicable in cosmetology. When considering biologically active compounds from different sources as elements to promote human health, many factors must be taken into account, such as: chemical structure and bioavailability, genetic factors, cultural and production factors, stability , extraction, concentration, etc.

B. Lavender flowers are rich in biologically active compounds with high physiological potential, an important aspect being given by maintaining the physiological activity of these compounds and after separation from the natural matrix, processing, conservation.

C. Lavender flowers have been suitable for extractions by different, conventional, assisted techniques, with supercritical fluids, by manipulating the extraction conditions being able to control the phytochemical profile of the extracts, thus controlling the target compounds and antioxidant properties.

D. Different extraction techniques were analyzed in terms of content in biologically active compounds, focusing on carotenoid, chlorophyll, polyphenolic, flavor and polyunsaturated fatty acids.

E. Once extracted from the basic matrix, biologically active compounds are unstable to heat processing specific to industrial applications, an effect observed for the vast majority of compounds studied, except for flavonoids, which showed high stability.

F. Thermoinstability of biologically active compounds in lavender invites the development of stabilization methods, the most widely used method in this regard being microencapsulation. The complex coacervation and lyophilization allowed the development of fine powders, with remarkable biological activities, especially antioxidant and selective antimicrobial. The powders had different morphological structures and sizes, depending on the type of extract used and the encapsulation matrix.

G. Microencapsulated powders were tested for cytocompatibility properties, with a proliferating effect on cell viability depending on concentration.

H. The functional properties of the powders were tested in ice cream and almonds. It has been shown that variants of products with the addition of microencapsulated powders have added value in terms of antioxidant activity.

I. The textural analyzes performed on the products concluded that the addition of microencapsulated powder with lavender extract in the products has beneficial effects on human health and improves the rheological and textural properties of the finished product.

J. A technological scheme has been proposed for obtaining a ice cream with the addition of microencapsulated powder with lavender flower extract, which can contribute to the development of innovative, patentable food products with technological transfer potential.

CHAPTER 8.
PERSONAL CONTRIBUTIONS AND PERSPECTIVES FOR CONTINUATION OF STUDIES

The novelty elements of the approaches presented in the doctoral thesis entitled Characterization in bioactive compounds of some plants for use in the food industry derived from the following:

The composition of biologically active compounds and the antioxidant activity of different types of extracts obtained from lavender flowers were compared, in terms of chlorophyll content, carotenoids, fatty acids, volatile compounds, flavonoids, and antioxidant activity. So far, no similar profile or study has been identified in the literature and hence the originality of the doctoral thesis.

Different techniques, spectrophotometric, chromatographic (liquid and gaseous) techniques were used and compared for the comparative characterization of the profiles, which allowed a detailed analysis, coupled with the study of the kinetic parameters of degradation of biologically active compounds in the perspective of structure-function-process correlation. , to maintain the biochemical properties in the processed finished products.

The kinetic parameters of degradation of biologically active compounds from the extracts obtained are necessary for the optimization of industrial processing conditions, from the perspective of minimizing losses and/or degradation of bioactive components.

Thermal instability opened the premises for identifying methods for stabilizing biologically active compounds, being selected coacervation, and lyophilization as methods for the development of stable powders. The results obtained provide sufficient scientific information, which has been exploited in the design of innovative ingredients for functional foods.

Therefore, the thesis stands out as elements of originality through a complete approach of the process-structure-function-product relationship, which opens as perspectives for further studies new directions, exploitation and other aromatic plants (mint, lemon, rosemary, basil, etc.), insufficiently studied so far, which is of major importance in increasing the quality of life, fermented dairy products, pastries and confectionery, sugar products, etc.).

CHAPTER 9. LIST OF PUBLICATIONS

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:

9.1. Articles in ISI journals with impact factor

1. **Radu (Lupoae), D.**, Mihalcea, L., Aprodu, I., Socaci, S.A., Cotârleț, M., Enachi, E., Crăciunescu, O., Barbu, V., Oancea, A., Dulf, F.V., Alexe, P., Bahrim, G.E., Râpeanu, G., Stănciuc, N. (**2020**). *Fostering lavender as a source for valuable bioactives for food and pharmaceutical applications through extraction and microencapsulation*. Molecules, *25*, 5001, doi:10.3390/molecules25215001, **Impact factor 3,267.**

9.2. Articles in ISI journals without impact factor

1. **Radu (Lupoae), D.**, Alexe, P., Stănciuc, N. (**2020**). *Overview on the potential role of phytochemicals from lavender as functional ingredients*. The Annals of the University Dunarea de Jos of Galati, Fascicle VI - FOOD TECHNOLOGY, *44*.

2. **Radu (Lupoae), D.,** Râpeanu, G., Bahrim, G.E., Stănciuc, N. (**2019**), Investigations on thermal degradation of phytochemicals from lavender extract.The Annals of the University Dunarea de Jos of Galati, Fascicle VI - FOOD TECHNOLOGY *43(2)*, 33-47.

9.3. Presentations at international conferences and symposia

Radu (Lupoae), D., Enachi, E., Aprodu, I., Mihalcea, L., Socaci, S.A., Cotârleț, M., Barbu, V., Dulf, F.V., Stănciuc, N. (**2019**). *Lavander as a Valuable Source of Biologically Active Compounds*, The 9th International Symposium EuroAliment, 2019, http://www.euroaliment.ugal.ro/Programme-EuroAliment-2019-B5_03.09.pdf, oral presentation.

9.4. Presentations at the Doctoral Schools conference

Radu (Lupoae), D., Alexe, P., Stănciuc, N. (**2017**), *Exploring the functional potential of lavender*, 5th Edition of CSSD-UDJG, 8th and 9th of June, Book of abstracts, p.120.

Radu (Lupoae), D., Alexe, P., Aprodu, I., Râpeanu, G., Stănciuc, N. (**2018**), *Phytochemical profile and thermal degradation kinetics of Chlorophylls and Carotenoids from lavander Extracts*, 6th Edition of CSSD-UDJG, 7th and 7th of June, Book of abstracts, p.174.

Radu (Lupoae), D., Râpeanu, G., Alexe, P., Stănciuc, N. (**2019**), *Microencapsulation biologically active compounds from lavander extract*, 6th Edition of CSSD-UDJG, 13th and 14th of June, Book of abstracts p. 224.

Radu (Lupoae), D., Mihalcea, L., Râpeanu, G., Stănciuc, N. (**2020**). Supercritical CO_2 fluid extraction of selected phytochemicals from lavender flowers: focus of volatiles and fatty acids composition of the extracts, 8th Edition of SCDS-UDJG, 18th and 19th of June 2020, p. 230.