IOSUD-„Dunărea de Jos" University of Galaţi

Doctoral school of Fundamental Sciences and Engineering

PhD thesis

Functional composites based on oleoresins and proteins for use in food industry

(PhD thesis summary)

PhD student,

Ionica (GHEONEA) DIMA

Scientific coordinator,

Prof.univ.dr.eng Nicoleta STĂNCIUC

Series I.7: Food Engineering No. 15

Galati

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The series of doctoral thesis publicly defended in UDJG starting with the 1st October 2013 are:

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Content:

Introduction

Tomato peel is obtained as a by-product in the tomato processing industry, with an abundant flow of solid waste worldwide. A large amount of waste is sent to landfills or as feed. From a quantitative point of view, but especially in terms of phytochemical profile, tomato peels presents an opportunity to capitalize on biologically active compounds, with well-defined functions for the human body and also creates an incentive for industries that facilitate the transition to bioproducts. renewable.

The doctoral thesis entitled "***FUNCTIONAL COMPOSITES BASED ON OLEORESINS AND PROTEINS FOR USE IN FOOD INDUSTRY***" aimed at studying the biochemical and functional behavior of biologically active compounds in tomato skin, mainly carotenoids, in order to obtain them.

The main scientific objectives pursued during doctoral studies are:

- Testing of different extraction methods to obtain oleoresins enriched in biologically active compounds, with determined functions, such as antioxidant activity;
- Phytochemical profiling of oleoresins from native tomato peels (Solanum lycopersicum), with the identification of biologically active compounds with an impact on antioxidant activity and evaluation of heat treatment behavior, in order to elucidate degradation mechanisms and optimize the conditions for obtaining and storing rich products carotenoids.
- Evaluation of the binding mechanisms between carotenoid compounds and whey proteins through molecular docking and molecular modeling experiments from the perspective of optimizing experimental conditions for microencapsulation of biologically active compounds in tomato peels extracts.
- Development of strategies for capitalizing on oleresins obtained by extracting biologically active compounds from tomato peels through combined microencapsulation techniques, with obtaining ingredients with multiple functional roles.
- Applied research activities by developing a technology for obtaining a functional product with added value by capitalizing on microencapsulated ingredients.

The doctoral thesis includes:

I. THE DOCUMENTARY STUDY, includes three chapters and presents recent data from the literature on the characteristics of bioactive compounds (mainly carotenoids) and the impact they have on the food industry, focusing on the beneficial effects on health.

II. **ORIGINAL CONTRIBUTIONS** includes the results of investigations carried out throughout the doctoral internship, and includes four chapters:

CHAPTER 4, entitled ***'COMPARATIVE EVALUATION OF SOME EXTRACTION METHODS APPLIED TO TOMATO PEELS FROM THE PERSPECTIVE OF CONTENTS IN BIOLOGICAL***

ACTIVE COMPOUNDS', presents the results obtained from experiments on the extraction, separation, identification and quantification of tomatoes (Solanum lycopersicum), using spectrophotometric methods, high performance liquid chromatography (HPLC) and gas chromatography (GC-MS) techniques.

CHAPTER 5, entitled "**DETERMINATION OF BINDING MECHANISMS BETWEEN BIOLOGICALLY ACTIVE COMPOUNDS FROM EXTRACTS FROM TOMATO PEELS AND WHEY PROTEINS FROM THE PERSPECTIVE OF MICRO-ENCAPSULATION**" presents the results obtained from the description of lycopene) and whey proteins, from the perspective of micro- and nano-encapsulation. Fluorescence quenching experiments were used, as well as molecular docking and molecular dynamics methods.

CHAPTER 6, entitled "***DEVELOPMENT OF HIGH-FUNCTIONAL INGREDIENTS FOR POTENTIAL USES IN FOODS***" presents the results obtained in the stages of microencapsulation and development of variants of functional ingredients and the characterization of the resulting powders, from a biological and structural point of view.

CHAPTER 7, entitled "***APPLICATIVE RESEARCH THROUGH THE DEVELOPMENT OF TECHNOLOGICAL VARIANTS FOR OBTAINING VALUE-ADDED FOOD PRODUCTS***" presents the results obtained that have contributed to the development of a technology for obtaining a product with functional potential and added value for obtaining a dressing product.

Each chapter of the experimental study is structured as follows: General aspects, Study objectives, Materials and methods, Results and discussions, Partial conclusions and Bibliographic references.

CHAPTER 8, ***FINAL CONCLUSIONS***, presents the main conclusions resulting from the investigations carried out.

CHAPTER 9, *ORIGINAL CONTRIBUTIONS AND FUTURE PERSPECTIVES* describes the main contributions to the development of knowledge in the subject and opens new perspectives for further studies.

CHAPTER 10, ***DISSEMINATION AND RESEARCH RESOULTS*** reviews the main publications and participations in national and international scientific events, which aimed to capitalize on the results obtained in the doctoral thesis.

The doctoral thesis comprises 133 pages, which includes 29 figures and 21 tables. The documentary study represents 21% and the experimental part 79%.

Finally, the original contributions of the doctoral thesis are presented, as well as the dissemination of the results obtained in the researched field. Thus, the research results were capitalized by the elaboration of 4 scientific articles published in ISI-listed journals (Journal of Food Engineering, Journal of Food Processing and Preservation, Antioxidants, Journal of Luminescence) and 10 papers at scientific events representative of the field of product engineering food, from abroad and from the country.

The research activities within the doctoral thesis were carried out with the help of the modern research infrastructure of the Integrated Center for Research, Expertise and

Technology Transfer (BioAliment-TehnIA) (www.bioaliment.ugal.ro), within the Faculty of Food Science and Engineering , “Dunărea de Jos” University of Galaţi.

The thesis was made under the scientific coordination of Prof.dr.eng. Nicoleta STĂNCIUC, as doctoral supervisor and of the guidance commission composed of: Prof.dr.eng. Gabriela RÂPEANU - coordinator of conventional extraction studies and degradation kinetics of biologically active compounds, Prof.dr.eng. Iuliana APRODU - coordinator of modeling and molecular docking studies, and Assoc. Liliana MIHALCEA - extraction coordinator with supercritical fluids.

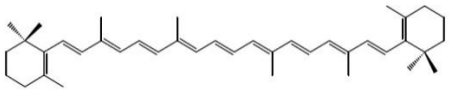
CHAPTER 1. BOTANICAL, MORPHOLOGICAL AND COMPOSITIONAL ASPECTS OF TOMATOES

1.1. Botanical and morphological aspects

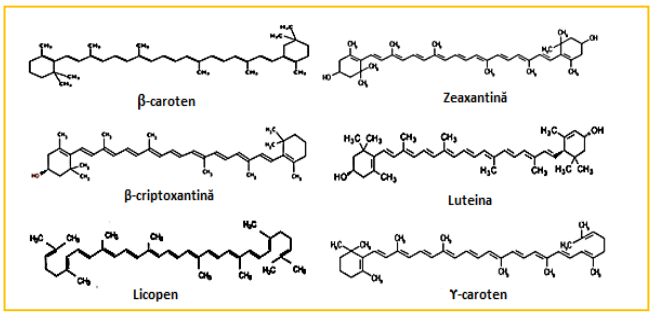
The tomato (Solanum lycopersicum) is an important shrub in the Solanaceae family. *S. lycopersicum* varieties are red in color and show great variability in terms of quality, shape, intensity of fruit color, plant habitat and leaf morphology. S. lycopersicum undergoes self-pollination processes and therefore does not require the help of insects or birds to perform pollination.

1.2. The main classes of biologically active compounds in tomatoes

Carotenoids are lipophilic pigments that give the color orange, yellow or red to many fruits and vegetables, crustaceans, some fish or egg yolks. They are biosynthesized by plants, algae, fungi and bacteria or are simply accumulated through unchanged or slightly modified diets in some animal species.



General chemical structure of carotenoids



The structure of the main carotenoids in tomatoes

Carotenoid compounds are recognized for their beneficial effect on eye health, but studies have also been reported that reduce the risk of stroke, breast cancer and improve skin health.

1.3. Health effects of biologically active compounds in tomatoes

Prevention of oxidative stress and inflammation

Carotenes and xanthophylls are important sources of singlet oxygen. Singlet oxygen results from the exposure of chromophores to sunlight. These activated molecules can degrade DNA, proteins and lipids.

Photoprotection and skin health

Sunlight is a harmful factor for the health of human skin. Ultraviolet, visible light and infrared are responsible for singlet oxygen and for the formation of radicals, especially in the presence of natural photosensitizers, such as porphyry and riboflavin. This can lead to photoaging (rough skin and wrinkles), UV-induced erythema (sunburn) and skin cancer.

Improving vision and preventing eye disease

In addition to the conversion of some carotenoids into vitamin A, evidence that humans need these compounds in their diet emerges from studies of the eye and diseases associated with aging. At the level of the retina, in its center, a yellow spot with a diameter of 5-6 mm may appear, where lutein and zeaxanthin with a high concentration found in plasma accumulate.

Cognitive decline and Alzheimer's disease

Many studies in the middle-aged population have explored the relationship between plasma β-carotene levels and cognitive decline. Clinical trials to evaluate supplements containing vitamins C and E and β-carotene in the case of heart disease and / or cancer have been supplemented with tests for cognitive decline.

Cancer prevention and treatment

Retionoid compounds have a potential anticancer effect against a wide range of experimental cancers, but toxic side effects are inseparable from their mode of action. These studies showed a low risk associated with high consumption of β-carotene or lycopene or their high blood levels and occasionally xanthophylls.

Metabolic syndrome, obesity, cardiovascular disease and diabetes

Metabolic syndrome is a cluster that includes abdominal obesity, high blood pressure, hyperglycemia, high triglycerides and low HDL cholesterol. These metabolic abnormalities increase the risk of cardiovascular disease and diabetes. The same can be observed in the case of obesity which is correlated with cardiovascular mortality, a fact highlighted by hypothetical investigative factors such as increased chronic inflammation and oxidative stress.

Carotenes through their antioxidant properties can help prevent the progression of chronic diseases related to obesity and metabolic syndrome, thus lowering mortality and morbidity.

1.4. Industrial processing of tomatoes

The industrial processing of tomatoes leads to obtaining significant quantities of by-products, of which tescovina represents 5-10%, being considered an important source of compounds with nutritional functions, such as proteins, amino acids, fatty acids, fibers and biologically active compounds with properties outstanding nutraceuticals. After the tomatoes are harvested from the crop, they are washed and sorted. Field materials are removed and the fruits

are sorted manually or electronically, then washed with steam and / or hot water. After peeling, the tomatoes are again sorted and graded for final processing, which consists of preparing various products such as jams, purees, juices and sauces. During these processes, the seeds and shells are obtained as by-products. These by-products can be recovered by various treatments by turning them into fine powders and can then be subjected to solvent-based or assisted extractions.

CHAPTER 2. THEORETICAL AND PRACTICAL ASPECTS REGARDING THE CONCEPT OF OLEORESINS AND METHODS OF OBTAINING THEM

2.1. Introduction

Being one of the richest sources of bioactive components, oleoresins find their wide application in the food and pharmaceutical industry.

2.2. Extraction of oleoresins by conventional methods

Recovery of oleoresins from plant sources can be performed by applying conventional extraction techniques, which consist of maceration with organic solvents, Soxhlet extraction, etc. These methods for conventional extraction are two-step processes that involve the use of organic solvents such as ethyl acetate, alcohols, acetone and hexane to extract oleoresin, followed by solvent removal steps. Conventional techniques have several disadvantages that need to be considered, such as the long extraction time and, more importantly, the risk of the presence of traces of solvent in the final product, with negative effects on the environment and human health.

2.3. Extraction of oleoresins from tomatoes by extraction with supercritical fluids

A frequently used technique for obtaining oleoresins from tomatoes is the extraction with supercritical fluids carried out, for example at pressures of 20-55 MPa, at temperatures of 40-80° C, at different CO_2 flows and different extraction times, which allowed to obtain variable yields of 5% -33%.

2.4. Extraction of oleoresins by enzyme-assisted supercritical fluid extraction technique

Enzyme-assisted supercritical fluid extraction may be one of the techniques to improve the extraction efficiency of biologically active compounds. Studies in the literature have shown that pretreatment of plant material with various enzymes has improved the extraction yield of polysaccharides, edible and inedible oils, proteins and biologically active compounds. This is because an enzyme can hydrolyze the cellulosic composite structure of the plant cell wall and therefore improves the recovery of both bound and free compounds.

2.5. Extraction of oleoresins by ultrasound-assisted supercritical fluid extraction technique

This technology integrates the principles of supercritical fluid extraction with ultrasonic-assisted one. The principle of ultrasound-assisted extraction is sonochemical, a phenomenon associated with cavitation and microbubble formation, when high pressure is applied to a liquid. These bubbles burst with the release of intense local energy due to important chemical and mechanical effects. Ultrasound-assisted extraction has emerged as a promising extraction technique with various benefits. The main advantage of the ultrasonic application is that it allows the extraction of both polar and non-polar compounds.

2.6. Extraction of oleoresins by extraction technique with supercritical fluids assisted by high hydrostatic pressure

The combination of high hydrostatic pressure (HHP) and supercritical fluid extraction is promising. HHP treatment is mainly used to ensure the preservation of food and the preservation of functional and organoleptic properties of food. The process has been used for the extraction of bioactive compounds from several fruits, as it can increase the permeability of cell walls and allow the diffusion of metabolites into the extraction fluid.

CHAPTER 3. THEORETICAL ASPECTS REGARDING MICRO-ENCAPSULATION OF BIOLOGICALLY ACTIVE COMPOUNDS

3.1. General aspects

Microencapsulation and nanoencapsulation are defined as a set of technologies that allow the incorporation of biologically active compounds known as "base material" using an encapsulating or "coating" material.

The major difference between micro- and nanoencapsulation is given by the particle size.

3.2. Classification criteria for microencapsulation techniques

New encapsulation techniques continue to appear and many companies in the food industry market products registered through patented technologies. The encapsulation of biologically active compounds is accomplished by a variety of methods. The 2 most used industrial processes are spray drying and extrusion**.**

3.3. The main methods used in the food industry. Benefits.

Coacervation encapsulation

The term coacervation is a process by which aqueous colloidal solutions have been separated into 2 liquid phases:

- one rich in colloids (coacervate),

- the other poor in colloids.

Parameters that influence the shape of coacervates:

The interactions between the participating biopolymers, temperature, ionic strength, pH of the reaction medium, the mixing ratio of the polymers, their molecular weight, total concentration, charge densities, all play an important role in initiating, continuing but also stopping the coacervation process. . Pressure also plays an important role, especially when using the method of extraction with supercritical fluids to obtain coacervates. Stirring speed is important in the control and size of coacervate formation. The degree of ionization of amino groups in proteins and carboxylic groups in polysaccharides depends on the pH of the medium in which they exist. Therefore, pH adjustment is essential in the coacervation process. The amount of salt present in the environment affects the ionic strength of the solution, which in turn influences the complex coacervation process.

CHAPTER 4. COMPARATIVE ASSESSMENT OF SOME EXTRACTION METHODS APPLIED TO TOMATO SKIN FROM THE PERSPECTIVE OF CONTENT IN BIOLOGICAL ACTIVE COMPOUNDS

4.1. General aspects

An efficient extraction technique is ultrasound-assisted extraction, which can be applied to the extraction of biologically active compounds. The major impact of ultrasound in a liquid medium is attributed to the acoustic cavity, which leads to cell rupture which improves the mass transfer of extractants. The power of ultrasound, intensity, temperature and density (sample to solvent ratio) are decisive factors for optimizing the extraction of biologically active compounds.

Supercritical Fluid Extraction (SFE) is a non-destructive separation process of High Pressure Extraction, performed at high pressures, based on the solvating power of fluids at temperatures and pressures above the critical point. The method is included in emerging technologies that allow obtaining solvent-free extracts. The most widely used supercritical fluid is carbon dioxide (CO_2), sometimes in the presence of cosolvent (ethanol or methanol).

4.2. Objectives of the study

The aim was to test conventional and assisted solid-liquid extraction methods (ultrasound-assisted extraction and supercritical fluid extraction - SC-CO_2) from the perspective of establishing comparative phytochemical profiles and selecting the extraction method that allows efficient extraction of carotenoid compounds, both from in terms of feasibility and phytochemical profile. Therefore, three extraction methods were selected:

- solid-liquid extraction with different solvents;
- combined solid-liquid extraction with solvents, assisted by ultrasound;
- extraction with supercritical fluids;

4.3. Materials and methods

The tomatoes (Solanum lycopersicum L.) were purchased from the local market (Galati) in August 2018 (during the maximum ripening period), were washed, the moldy or altered parts were removed, cut into large pieces and then processed to level laboratory, using a tomato shredder. Thus, when obtaining the tomato juice, the tomato seeds and skin were collected, lyophilized and then stored at an average temperature of 20 ° C, in the dark

4.3.1. Materials

Reagents

- ABTS • + (2,2'-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid))
- Potassium persulfate, K2S2O8
  - Hexan
  - Acetone (HPLC grade)
  - Ethanol 70% (HPLC grade)
  - Methanol (HPLC grade)
  - Ethyl acetate (HPLC grade)
  - Ultrapure water

Equipment:

 High precision analytical balance, XS 403 SM, METTLER TOLEDO, Switzerland

 Ultracentrifuge with cooling, HETTICH Universal 320 R, Germany

 UV-VIS Spectrophotometer Biochrom Libra S22, 2017

 Vacuum concentrator AVC 2-18, CHRIST

 Freeze dryer

 Thermo Finnigan Surveyor HPLC system coupled with a UV-visible DAD detector (Finnigan Surveyor LC, Thermo Scientific, USA)

 Ultrasound bath (MRC Scientific Instruments)

 Vortex

 Block heater (Stuart SBH200D, UK)

 The supercritical CO2 extraction installation endowed by the University of the Lower Danube in Galați, Faculty of Food Science and Engineering, NATEX Prozesstechnologie GesmbH, Ternitz, Austria (http://www.sia.ugal.ro/respia/imbunatatire.html)

 Gas Comatograph (GC) coupled with mass spectrometer (MS), PerkinElmer Clarus 600 T GC-MS (PerkinElmer, Inc., Shelton, CT, USA)

 Rancimat mode 892 (Metrohm LTD, Herisau, Switzerland)

 Colorimeter CHROMA Meter CR - 410 (Konica Minolta, USA)

 Software HyperChem release 8.0 (Hypercube, Inc., Ontario, Canada)

4.3.2. Extraction of biologically active compounds of freeze-dried tomato peels by solid-liquid extraction with various solvents

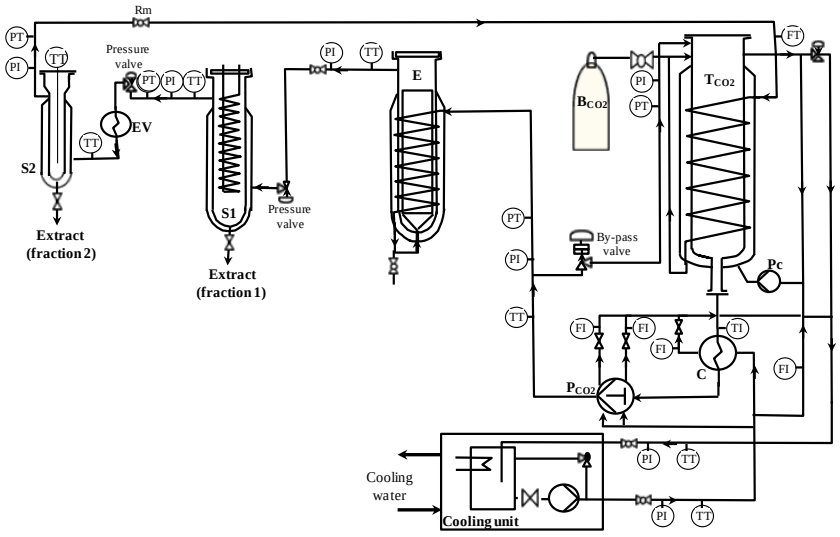
The sample (1 g) was homogenized (in turn, with each solvent) with 10 mL, acetone, hexane, methanol, 70% ethanol, ethyl acetate, hexane / acetone mixture in a ratio of 1: 1 (v / v) and hexane / acetone, ratio of 3: 1 (v / v) and homogenized using a vortex at room temperature for 5 min, in order to extract the carotenoid compounds then rest for 40 min. The samples were centrifuged at 9000 rpm at 10 ° C for 10 minutes, then the supernatant was collected. The resulting supernatant was concentrated at 40 ° C under reduced pressure to dryness (AVC 2-18, CHRIST).

4.3.3. Extraction of biologically active compounds from lyophilized tomato peels by combined solid-liquid extraction with various solvents and ultrasound

The sample (1 g) was homogenized, in turn, with 20 ml of each solvent (hexane, acetone, 70% ethanol, ethyl acetate, methanol, hexane / acetone mixture, 3: 1 ratio (v / v) and hexane / acetone mixture, 1: 1 (v / v) ratio, then homogenized with a vortex at room temperature for 2 min after which they were at rest for 40 minutes for the extraction of carotenoid compounds. ultrasound, for 30 min and maintained at an optimum temperature of 40 ± 3 ° C. Each sample was then centrifuged at 9000 rpm at 10 ° C for 10 minutes. temperature 40˚C under reduced pressure until dry (AVC 2-18, CHRIST).

4.3.4. Extraction of biologically active compounds from lyophilized tomato peels by extraction with supercritical fluids

Obtaining selective extracts in terms of target biologically active compounds will involve variations in extraction parameters: pressure, temperature, time, solvent and / or cosolvent flow in order to establish the optimal conditions for obtaining extracts with high concentrations of biologically active compounds target (lycopene, total carotenoids, β-carotene, etc.). The equipment used in the experimental development component for SC-CO2 extraction is shown schematically in the figure below:



The installation within University of "Dunarea de Jos" Galati, Faculty of Food Science and Engineering

TCO2 - CO2 tank; BCO2 - CO2 cylinder; E - extractor (C30); S1 - separator 1 (S40); S2 - separator 2 (S45); PCO2 - high pressure pump for CO2; Pc - circulation pump for CO2: 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.

4.3.5. Determination of total carotenoids, β-carotene and lycopene content

All extracts obtained were analyzed to determine the content of total carotenoids, β-carotene and lycopene by spectrophotometric methods, which involved dissolving an amount of the extract in the extraction solvent and determining the absorbance at wavelengths of 470 nm for total carotenoids, 450 nm for β-carotene and 503 nm for lycopene. For the calculation of the carotenoid concentration the relation was used:

$$Carotenoids\ \left({}^{mg}/_{g}\right) = A\ x\ M_W\ x\ V/(\varepsilon\ x\ M_a\ x\ L)$$

where: A = Absorbance at wavelengths of 470 nm, 450 nm and, respectively, 503 nm, Mw: molecular mass of lycopene and β-carotene, respectively (536,873 g / mol, 536,826 g / mol); V = volume of the solution; Ma = mass of extract taken into account (g), L = cuvette length (1 cm), fd = dilution factor, ε = extinction coefficient (εCT = 2590, εβ-carotene = 2500, εlic = 3450 (L / mol * cm).

4.3.6. Determination of antioxidant activity

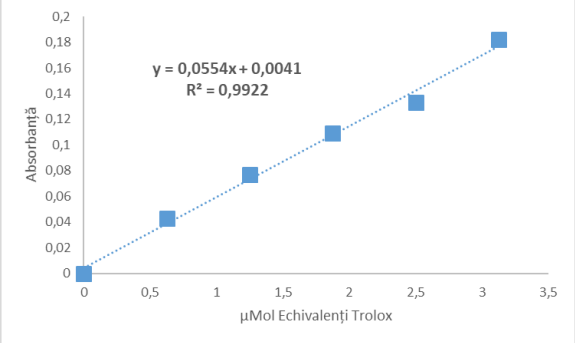
The antioxidant activity of the extracts obtained was measured using the ABTS radical scavenging test [2,20 azinobis (3-ethylbenzothiazole-6-sulfonic acid)]. The determination of the antioxidant activity was performed using the ABTS • + cation radical method.

Materials:

Dissolve ABTS in deionized water to 7 mM. ABTS • + is generated by oxidation of ABTS (2,2 azinobis 3-ethylbenzothiazoline-6-sulfonic acid) with 2.45 mM potassium persulfate. The reaction to form the ABTS radical takes place in the dark, at room temperature for at least 12-16 hours. Thus the stock solution of radical ABTS is obtained. The stock solution of ABTS is diluted with ethanol to an absorbance of 0,700 ± 0,020 at 734 nm (usually 1/90).

Working protocol:

To determine the antioxidant activity, over 0.15 mL of extract dissolved in the extraction solvent was added over 2.85 mL of ABTS • + reagent. The reaction takes place in the dark for two hours. The standard curve shown in the figure below was used to express the antioxidant activity in μMol Trolox / mL:



Standard curve used to determine antioxidant activity in µMol Trolox / mL

4.3.7. Identification of carotenoid compounds in selected extracts by high performance liquid chromatography (HPLC) of carotenoids

HPLC analysis was performed by comparing the retention times of the carotenodes in the samples studied with those of the standards, as well as with data from the literature. For the chromatographic profile of the obtained extract, a Thermo Finnigan Surveyor HPLC system was used coupled with a UV-visible DAD detector (Finnigan Surveyor LC, Thermo Scientific, USA), controlled by Xcalibur software. The carotenoid compounds in the tomato extract were analyzed at 450 nm using a Lichrosorb RP-18 (5 pm) Hibar RT 125-4 column. The mobile elution phase consisted of two solvents, namely 90% acetonitrile (A) and 100% ethyl acetate (B). The injection volume was 10 µL, while the flow rate was maintained at 1000 ml / min. The elution gradient was: 0-16 min, 15% B; 16-54 minutes, 15-62% B, 54-56 min, 62% B; 56-60 min, 62-15% B; 60-70 min, 15% B. Quantification of lycopene and β-carotene was done using the calibration curves for each compound.

4.3.8. Determination of total lipid fatty acids in selected extracts

To determine the fatty acids from the total lipids, the extract obtained by solid-liquid extraction with hexane and acetone was selected, 3: 1 ratio followed by ultrasound at 40 ° C, 30 minutes.

4.3.9. Evaluation of the behavior of carotenoids in selected extracts for heat treatment

For the heat treatment experiments, the extract was weighed and dissolved in commercial sunflower oil at a concentration of 25 mg / mL. The volumes of 0.20 ml of oil were distributed in glass tubes (1 cm in diameter) and subjected to heat treatment in the temperature range between 100 and 145 ° C, for variable time intervals (0-40 min), using a block heater (Stuart SBH200D, UK). A heating time of 30 s was used for each experiment. After heat treatment, the tubes were immediately cooled in ice water to prevent further degradation.

4.3.10. Kinetics of denaturation reactions of biologically active compounds

The degradation kinetics of total carotenoids, β-carotene and lycopene, as well as antioxidant capacity, were described by the first order kinetic model, described by the equation:

$$\frac{C}{C_0} = e^{-kt}$$

Where: C is the parameter to be estimated, the index 0 indicates the initial value of the parameter, t is the heating time and k is the rate of degradation constant at temperature T (1 / min).

The half-life (t1 / 2) of the reaction was calculated assuming first-order kinetics according to the equation:

$$t_{1/2} = -\ln 0.5 / k$$

The Arrhenius model was used to describe the temperature dependence of both degradation rate constants as described by the equation:

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

where

E_a is the activation energy (kJ / mol),

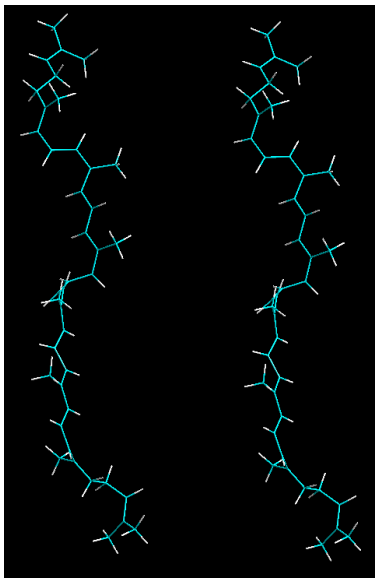
k_{ref} is the rate of degradation constant at the reference temperature (1 / min),

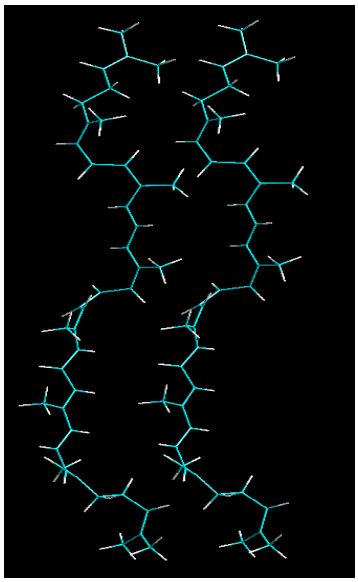
T is the absolute temperature (K) and

R universal gas constant (8,314 J / mol K).

4.3.11. Molecular modeling investigations on the behavior of lycopene at different temperatures

HyperChem release 8.0 software was used to simulate the thermal behavior of the lycopene molecule. (Hypercube, Inc., Ontario, Canada).





Optimized models of complex 1 (a) and complex 2 (b) consisting of two lycopene molecules each

4.3.12. Evaluation of the oxidation stability of oils enriched with tomato peels extract by the Rancimat method

The evaluation of the oxidation stability of some oils enriched with tomato skin extract by the Rancimat method was performed according to a method described by Nour et al. (2018), using Rancimat mode 892 equipment (Metrohm LTD, Herisau, Switzerland).

4.3.13. The color of the extracts in cotton oil and peanut oil

CieLab parameters (L *, a *, b *) for peanut and cotton oils enriched with carotenoid extracts from tomato peels were determined with a CHROMA Meter CR-410 colorimeter (Konica Minolta, USA). Measurements were performed in triplicate.

The L * parameter represents the brightness that varies from 0 (black) to 100 (white), the a * parameter provides information about the red / green axis and varies from -100 (green) to +100 (red) and the b * parameter evaluates the axis yellow / blue and ranges from -100 (blue) to +100 (yellow). The experiments were performed in triplicate.

4.3.14. Statistical analysis

All experiments were performed in triplicate. The results were expressed in terms of mean values. Statistical data analysis was performed using the data analysis toolkit from the Microsoft Excel software. The effects of temperature on kinetic parameters were evaluated by a univariate analysis of variance (ANOVA) with a significance level of 95% (p = 0.05) using the Tukey test. The coefficient of determination (R2) and the mean error were used as a criterion for the adequacy of the adaptation of the experimental values to the first order kinetic model.

4.4. RESULTS AND DISCUSSIONS

4.4.1. Comparative evaluation of the overall phytochemical profile of extracts from tomato peels obtained by solvent extraction, ultrasound-assisted extraction and extraction with supercritical fluids by spectrophotometric methods

For extraction with organic solvents, the following were used: acetone, hexane, 70% ethanol, methanol, ethyl acetate, hexane: acetone (1: 1, v / v) and hexane: acetone mixture (3: 1, v, v). The results obtained by extraction with organic solvents are presented in the table below:

Solvent used	Total Carotenoids, g %	Lycopene, g %	β-carotene, g%	Antioxidant activity µMol Trolox/g su
Ethanol 70%	50,47±0,77	30,81±1,43	52,65±0,55	20,80±0,28
Methanol	25,13±0,94	11,27±0,48	26,81±0,84	50,28±1,78
Hexane	89,91±3,23	53,04±1,86	62,32±2,05	**90,90±0,38**
Acetone	115,32±0,43	67,05±2,38	87,41±0,24	73,03±1,85
Athyl acetate	117,53±0,55	74,08±2,18	90,86±0,32	75,25±0,79
Mixure of	125,62±2,80	77,41±2,93	94,38±2,24	69,30±2,84

hexane:acetone (1:1, v/v)				
Mixure of hexane:acetone(3: 1, v/v)	**131,21±1,09**	**81,80±0,15**	**98,18±0,43**	66,71±0,55

Content of biologically active compounds and antioxidant activity of extracts obtained by solid-liquid extraction with organic solvents

Conventional solid-liquid extraction was combined with ultrasonic-assisted extraction at a constant frequency and power of 40 kHz for 30 minutes. The results obtained by extraction with organic solvents in combination with ultrasonic assisted extraction are presented in the table below:

Solvent used	Total carotenoids, g/100 g	Lycopene, g/100 g	β-carotene, g/100 g	Antioxidnt activity µMol Trolox/g su
Ethanol 70%	17,55±1,14	9,52±0,35	16,46±0,66	85,12±0,91
Methanol	25,01±0,84	11,12±0,46	23,55±0,89	49,38±1,15
Hexane	78,24±0,89	44,86±2,21	53,34±2,40	**87,65±1,03**
Acetone	89,55±4,47	55,78±0,40	68,20±6,90	68,27±1,37
Ethyl acetate	89,12±2,81	53,19±1,73	68,54±2,22	73,33±0,99
Mixure of hexane:acetone (1:1, v/v)	108,45±0,77	64,70±0,31	82,50±0,55	73,87±0,28
Mixure of hexane:acetone (3:1, v/v)	**102,15±4,80**	**62,76±1,08**	**78,22±1,58**	74,89±0,65

The table below shows the experimental results obtained in the extraction with supercritical fluids:

The content of biologically active compounds and the antioxidant activity of extracts obtained by supercritical CO2 extraction

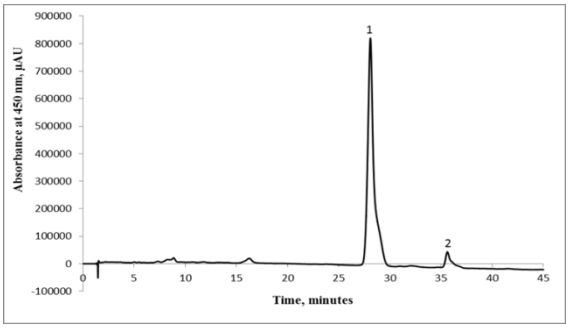
Extract	Biological active compounds					
	Lycopene (mg/g su)		β-carotene (mg/g su)		Antioxidant activity (mMol Trolox/g su)	
	S40	S45	S40	S45	S40	S45
Extract (70 °C)	6.06 ± 0.06^{b}	9.48 ± 0.41^{c}	10.88 ± 0.33^{b}	18.93 ± 0.73^{c}	48.52 ± 3.63^{b}	24.35 ± 0.71^{c}
Extract (74 °C)	5.28 ± 0.07^{b}	39.11 ± 0.59^{a}	12.57 ± 0.11^{a}	68.24 ± 0.71^{a}	77.61 ± 0.99^{a}	62.74 ± 1.74^{a}
Extract (80 °C)	8.11 ± 0.65^{a}	30.59 ± 0.63^{b}	11.85 ± 0.59^{ab}	47.08 ± 1.05^{b}	39.99 ± 1.02^{c}	38.34 ± 2.13^{b}

4.4.2. Comparative evaluation of the individual phytochemical profile of extracts from tomato peels obtained by solvent extraction, ultrasound-assisted extraction and extraction with supercritical fluids by chromatographic methods

From the analysis of the results presented in subchapter 4.4.1, the following extracts were selected for chromatographic analysis:

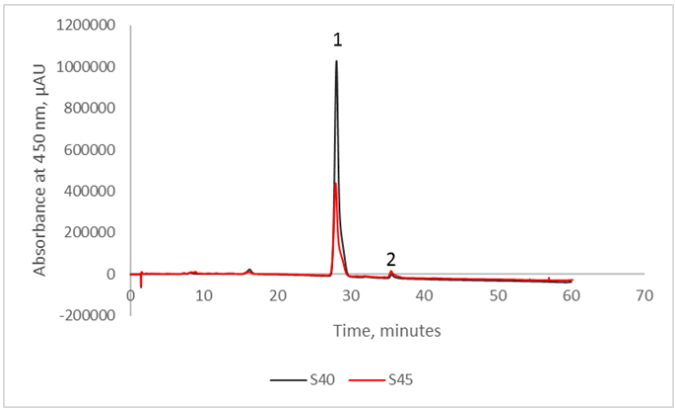
- The extract obtained by combined solid-liquid extraction and ultrasound, using as solvent the mixture of hexane: acetone 3: 1 and ultrasound at a constant frequency of 40 kHz, power of 100 W, for 30 minutes at 40 ° C.

Chromatographic profile of the extract obtained by combined solid-liquid extraction and



ultrasound, using as solvent the mixture of hexane: acetone 3: 1 and ultrasound for 30 minutes at 40 ° C (peak 1 - lycopene, peak 2 - β-carotene)

- The two fractions (S40 and S45) obtained from the extraction with supercritical fluids, obtained at a temperature of 74 ° C.

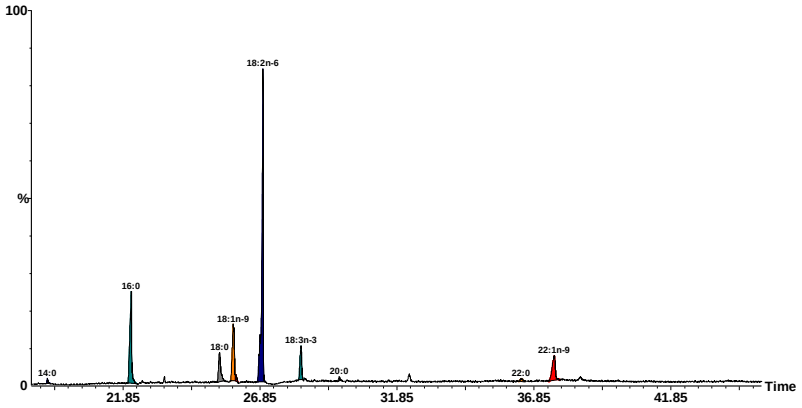


Chromatographic profile of tomato skin extracts obtained by extraction with supercritical CO2 at 450 nm (S40 - separate extract fraction at 20MPa in the first separator, S45 - separate extract fraction at 5MPa in the second separator).

4.4.3. Evaluation of the fatty acid profile of extracts from tomato peels obtained by solvent extraction combined with ultrasound-assisted extraction

To identify the fatty acid content of the extracts obtained from tomato peels, the extract obtained by combined solid-liquid and ultrasonic extraction was selected, using as solvent the mixture of hexane: 3: 1 acetone and ultrasound at a constant frequency of 40 kHz, power of 100

W, for 30 minutes at 40 ° C, with depletion in biologically active compounds by repeating the extraction four times. The following figure shows the chromatogram for identification and quantification of the fatty acid profile:



GC-MS chromatogram to identify and quantify the profile of total fatty acids in tomato pomace extract, obtained by solid-liquid extraction in combination with ultrasound

Fatty acid composition (% of total fatty acids) in tomato pees extract, obtained by solid-liquid extraction in combination with ultrasound

Fatty acid	% fatty acid (percentage of area)
Miristic acid(14:0)	0,49±0,02
Palmitic acid(16:0)	17,26±0,89
Stearic acid (18:0)	6,56±0,23
Oleic acid (18:1n-9)	12,64±0,56
Vaccenic acid (18:1n-7)	0,75±0,05
Linoleic acid (18:2n-6)	47,42±1,02
α-linolenic acid (18:3n-3)	5,73±0,11
Arahidic acid(20:0)	0,50±0,09
Behenic acid(22:0)	0,71±0,05
Erucic acid (22:1n-9)	7,94±0,34
AGSs	25,52
AGMSs	21,32
AGPNs	53,15
n-3 AGPN	5,73
n-6 AGPNs	47,42
n-6/n-3	8,28
AGPN/AGM	2,08

Abbreviations: AGS- saturated fatty acids, AGMN- monounsaturated fatty acids, AGPN-polyunsaturated fatty acids.

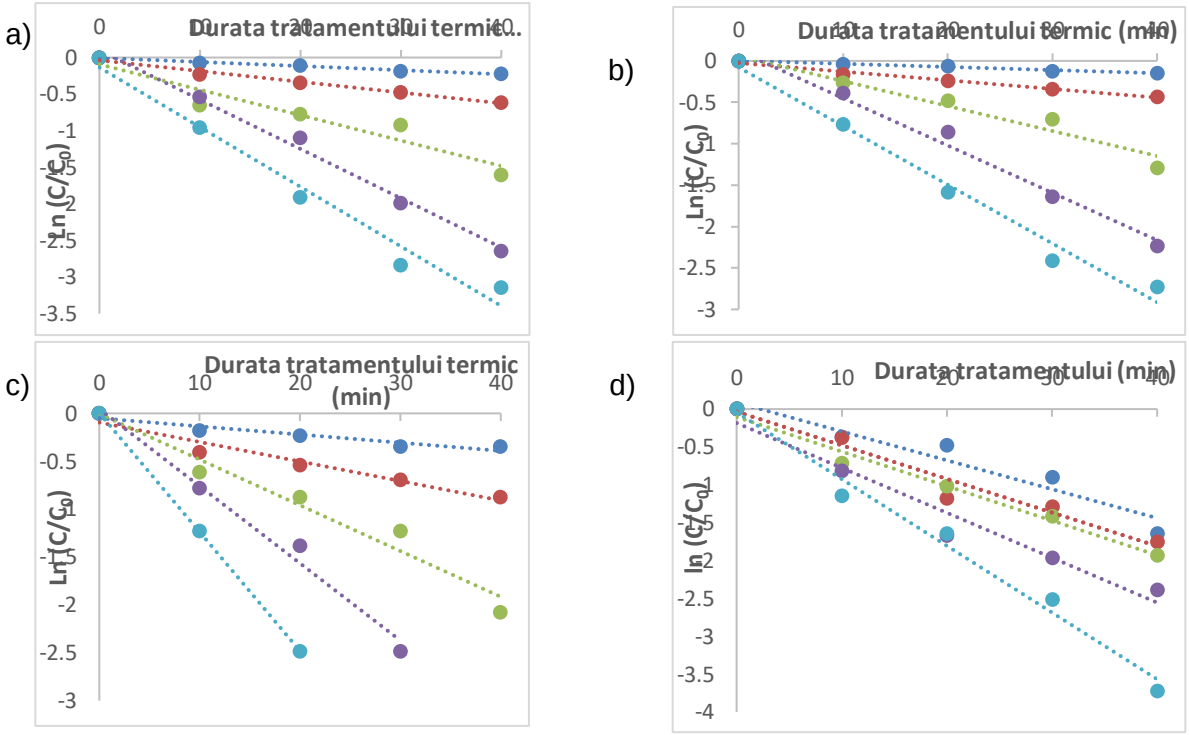
4.4.4. Evaluation of the stability of biologically active compounds and antioxidant activity in heat treatment

Kinetic parameters for estimating the behavior at different thermal regimes of biologically active compounds, described by the constant rate of thermal degradation, half-life and activation energy are extremely necessary to optimize heat treatment treatments at the industrial level, to

reduce the impact of processing thermal effects on the nutritional and functional potential of foods.

The figure below shows the linear regression lines, which show a decrease in the concentration of biologically active compounds at constant temperature as a function of heating time.

Graphical representation of linear regression lines for first-order thermal degradation of total carotenoids (a), β-carotene (b), lycopene (c) and antioxidant activity (d) of tomato pomace extract in the temperature range of 100-145 ° C with holding time 0- 40 min (□100 ° C, □115 ° C, □125 ° C, □135 ° C and □145 ° C). The values represent the arithmetic mean of three independent determinations



The estimated kinetic parameters are presented in the table below:

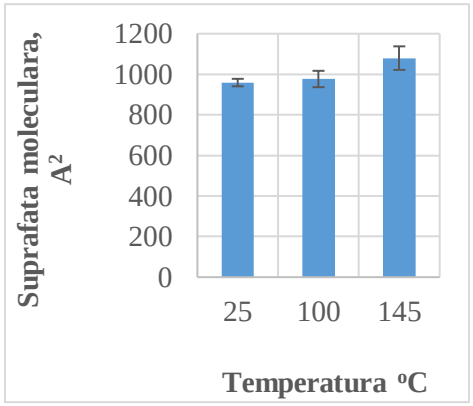
Temperature °C	Total carotenoids		β-carotene		Lycopene		Antioxidant activity	
	$k \cdot 10^{-2}$ (1/min)	$t_{1/2}$ (min)	$k \cdot 10^{-2}$ (1/min)	$t_{1/2}$ (min)	$k \cdot 10^{-2}$ (1/min)	$t_{1/2}$ (min)	$k \cdot 10^{-2}$ (1/min)	$t_{1/2}$ (min)
100	0.56±0.02	123.77±4.13	0.38±0.02	182.40±11.53	0.85±0.04	81.54±2.79	3.82±0.12	18.14±1.12
115	1.47±0.02	47.15±1.85	1.04±0.03	66.64±3.60	2.04±0.02	33.97±2.00	4.44±0.18	15.61±0.75
125	3.47±0.01	19.97±1.16	3.01±0.04	23.02±1.77	4.78±0.01	14.50±0.98	4.56±0.32	15.20±1.38
135	6.47±0.18	10.28±0.63	5.71±0.11	12.13±0.99	8.06±0.40	8.59±1.65	5.93±0.33	11.68±1.74
145	8.15±0.23	8.50±1.51	7.09±0.14	9.77±0.09	12.42±0.20	5.58±1.58	8.81±0.45	7.86±1.25
E_a (kJ·mol^{-1})	**81.76±6.82**		**89.81±8.48**		**79.66±4.03**		**22.20±5.79**	

Estimated kinetic parameters (degradation rate constant - k, half-life - t1 / 2 and activation energy - Ea) for the degradation of biologically active compounds from tomato skin extract into vegetable oil

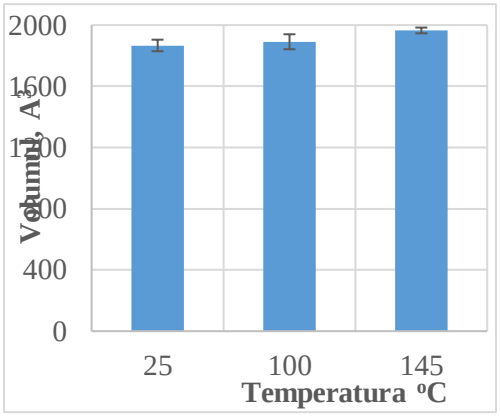
4.4.5. The temperature behavior of lycopene molecules estimated by molecular modeling techniques

The silico approach was adopted to collect information at the molecular level on the behavior of lycopene in heat treatment. Analysis of QSAR properties after performing molecular dynamics steps at different temperatures between 25 ° C and 145 ° C indicated that, regardless of the distance between molecules of the same type in the initial optimized models, heat treatment causes gradual tension of covalent bonds in the structure. lycopene. Thus, when the temperature was raised from 25 ° C to 100 ° C, a slight change in molecular surface area was recorded from 959.16 ± 18.44 to 976.77 ± 40.29 Å2.

a)



b)



Effect of heat treatment on van der Waals surface (a) and volume of lycopene molecules (b)

Total carotenoid content, antioxidant activity and protection factor Rancimated of control and enriched oils, as well as color results.

4.4.6. Evaluation of the potential use of extracts as antioxidants in various oils

	Umiditate %	Total carotenoids (µg/g)	Antioxidant activity(%)	Protction factor Rancimat
Peanuts oil				
Control	0.81±0.01	272.84±3.28	89.61±0.52	1.00±0.01
TPE:PO (1:2)	1.51±0.12	377.25±9.04	81.74±2.09	1.01±0.02
TPE:PO (2:1)	1.37±0.01	481.66±14.81	94.82±1.49	1.06±0.04
TPE:PO (3 g)	2.40±0.01	586.07±20.59	89.60±0.53	1.04±0.01
Cotton oil				
Control	1.23±0.01	327.80±6.70	86.70±0.75	1.00±0.00
TPE:CO (1:2)	1.22±0.01	434.07±9.70	81.74±2.09	1.01±0.01
TPE:CO (2:1)	0.81±0.01	556.70±23.11	99.54±0.16	1.02±0.02
TPE:CO (3 g)	2.025±0.01	646.64±36.94	86.71±0.75	1.03±0.01

4.4.7. Colour

The values of the color parameters for the oils enriched with tomato peels extract

Oils enriched with different extract ratios showed brightness values (L *) which ranged from 65.86 to 83.36 for enriched peanut oils and from 66.27 to 83.95 for cotton oils, which shows the transition from lighter oils to darker oils, corresponding to those with added extract.

	L*	***a****	***b****
Peanuts oil			
Control	94.23±0.01	-1.26±0.01	6.97±0.01
TPE:PO (1:2)	83.36±0.03	11.90±0.22	59.74±0.88
TPE:PO (2:1)	79.84±0.17	18.62±0.39	80.15±0.68
TPE:PO (3 g)	65.86±0.21	22.15±0.10	65.65±0.41
Cotton oil			
Control	91.19±0.16	-6.55±0.16	34.03±1.71
TPE:CO (1:2)	83.95±0.57	8.00±0.82	56.68±2.95
TPE:CO (2:1)	80.60±0.22	15.26±0.32	75.05±0.57
TPE:CO (3 g)	66.27±0.13	21.92±0.19	65.89±0.29

(Abbreviations: CO, cottonseed oil; PO, peanut oil; TPE, tomato peels extract).

4.5. Partial conclusions

The experimental results obtained allow the elaboration of partial conclusions, as follows:

1) For the solid-liquid extraction, the efficiency of different solvents was tested: ethanol, methanol, acetone, ethyl acetate, hexane: acetone mixture, in different ratios of 1: 1 and 3: 1, respectively.
2) The experimental results showed that the use of the mixture of hexane: acetone, in a ratio of 3: 1 allows the extraction of a high content of total carotenoids (131.21 ± 1.09 g / 100 g), lycopene (81.80 ± 0, 15 g / 100 g), β-carotene (98.18 ± 0.43 g / 100 g),

compounds that contributed to the quantification of an antioxidant activity of the extracts of 66.71 ± 0.55 µMol Trolox / g su.

3) Regarding the antioxidant activity, hexane determined the extraction of various compounds, in addition to carotenoid compounds, which had a greater contribution to the antioxidant activity ((90.90 ± 0.38 µMol Trolox / g su).
4) Solid-liquid extraction in combination with ultrasound-assisted extraction tested the same solvents. And in this case, the combination of hexane: acetone, in a ratio of 3: 1 determined the highest concentration of extracted compounds (total carotenoids - 102.15 ± 4.80 g / 100g, lycopene - 62.76 ± 1.08 g / 100 g and β-carotene, respectively - 78.22 ± 1.58 g / 100g), hexane in a single combination leading again to an extract with superior antioxidant activity (87.65 ± 1.03 g / 100 g).
5) 5) The comparison between the two tested methods showed a higher efficiency of the conventional extraction to recover the biologically active compounds tested.
6) 6) Because the initial testing of the methods did not provide a stage of depletion of tescovine in biologically active compounds by repeated extractions, but only a quantitative analysis to allow the selection of working parameters, following the study, the favorable extraction conditions were selected, the combined solid-liquid extraction using as extraction solvent the mixture of hexane: acetone in a ratio of 3: 1 was applied repeatedly (4 times), in order to deplete in biologically active compounds.
7) In the case of extraction with supercritical fluids, the extraction conditions were tested based on the literature, such as: pressure 40 MPa, temperatures 70 ° C, 74 C and 80 ° C and extraction time of 155 min.
8) 2 fractions were obtained for each temperature, the two separate parts, which were analyzed from the point of view of the global phytochemical profile. In the case of the S40 separator, at a pressure of 20 MPa and the increase of the temperature from 70 ° C to 80 ° C there was an increase in the degree of extractability of lycopene, obtaining extracts with a content of 6.06 ± 0.06 mg / g su and, respectively, 8.11 ± 0.65 mg / g su, with an increase of about 34%. In the case of β-carotene extraction, the concentration in the extract was positively correlated in the temperature range 70 ° C - 74 ° C.
9) The increase in temperature from 70 ° C to 80 ° C did not have a significant influence on the gravimetric efficiency (3.39% for S40 and 6.18% for S45 at 70 ° C and 3.81% and 5, respectively).). , 99% at 80 ° C).
10) The results obtained indicated that the extraction with supercritical CO2 was more favorable for the extraction of bioactive compounds at a temperature of 74 ° C, and a significantly higher concentration was obtained in the second separator (S45). From the analysis and correlation of the results, two extracts were selected for further experiments:
11) Extract obtained by combined solid-liquid extraction and ultrasonication, using as solvent the mixture of hexane: 3: 1 acetone and ultrasound at a constant frequency of 40 kHz, power of 100 W, for 30 minutes at 40 ° C. The two fractions (S40 and S45) obtained from the extraction with supercritical fluids, obtained at a temperature of 74 ° C. These extracts were used for chromatographic analysis (carotenoids), but also for microencapsulation experiments.

12) Chromatographic analysis revealed the presence of lycopene as the majority, with different concentrations depending on the type of extraction. The highest concentration was presented by the S40 fraction, with a lycopene concentration of 0.875 mg / mL, followed by the extract obtained by combining conventional extraction and ultrasound (0.449 mg / mL), and the lowest lycopene concentration was identified and quantified. in the fraction S45 (0.345 mg / mL).
13) The fatty acid profile showed a concentration of about 48% linoleic acid (18: 2n-6), followed by palmitic acid (16: 0) with 17% and oleic acid with about 13%. The lipid fraction of the extract under analysis was highlighted by a higher concentration of polyunsaturated acids, of 53% and a lower value of monounsaturated fatty acids, of 21%. N-3 acids had a contribution of about 6% of the total fatty acid content, while n-6 acids had a significantly higher contribution of about 48%.
14) The thermal degradation of the bioactive compounds from tomato peels extract followed a first order kinetic model, highlighting a very high tendency of degradation of lycopene at lower temperatures, of 100 ° C. The increase of the temperature up to 145 ° C determined a significant increase of the values of the degradation rate constants for all the studied compounds.
15) The values of the estimated heat degradation rate constants, which evaluated the impact of heat treatment on antioxidant activity, were significantly higher compared to the corresponding values of carotenoid degradation throughout the studied temperature range, which indicates that antioxidant activity is determined cumulatively by the compounds studied.
16) The half-lives of the compounds were estimated at each temperature, which showed the following interrelationship: β-carotene> total carotenoids> lycopene >> antioxidant activity.
17) Activation energy values for total carotenoids, β-carotene, lycopene and antioxidant activity were estimated at 81.76 ± 6.82 kJ / M, 89.81 ± 8.48 kJ / M, 79.66 ± 4, 03 kJ / M and 22.20 ± 5.79 kJ / M, respectively.
18) The silico approach allowed the detailed analysis of the conformation of the balanced molecular models at different temperatures and revealed that the increase of the temperature determined the gradual bending of the tetraterpenic chains. Consequently, at temperatures above 100 ° C, the isomerization of all trans-lycopene molecules tends to form a 9.9 'cis conformation.
19) The initial distance between the tetraterpene chains played an important role in modulating the behavior of lycopene, influencing the dynamics of molecules at all temperatures investigated. When simulating the molecular behavior at 25 ° C, the interaction energy between the two lycopene molecules was three times higher for Complex 1 compared to Complex 2, which suggested the development of more intense forces of attraction between molecules when the concentration is not very high and the available space allows the free rearrangement of the relative position of the two molecules.
20) The functionality of the extracts was tested as an antioxidant ingredient for two commercial oils, peanut and cotton, and the results showed that the oxidative stability of

cotton oil increased with increasing concentrations of added extract, in a linear dose-dependent response . The use of tomato peels extract as an antioxidant was less effective in cotton oil.

21) The color parameters of the enriched oils showed the influence of adding the oil color extract, resulting in darker shades, with high degrees of red and yellow.
22) The results obtained certify the biologically active potential and the functional value of these by-products resulting from the processing of tomatoes, which can be exploited as a particularly valuable and alternative resource of biologically active compounds.

CHAPTER 5. ESTABLISHMENT OF BINDING MECHANISMS BETWEEN BIOLOGICALLY ACTIVE COMPOUNDS FROM TOMATO PEELS AND WHEY PROTEIN EXTRACTS FROM THE PERSPECTIVE OF EFFICIENT MICROCAPSULATION

5.1. General aspects

Proteins have a potential role in the encapsulation, protection and targeted delivery of bioactive components in functional foods, due to their ability to form protein-ligand complexes, protecting components related to oxidation and degradation processes and also providing means of release induced by stimuli.

5.2. Objectives of the study

The main objective of the study was to evaluate the binding mechanisms between lycopene and whey proteins, from the perspective of establishing binding parameters, thermodynamic parameters and identifying the forces involved in the interaction, to make encapsulation more efficient. The investigations involved the use of experiments to quenching (quenching) the fluorescent intensity of proteins in the successive titration with lycopene-enriched extracts of tomato peels and of simulation methods by molecular docking and molecular dynamics.

The stages of the study were:

- advanced extraction and characterization of carotenoids from tomato peels, with emphasis on lycopene, from the perspective of determining the content of total carotenoids, β-carotene and lycopene, as well as the antioxidant activity of extracts from tomato peels by spectrophotometric methods;
- study of binding mechanisms by fluorescence spectroscopy (quenching studies);

- calculation of binding parameters and thermodynamic parameters;
- study of binding mechanisms by molecular docking and methods of simulation of molecular dynamics;
- description of the binding mechanisms from the perspective of optimizing the microencapsulation conditions.

5.3. Materials and methods

5.3.1. Materials

β-LG (purity> 90%, genetic variants A and B) from bovine milk was purchased from Sigma (Sigma - Aldrich Co., St. Louis, MO). All reagents used in the analyzes had analytical purity.

The ripe tomatoes were purchased from a local market and stored at 4 ° C for a maximum of 2 days before use. The peels was dried at room temperature (20-22 ° C) in the dark for 4-5 hours. They were then wrapped in aluminum foil and stored at 4 ° C for up to 24 hours before use.

5.3.2. Equipment

The Perkin Elmer LS 55 luminescence spectrofluorimeter (PerkinElmer Life Sciences, Shelton, CT) from the Integrated Research, Expertise and Technology Transfer Center (BioAliment-TehnIA, https://www.unicer.ugal.ro/) was used to evaluate the fluorescent properties.

5.3.3. Lycopene extraction

Five grams of dried plant material were mixed for the extraction of lycopene with 35 ml of ethanol: hexane (4: 3, v / v) solutions containing 0.05 g of magnesium carbonate on an orbital shaker for 1 hour at room temperature. After extraction, the supernatant was separated and the residue was re-extracted with 70 ml solutions of ethanol: hexane (4: 3, v / v). The resulting residue was washed with 25 ml of ethanol and then with 12.5 ml of hexane. The residue was washed again with 100 ml of 10% NaCl and 150 ml of water. The lycopene extract was concentrated at 40 ° C to dryness, dissolved in 10 ml of ethanol (70%) and filtered through 0.45 μm membranes. Lycopene was quantified using a colorimetric method. To quantify the lycopene content of the extract, the absorbance of 503 nm was determined. The concentration of lycopene in the extract was calculated using the extinction coefficient of 3150 1 / M * cm.

5.3.4. Determination of total carotenoids, β-carotene and lycopene content

The resulting extracts were diluted in the extraction solvent at a rate of 1 mg / ml and the absorbance was read at 470 nm for total carotenoids, 450 nm for β-carotene and 503 nm for lycopene. The concentration was expressed in mg / g.

5.3.5. Chromatographic quantification of lycopene in tomato extract

To identify and quantify the carotenoid pigments in the tomato peels extract, a chromatographic analysis was performed. The system used was an HPLC from Thermo Finnigan Surveyor (Finnigan Surveyor LC, Thermo Scientific, USA), controlled by the Xcalibur

software system. Carotenoids were analyzed at 450 nm on a Lichrosorb RP-18 (5 μm) Hibar RT 125-44 column. The elution solvents were 90% acetonitrile (A) and 100% ethyl acetate (B). The injection volume was 20 μL and the flow rate was maintained at 0.800 ml / min.

5.3.6. Heat treatment of protein solutions

The plastic tubes (1 cm in diameter) were filled with 0.150 ml of 1 mg / mL β-LG solution. The samples were heated at different temperatures between 25 ° C and 90 ° C for 15 minutes, using a thermostatic water bath (Digibath2 BAD 4, Raypa Trade, Barcelona, Spain). The samples were then cooled in ice water to avoid any further thermal denaturation of the protein.

5.3.7. Quencing experiments using tomato extract

The untreated and heat-treated protein samples (0.100 mL of 1 mg / mL β-LG in 0.01 M Tris-HCl solution at pH = 7.7) were diluted in 3 mL of appropriate buffer and titrated by successive addition of lycopene extract diluted (1:10) in ethanol. The excitation wavelength was set at 292 nm, while the emission spectra were collected from 310 nm to 400 nm with steps of 0.5 nm.

5.3.8. Molecular modeling studies

The crystal structure of β-LG of bovine origin was taken from the RCSB Protein Database. The β-LG model was defined by the elimination of all other compounds accompanying the protein, optimized in vacuo, solvated using water molecules and optimized in the aqueous medium. To simulate the effect of the heat treatment applied on β-LG in the experiment, the solvated model was further heated to 25 ° C, 60 ° C and 90 ° C using a Berendsen thermostat and definitively balanced by the procedure reported by Aprodu et al. (2017), so as to reduce the temperature and energy oscillations of the systems.

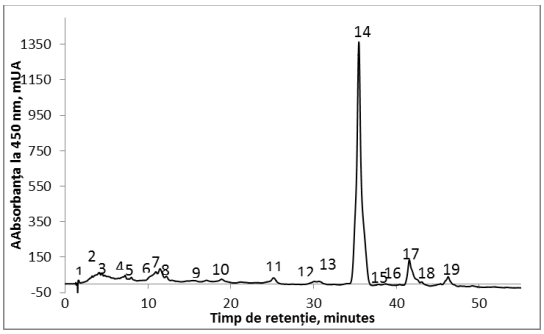
5.3.9. Statistical analysis

All experiments were performed in triplicate with duplicate samples. The data obtained were analyzed using Microsoft Office version Excel 2007. Values are represented as means ± standard deviation. For multiple comparisons, the unidirectional ANOVA test was used and the p-value was less than 0.05.

5.4. RESULTS AND DISCUSSIONS

5.4.1. Characterization in biologically active compounds of tomato peels extract

Prior to the quenching experiments, a detailed characterization of the lycopene extract was performed by chromatographic techniques. The typical HPLC chromatogram for carotenoids is shown in the figure below:

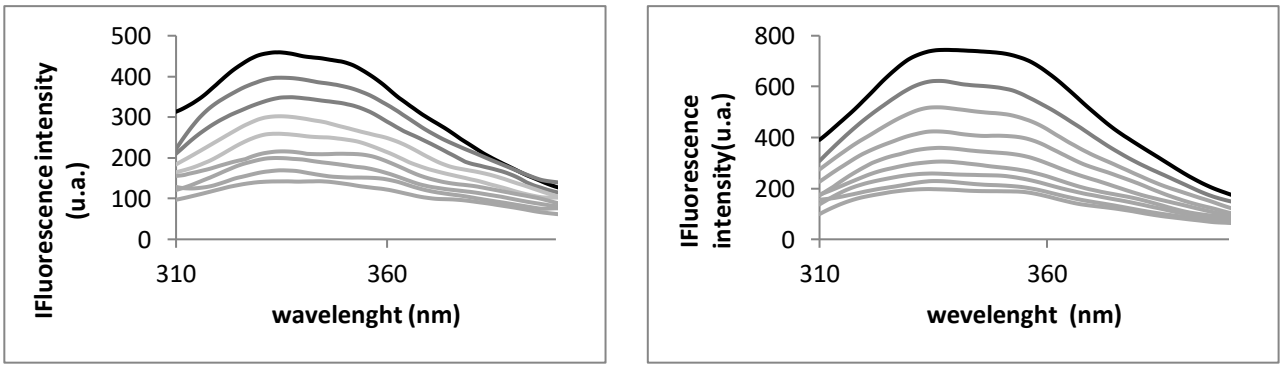


Chromatographic profile of carotenoids in tomato peels extract

5.4.2. Evaluation of the binding mechanisms between lycopene and whey proteins by fluorescence spectroscopy

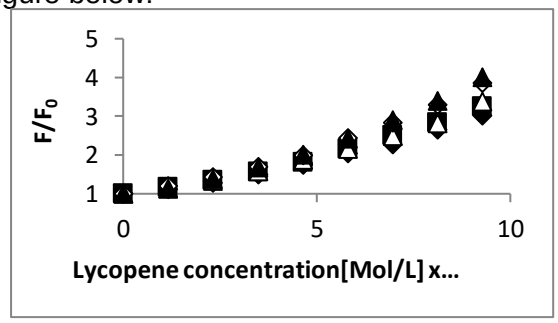
Emission spectra at two different temperatures (25 ° C and 90 ° C) are shown in the figure below. The fluorescence of β-LG molecules was extinguished successively with increasing concentration of the extract, as a consequence of the interaction between proteins and carotenoids in the extract.

a) b)



Emission spectra showing the interaction between β-lactoglobulin molecules and lycopene extract at 25 ° C and 90 ° C. The lycopene concentration (from a-f) ranged from 0 to 2.91 • 10−5 M / L.

To determine whether the binding mechanism between β-LG and lycopene extract is static or dynamic, the data were analyzed using the Stern-Volmer equation, the Stern-Volmer representations are given in the figure below:



Stern-Volmer representation for bovine β-LG quenching experiments and lycopene extract at 25 ° C (), 60 ° C (), 70 ° C (Δ), 80 ° C (□) 90˚C (▲)

The binding parameters for the interaction between β-LG and lycopene extract at different temperatures are shown in the table below:

T (˚C)	K_{SV} (10^{-7}/ mol)	K_q (10^{-15}L/mol/s)	K_b (10^{-7}L/mol)	*n*	K_a (10^{-7}L/mol)	f_a
25	0.22±0.03[a]	0.22±0.03	1.04±0.01	1.09±0.05	8.75±0.91	0.59±0.12
60	0.24±0.04	0.24±0.04	0.88±0.08	0.76±0.09	7.19±0.60	0.60±0.13
70	0.25±0.07	0.25±0.07	0.83±0.04	0.74±0.08	5.75±0.33	0.83±0.11
80	0.30±0.07	0.30±0.07	0.79±0.03	0.71±0.06	5.69±0.17	0.89±0.09
90	0.32±0.07	0.32±0.07	0.72±0.09	0.67±0.06	3.26±0.17	1.03±0.34

[a] – standard deviation
K_{SV} – Stern-Volmer constant estimated
K_q – constant extinction rate;
K_b și *n* – binding constant and estimated number of binding sites

*K*a – affinity constant și f_a -the accessible fraction calculated

5.4.3. The calcul of binding and thermodynamic parameters

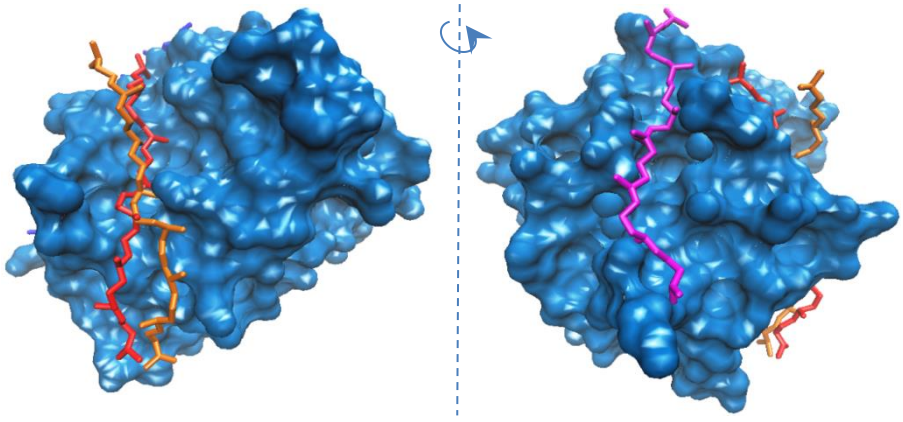
Different forces can appear in the interaction between a ligand and biomolecules, such as: van der Waals, hydrogen bonds, electrostatic forces and hydrophobic interactions. The thermodynamic study allows obtaining useful information about the forces involved in these interactions, based on the values and sign of the thermodynamic parameters.

The thermodynamic parameters for the interaction between β-LG and lycopene are presented in the table below:

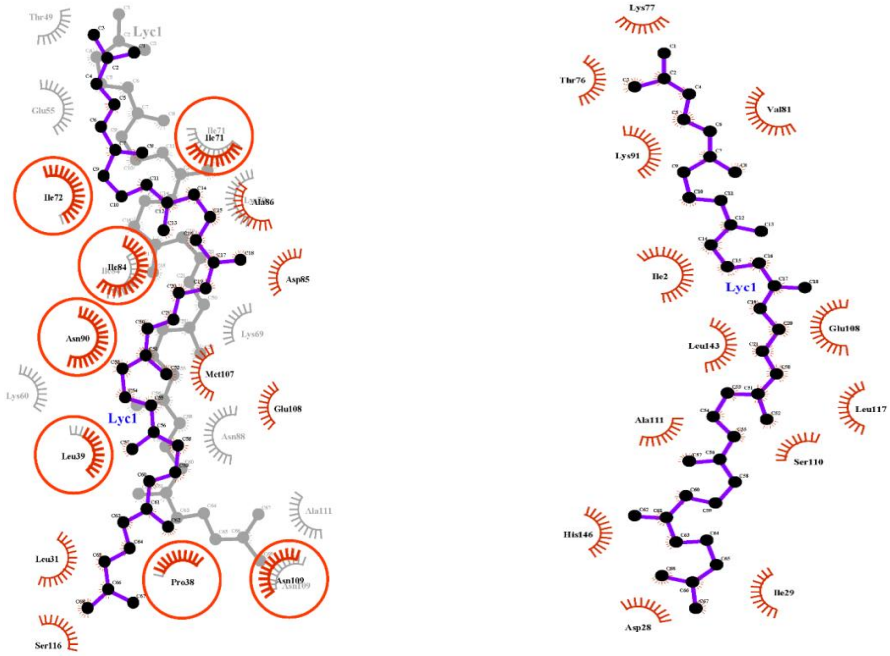
T(K)	*ΔH* (J/mol)	*ΔS* (J/mol/K)	*ΔG* (J/mol)
298	-68.19±1.50	-2.16±0.54	575.68±11.25
333			651.30±10.69
343			672.91±10.47
353			694.51±10.12
363			716.12±11.17

5.4.4. Evaluation of the binding mechanisms between lycopene and whey proteins through molecular docking and molecular modeling experiments

Molecular modeling was further used to detail the binding mechanism between the two compounds at the molecular level. Molecular dynamics was first used to heat and balance the protein to 25 ° C, 60 ° C and 90 ° C.



Details of β-LG lycopene binding patterns balanced at different temperatures. β-LG is represented in Surf style in blue, while the ligand is represented in Licorice style in red, orange and magenta at 25 ° C, 60 ° C and 90 ° C respectively. Complex overlap was prepared using Visual Molecular Dynamics (VMD) software



Hydrophobic contacts established between the amino acids β-lactoglobulin (represented by arcs that radiate to the ligand atoms with which they are in contact) and the lycopene molecule. (a) Overlapping molecular models balanced at 25 ° C (colored) and 60 ° C (gray) (b)

5.5. Partial conclusions

The results obtained allowed the following partial conclusions to be described:

1) The application of the solvent extraction technique allowed to obtain an extract with a high content of biologically active compounds, and the chromatographic technique allowed the identification of 19 compounds, with the following quantitative distribution: lycopene> β-carotene> zeaxanthin di-palmitate> astaxanthin > zeaxanthin> lutein> γ-carotene> cis lycopene> α-carotene> cis β-carotene> cis γ-carotene> 15 - δ-carotene.
2) The application of heat treatments to β-lactoglobulin solutions determined the appearance of partial conformational changes in the entire temperature range studied, monitored by successive red-shifts in the maximum wavelength at emission (λmax), suggesting an increase in hydrophobicity in the vicinity of fluorophores. Thus, the heat treatment determined a red-shift of 2 nm in λmax at temperatures up to 80 ° C and 4 nm by heating at 90 ° C, which suggests the relocation of Trp residues in a more polar environment, which In most cases, it involves exposure to the solvent.

3) Lycopene extract significantly extinguished the fluorescence of fluorophores in the protein structure, monitored by red-shifts of 7 nm and 10 nm at 80 ° C and 90 ° C, respectively, at the maximum emission wavelength (λmax), indicating the fact that the addition of lycopene extract caused the partial loss of compact protein conformation, exposing the hydrophobic subdomain where Trp19 is placed.
4) The fluorescence quenching mechanism is dynamic, with an increase in K_{SV} values as the temperature increases from 25 ° C to 90 ° C.
5) The values of binding constants were lower with increasing temperature, suggesting some changes in binding sites along with changes in the binding capacity of lycopene extract by β-lactoglobulin. Thus, Kb values decreased with increasing temperature from 1.58 ± 0.27 × 10−5 mol / L at 25 ° C to 1.06 ± 0.012 × 10–5 mol / L at 90 ° C, suggesting the change or modification of the binding sites together with changes in the binding capacity of the lycopene in the extract by β-LG.
6) The number of binding sites was approximately equal to 1 at 25 ° C and decreased with increasing temperature, indicating that heating led to the formation of a class of independent binding sites.
7) The thermodynamic parameters allowed the evaluation of the forces involved in the interaction. Thus, the negative values of enthalpy and entropy led to the conclusion that the interaction between β-lactoglobulin and lycopene is coordinated by van der Waals forces and hydrogen bonds.
8) Computational analysis allowed the identification of amino acids involved in the binding of lycopene to the β-lactoglobulin molecule. Due to the small rearrangements of the side chains when heating proteins from 25 ° C to 60 ° C, some differences in ligand binding above the protein calyx were observed, as well as local reorientations of the EF loop region, where the main ligand binding site is located. protein molecule.
9) Heat treatment at higher temperatures led to a significant disturbance of the conformation of β-lactoglobulin, which affects the binding affinity of lycopene. Docking studies performed with β-lactoglobulin balanced at 90 ° C showed that lycopene binds preferentially to the site at the interface between monomers.
10) The results obtained in this study described in detail the binding mechanism between the main protein in whey and the main carotenoid in tomato peels, facilitating the understanding of the ways of interaction and the appreciation of the stability of complexes under different conditions.

CHAPTER 6. DEVELOPMENT OF HIGH-FUNCTIONAL INGREDIENTS FOR POTENTIAL USES IN FOODS

6.1. General aspects

Efforts to produce functional foods at the industrial level are successful due to the ability of some compounds to benefit human health. Functional foods are products enriched with compounds with increased biological activity, with additional health benefits such as vitamins, minerals and bioactive compounds.

6.2. Objectives of the study

In this chapter, we studied the possibility of developing ingredients with multiple functionality, which capitalize on the potential added value of tomato peels as a valuable source of biologically active compounds. Two combined microencapsulation techniques of the extracts selected in the previous chapters were tested, namely emulsification, complex coacervation and lyophilization. The resulting powders were analyzed for the efficiency of microencapsulation of biologically active compounds, phytochemical content and antioxidant activity, antimicrobial activity, structural and morphological appearance as well as antiproliferative and cytotoxic properties.

6.3. Materials

Whey protein isolate (95% protein content) was purchased from Fonterra (Auckland, New Zealand). Hexane, acetone, acetonitrile, ethyl acetate and methanol were of HPLC purity; [2,2-azinobis- (3-ethylbenzothiazolin-6-sulfonic acid) diammonium salt] (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), acacia gum, and lycopene and β-carotene standards were purchased from Sigma-Aldrich (Steinheim, Germany).

Equipment:

- Libra S 22 biochrome spectrophotometer from the BioAliment platform equipment,
- High precision analytical balance, XS 403 SM, METTLER TOLEDO, Switzerland
- Ultracentrifuge with cooling, HETTICH Universal 320 R, Germany
- Vacuum concentrator AVC 2-18, CHRIST
- pH meter S 20 K, Mettler Toledo, Switzerland,
- Orbital shaker with analog control of stirring frequency and thermostat, Lab Companion Comecta SA,
- Orbital shaker IKA T18 Ultra Turrax,
- Martin Christ Alpha Freeze Dryer 1-4

6.4. Conventional solvent-liquid extraction with solvents combined with ultrasonic extraction

A quantity of 100 g of tomato peels was homogenized with 100 mL of magnesium carbonate, 20% with maintenance on the orbital stirrer at a temperature of 25 ° C for 2 hours. Then, 120 mL of hexane: acetone (3/1; v / v) mixture was added for extraction, followed by introduction into the ultrasonic bath for 30 min at a maximum temperature of 50 ° C. The sample was then centrifuged at 9000 rpm for 10 min at 10 ° C. The extraction was repeated 10 times, and the total supernatant obtained was collected and concentrated at a temperature of 40˚C under reduced pressure until dry (AVC 2-18, CHRIST).

6.5. Extraction of carotenoids with supercritical fluids

A quantity of 165 g of fresh and crushed tomato peels was introduced into the supercritical fluid extraction plant in the C30 feed compartment. The CO2 flow was maintained during the extraction period between 2.5 ~ 4.5 mL / min., The pressure maintained between 20 ~ 50 Mpa and the temperature of 70 °, 74 ° and 80ºC. Fractions S40 and S45 were collected for

their characterization into biologically active compounds and their microencapsulation in different protein matrices.

6.6. Experimental variants of microencapsulation of biologically active compounds in tomato peels

Various encapsulation methods and matrices were used for microencapsulation of carotenoid compounds in tomato peels extract.

12 microencapsulation variants were performed, in which carotenoid extract dissolved in oil (sunflower and grape seed oil), whey protein isolate in different concentrations, soybean vegetable proteins (lecithin), carboxymethylcellulose, transglutaminase, acacia gum. Methods for obtaining encapsulated powders are coacervation and lyophilization.

6.7. Determination of carotenoid and lycopene content in experimental variants

Lycopene and the total carotenoids were determined by the spectrophotometric method described in Chapter 4.3.5. An amount of 0.2 g of concentrated extract was dissolved in 11 mL of hexane / acetone mixture (3/1; v / v), followed by the determination of absorbance at different wavelengths, respectively 470 nm, 450 nm, 503 nm.

6.8. Determination of antioxidant activity using the ABTS method

The method for determining antioxidant activity has been described in subchapter 4.3.6.

6.9. Chromatographic determination of bioactive compounds in tomato peels extracts and powders

The method for determining the chromatographic profile of the analyzed samples was described in subchapter 4.3.7.

6.10. Efficiency of microencapsulation of carotenoid compounds in microencapsulated extracts

For variants 2-11 of microencapsulation, the total and surface content of the carotenoids in the microencapsulated powder by solvent extraction was determined.

The encapsulation efficiency was calculated according to the equation:

$$EE, \% = \frac{Total\ lycopene - Surface\ lycopene}{Total\ lycopene} * 100$$

6.11. Structure and morphology of microencapsulated powders using confocal microscopy

The structure and morphology of the obtained powders were determined using a Zeiss LSM 710 confocal laser scanning microscopic system (Carl Zeiss Microscopy GmbH, Cologne, Germany). The technical specifications of the LSM 710 microscope are: laser diodes (405 nm), Ar laser (458, 488 and 514 nm), DPSS laser (diode pump for solids 561 nm) and HeNe laser (633 nm). The distribution of bioactive compounds in the complex biopolymer matrix was observed using apochromatic target 20 and magnification 0.6. The obtained powders were observed both in their native state and fluorescently labeled with Congo Red (40 μM), in a ratio

of 3: 1. Confocal powder images were captured and analyzed with ZEN 2012 SP1 software (Black Edition, Carl Zeiss Microscopy GmbH, Jena, Germany).

6.12. Structure and morphology of microencapsulated powders using electron scanning microscopy

Examination of the structure of selected samples 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 5000 and 100,000X.

6.13. Kinetics of thermal degradation of bioactive compounds in microencapsulated powders

A volume of 1 mL solution with microencapsulated powder (8.5 mg / mL in 0.1 M sodium phosphate buffer at pH 6.9) was introduced into glass tubes and sealed. The tubes were then subjected to heat treatment in the range of 110-140 ° C, the time maintained for heat treatment varying between 0 and 40 minutes. After heat treatment of the samples, the glass tubes were immersed in ice water for about 2 minutes in order to prevent possible further thermal degradation. The samples thus obtained were diluted with 1 mL of 0.1 M sodium phosphate buffer at pH 6.9 at which the absorbances were measured at the wavelengths corresponding to total carotenes and lycopene, respectively 470 nm and 503 nm. The obtained results were adapted to a first order kinetic model.

6.14. α -amylase and α-glucosidase inhibition activity

Selected experimental variants of microencapsulated powders were dissolved in sodium phosphate buffer (0.1 M and pH 6.9) with a concentration of 8.5 mg / mL.

6.15. Antimicrobial activity

Testing of antimicrobial activity against indicator microorganisms (Aspergillus niger MIUG M5 and Bacillus subtilis MIUG B1) was performed using 0.5 g of microencapsulated powder, homogenized with 45 mL of sterile PDA culture medium (Potato Dextrose Agar), pH = 5.4 (for molds), PCA (Plate Count Agar) for B. subtilis and TSA (Tryptic Soy Agar) (for S. agona), tempered at 42 ° C, and then distributed in Petri plate. The solidified medium was inoculated, in the center of the plate, with 5 µL suspension of indicator microorganism, having a final concentration of 1x107 spores / mL. The plates were thermostated at 25 ° C for 5 days for mold and 37 ° C for 24 h (for bacteria). In parallel, control samples were performed, in conditions similar to the samples to be analyzed. After thermostating, the diameters of the colony of the indicator microorganism in the control sample (DM) and the sample to be analyzed (DP) were measured, and expressed in mm. The growth inhibition ratio was calculated using the following equation:

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

6.16. Study of the cytotoxicity of microencapsulated compounds

Cell culture assays were performed in accordance with SR EN ISO 10993-5 for the cytotoxicity of medical devices using the direct contact culture method and Neutral Red (RN) for the quantitative assessment of cell viability.

The cell line of mouse fibroblasts NCTC clone L929 were cultured on MEM medium supplemented with 10% (v / v) FCS, 2 mM L-glutamine and 1% (v / v) PSN mixture in humidified atmosphere with 5% CO2, at temperature of 37 ° C to subconfluence.

6.17. Study of the stability of the phytochemical content during storage

For the study of storage stability, the powders were stored in light-resistant glass containers at a temperature of 25 ° C for 21 days, and changes in the phytochemical content were observed.

6.18. Statistical analysis

All experiments were performed in triplicate, with samples in duplicate. The results were expressed in terms of mean values. Statistical analysis of the data was performed by univariate analysis of variance (ANOVA) with a significant level of 95% ($p < 0.05$) using the Tukey test.

6.19. RESULTS AND DISCUSSIONS

6.19.1. Comparative analysis of the encapsulation efficiency of microencapsulated experimental variants

Microencapsulation has been used in order to obtain high quality powders, minimizing the oxidative degradation of biologically active compounds in extracts. The production of microcapsules was performed by a combined method, which used emulsification, complex coacervation and lyophilization as a drying technique, obtaining fine, orange powders.

The table below shows the values recorded for the encapsulation efficiency of the main biologically active compounds from the experimental variants obtained.

Encapsulation efficiency (EE) for microencapsulated powder variants

Variants	Total carotenoids, %	β-carotene, %	Lycopene, %
V1	**89.78±0.41**	**89.61±0.31**	**83.6±0.20**
V2	6.49±0.69	0.41±1.44	5.06±0.001
V3	19.93±0.001	17.94±1.8	22.31±0.61
V4	21.64±7.51	13.13±3.71	12.85±4.39
V5	21.22±1.37	17.67±1.63	20.27±1.83
V6	-	-	-
V7	19.74±2.69	16.37±3.12	19.50±3.42
V8	-	-	-
V9	38.25±2.96	38.11±3.29	36.14±3.5
V10	46.87±9.73	46.31±8.49	46.08±8.46
V11	49.43±3.54	44.59±4.75	49.22±4.00
V12 (S40)	**51.94±1.94**	**51.63±1.61**	**37.08±4.3**
V12 (S45)	**51.16±1.31**	**50.07±1.55**	**40.52±2.14**

6.19.2. Phytochemical characterization of microencapsulated powders and evaluation of the stability of compounds in controlled storage

The table below shows the phytochemical profiles of the experimental variants in terms of the content of total carotenoids, lycopene and β-carotene, as well as the variation of the concentration of these compounds in storage for certain selected variants.

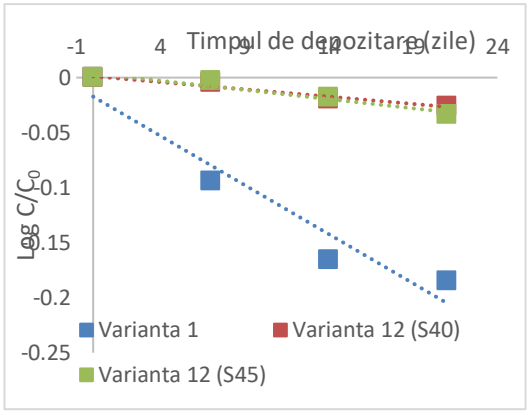
Stability of biologically active compounds in storage at 4 ° C, 21-28 days

Encapsulation variant	Total carotenoids, mg/g s.u.	β-carotene, mg/g s.u.	Lycopene, mg/g s.u.	ABTS µg Trolox/g s.u.
V1				
Initial	46.75±0.30	34.97±0.13	29.09±0.20	1.77±0.05
7 days	37.64±0.11	29.15±0.08	22.68±0.07	2.29±0.02
14 days	31.94±0.51	26.46±0.50	18.19±0.37	2.39±0.02
21 days	30,56±0,45	25,67±0,55	17,90±0,34	
V2				
Inițial	13.81±0.001	13.64±0.001	9.52±0.001	nd
7 days	5.36±0.45	5.09±0.47	3.35±0.34	nd
14 days	4.85±0.18	4.61±0.31	2.97±0.14	nd
21 days	5.60±0.25	5.49±0.42	3.38±0.31	nd
V3				
Inițial	16.38±0.001	16.22±0.12	11.57±0.001	nd
7 days	4.62±0.09	4.31±0.07	2.84±0.07	nd
14 days	4.64±0.11	4.42±0.05	2.87±0.07	nd
21 days	5.04±0.41	4.92±0.38	3.04±0.27	nd
V4	9.19±0.07	8.61±0.05	5.2±0.05	nd
V5	23.60±0.59	21.55±0.66	13.85±0.4	nd
V6	7.88±0.11	7.15±0.11	5.07±0.08	nd
V7	14.89±0.44	13.81±0.46	8.84±0.33	nd
V8	4.50±0.59	4.10±0.65	2.95±0.43	nd
V9	3.70±0.31	3.47±0.31	2.04±0.22	nd
V10				
Inițial	3.38±0.18	3.37±0.15	2.34±0.12	2.11±0.07
7 days	2.44±0.20	2.34±0.20	1.69±0.14	2.34±0.01
14 days	1.99±0.06	1.90±0.05	1.40±0.05	2.57±0.02
21 days	2.26±0.06	2.15±0.05	1.57±0.03	4.52±0.28
V11				
Inițial	7.14±0.62	5.68±0.60	4.86±0.48	2.82±0.06
7 days	8.63±0.51	7.07±0.53	5.73±0.33	2.96±0.03
14 days	3.35±0.02	2.95±0.03	2.24±0.01	2.67±0.11
21 days	4.16±0.10	3.59±0.11	2.76±0.08	3.92±0.27
V12 (S40)				
Inițial	26.28±1.28	25.57±1.14	12.03±0.81	10.10±0.87
7 days	25.99±0.49	25.32±0.44	11.70±0.28	11.12±0.33
14 days	24,88±1.03	29.39±0.80	15.39±0.67	12.15±0.60
21 days	24.76±0.48	23.73±0.48	10.82±0.32	9.71±0.08
V12 (S45)				
Inițial	21.95±0.47	21.60±0.69	11.97±0.27	10.42±0.21
7 days	21.81±0.46	21.37±0.5	11.81±0.16	10.78±0.20
14 days	21.41±1.41	31.35±1.15	18.82±0.90	11.40±1.20
21 days	20.30±0.15	20.05±0.12	10.85±0.08	8.86±0.38

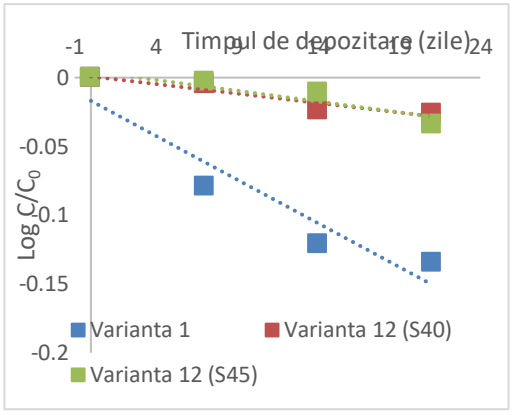
For the kinetic analysis of the data, three experimental variants were selected, respectively variants 1, 12 (S40) and 12 (S45).

The figure below shows the linear regression lines that describe the variation of the concentration of biologically active compounds during storage, described by the first order kinetic model.

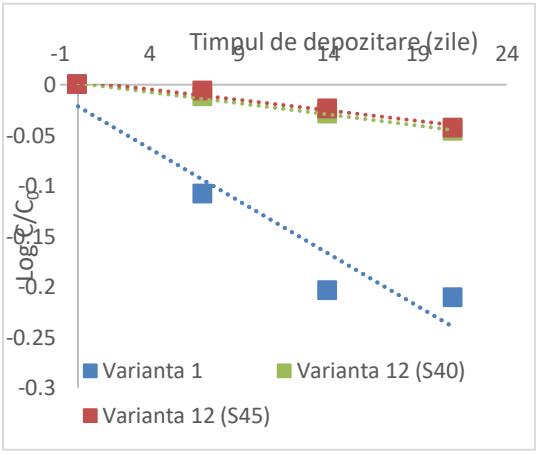
a)



b)



c)



Linear regression lines describing the variation of the concentration of biologically active compounds during storage (a - total carotenoids, b - β-carotene, c - lycopene)

Kinetic parameter values describing variation in total carotenoid, β-carotene and lycopene content during storage of experimental variants of microencapsulated powders

Variant	k (days^{-1})x 10^{-3}			$t_{1/2}$ (days)		
	Total carotenoids	β-carotene	Lycopene	Total arotenoids	β-carotene	Lycopene
V 11 (S40)	2,99±0,78	3,22±0,88	5,06±0,36	231,51±1,23	214,98±4,56	136,80±2,35
V 12 (S45)	3,91±0,67	3,68±0,97	4,83±0,78	177,04±1,89	188,10±3,21	143,32±2,78
V 1	20,49±0,99	14,73±1,02	23,95±1,45	33,81±0,86	47,02±1,23	28,93±1,09

Taking into account the results obtained for the efficiency of microencapsulation, variants no.1 and 12 were selected for further experiments.

6.19.3. Inhibitory activity on metabolically important enzymes.

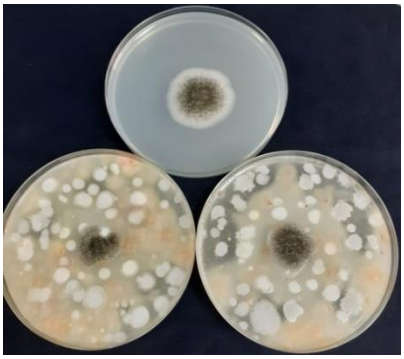
The microencapsulated V12 variants (S40 and S45) showed an inhibitory effect on α-amylase of 79.89-1.74%, while the inhibitory effect on α-glucosidase was lower for, with inhibition of only 5.80 0.62%. Therefore, variant V12 was more effective for inhibiting the enzymatic activity of α-amylase compared to α-glucosidase. This is an important issue, as it has been reported that simultaneous inhibition of both enzymes would lead to abnormal bacterial fermentation in the colon due to the presence of undigested carbohydrates.

6.19.4. Antimicrobial activity of selected microencapsulated variants

V12 microencapsulated variants (S40 and S45) were tested for antifungal and antimicrobial activity, using Aspergillus niger MIUG M5 and Bacillus subtilis MIUG B1 as indicator microorganisms.

Antifungal activity of experimental variants of microencapsulated powders

Microorganism indicator	**Inhibition ratio, %**	
	V12 (S40)	**V12 (S45)**
Aspergillus niger MIUG M5	38,23	35,29
Bacillus subtilis MIUG B1	0	0

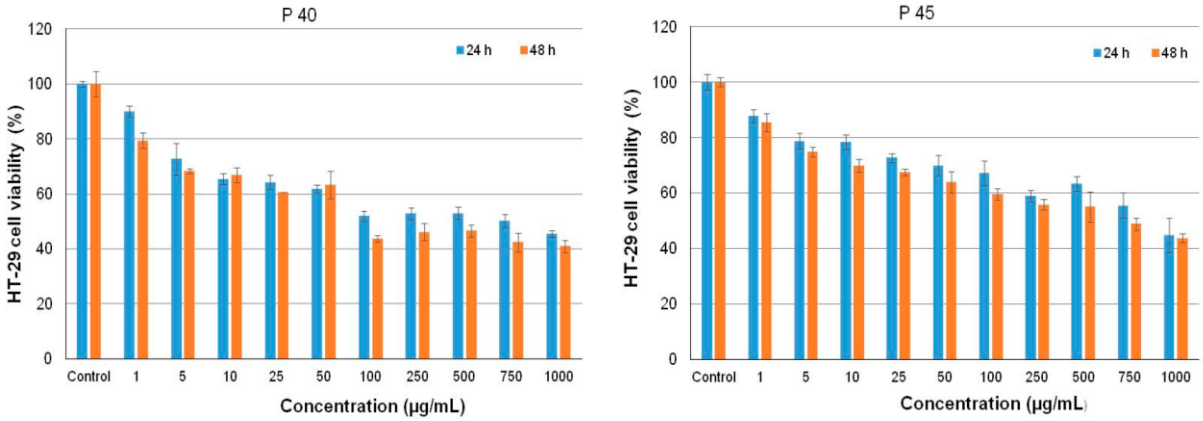


Inhibitory effect of the samples tested against Aspergillus niger MIUG M5

Higher antimicrobial activity in S40 and S45 samples may be associated with higher carotenoid content and probably with free fatty acids.

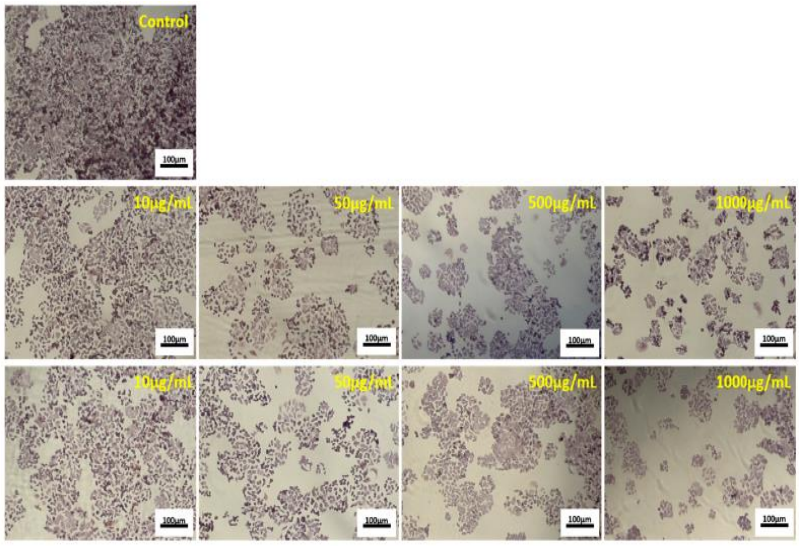
6.19.5. Cytotoxicological analysis of microencapsulated variants

The in vitro cytotoxicity of microencapsulated powders, variant V12 (S40 and S45) was evaluated on the HT-29 and L929 cell lines, after 24 hours and 48 hours of culture by the Neutral Red test. The results showed a decrease in HT-29 cell viability, which is quantity dependent, in both variants.



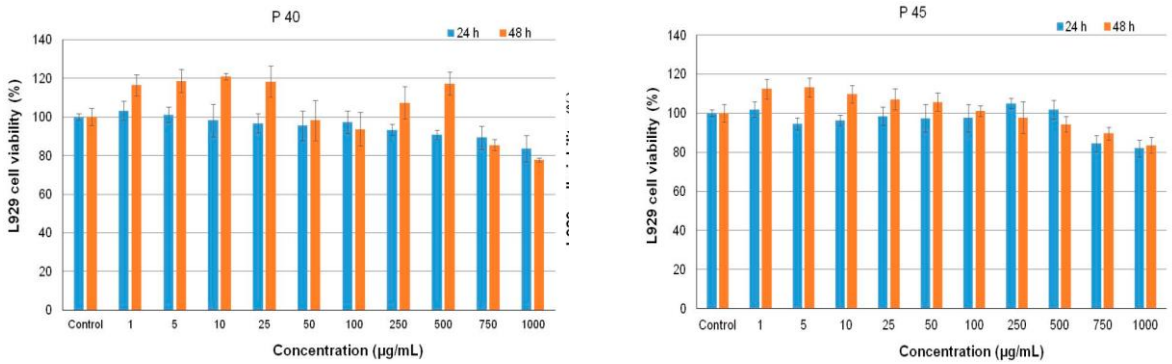
Cell viability on HT-29 cells cultured in the presence of different concentrations of microencapsulated powders after 24 hours (variant 1 blue) and 48 hours (variant 2 red), by the Red Red method. Results were expressed as mean ± SD values (n = 3). * p <0.05 compared to the control sample

Concentration values (IC50) were 100 µg / mL for V12 (S40) and 750 µg / mL for V12 (S45), indicating a higher proliferative effect of V12 (S40) than V12 (S45) in the line HT-29 cell. Observations on cell morphology following the Neutral Red quantitative data.

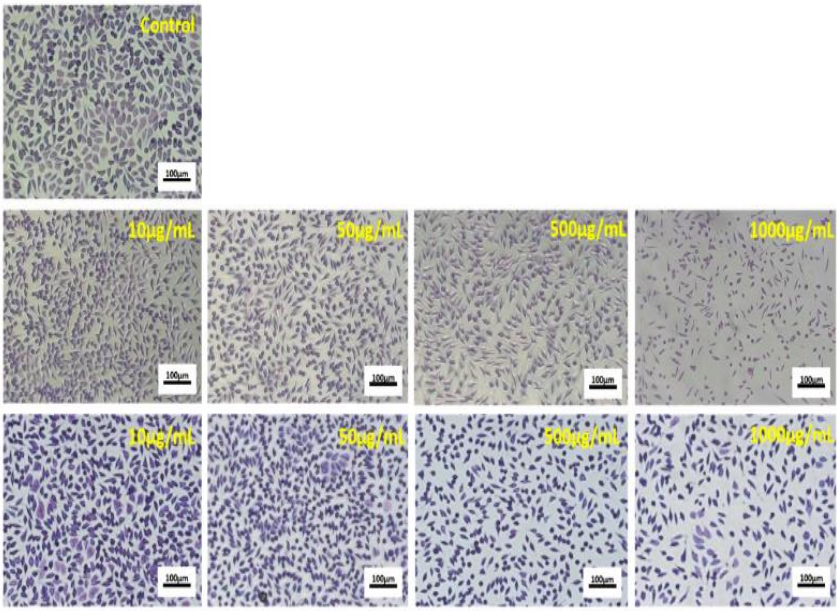


Optical microscopy images of the culture of HT-29 cells cultured in the presence of different concentrations from the experimental variant V12 with samples S40 (line 2) and S45 (line 3), for 48 h. The untreated culture served as a control sample (first line). (Giemsa coloring). Size scale = 100µm.

Optical microscopy showed that the cell morphology and density of L929 cell cultures treated with different concentrations of microencapsulated powders were in accordance with the quantitative data obtained by the Neutral Red test:



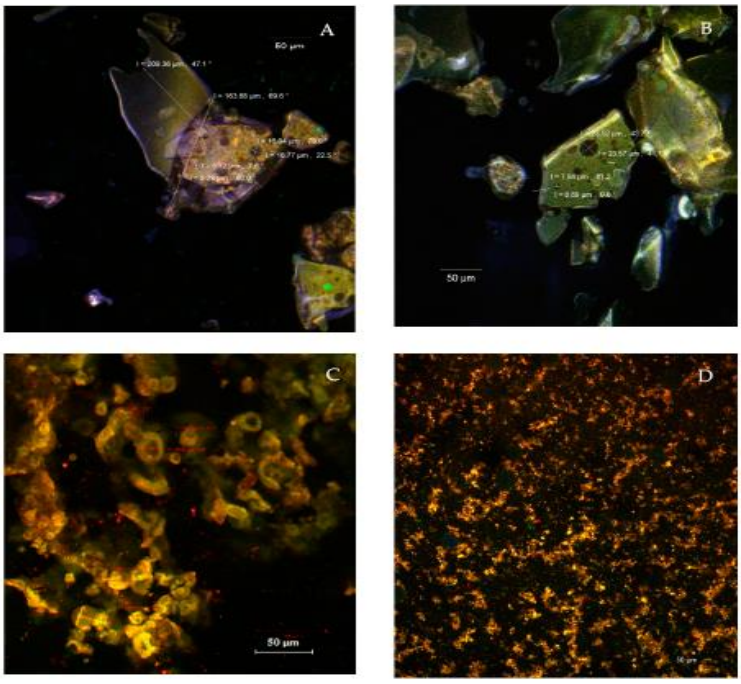
Cell viability on L929 cells cultured in the presence of different concentrations of microencapsulated powders S40 and S45, respectively, after 24 hours (variant 1 blue) and 48 hours (variant 2 red), by the method Red Red. Results were expressed as mean ± SD values (n = 3). * p <0.05 compared to the control sample



Optical microscopy images of L929 cell culture cultured in the presence of different concentrations of samples S40 (line 2) and S45 (line 3) for 48 h. The untreated culture served as a control sample (first line). (Giemsa coloring). Size scale = 100µm.

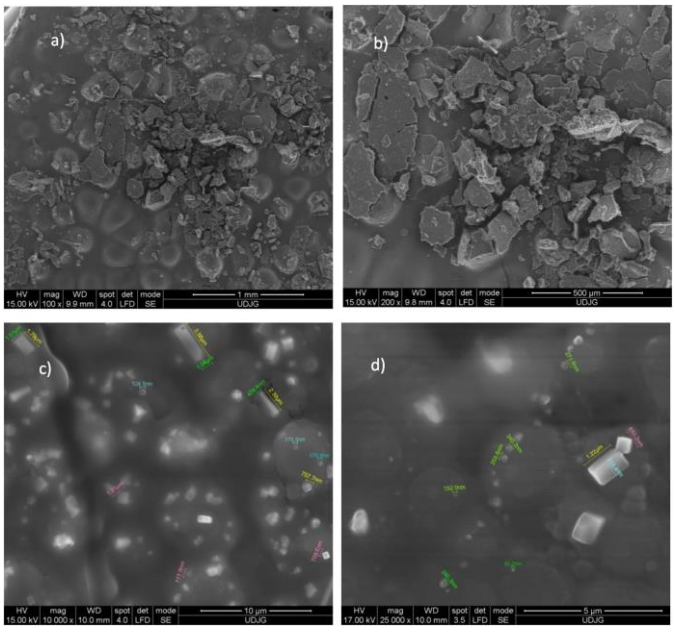
6.19.6. Morphological and structural analysis of microencapsulated variants

The images obtained from the native powders show the microencapsulation of phytochemical compounds in tomato peels in the form of large, polygonal scales, with dimensions between 163.68–209.36 µm inside which spherical vesicles (spherosomes) with diameters of 5.29 can be observed. –16.77 µm (in P40) or 7.94–23.5 µm (in P45). As the main compound of the extract, lycopene, with an auto-fluorescence in the range of 500–580 nm, was captured in the microencapsule matrix consisting of whey protein isolate (in green) and gum arabic (in blue).



CLSM images with microscopic preparations (20x apochromatic objective, zoom 1) from microencapsulated powder in native state a) sample S40; b) S45 test; and preparations to which dyes have been added c) sample S40; d) test S45

The SEM images of the microparticles are shown in the figure below:

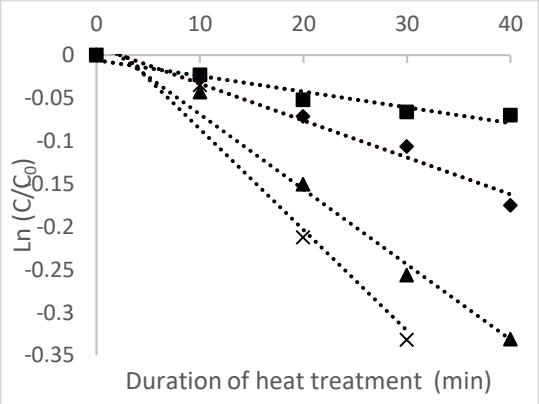


SEM images of microencapsulated variant 1 at magnitude 100 (a), 200x (b), 10000x (c) and 25000x (d).

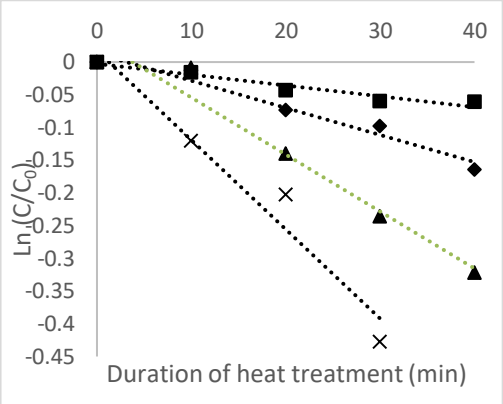
6.19.7. Stability to heat treatment of biologically active compounds in the microencapsulation variant no.1

To test the thermal behavior of lycopene and total carotenoids, a kinetic study of biologically active compounds in microcapsules in heat treatment was performed. The first order kinetic model was used to determine the rate of degradation constant figure a) and b).

a)



b)



1st order kinetic model describing the thermal degradation of total carotenoids (a) and lycopene (b) in microencapsulated variant no.1. The results are presented as the arithmetic mean of three determinations. Temperature: ■ 110 ° C, □120 ° C, ▲ 130 ° C and x140 ° C.

Estimated kinetic parameters (k) and activation energy for thermal degradation of phytochemicals in the skin extract of microencapsulated tomatoes in whey and acacia gum protein isolate

Temperature, °C	Total carotenoids	Lycopene
	$k \cdot 10^{-3}$ (min^{-1})	$k \cdot 10^{-3}$ (min^{-1})
110	4.14 ± 0.24^{d}	3.91 ± 0.17^{d}
120	9.90 ± 0.98^{c}	9.44 ± 0.27^{c}
130	20.26 ± 0.99^{b}	20.03 ± 0.87^{b}
140	27.17 ± 1.02^{a}	31.32 ± 1.45^{a}
E_a (kJ·mol^{-1})	**83.95±1.96**	**92.19±7.73**

6.20. Partial conclusions

The results obtained allowed the following partial conclusions to be described:

1) Using combined microencapsulation techniques, based on emulsification, complex coacervation and lyophilization, 12 variants of biopolymer matrices and two extracts obtained from tomato peels were tested, respectively the extract obtained by solvent-liquid extraction with solvents, assisted by ultrasound and repeated for depletion of the plant matrix in functional compounds and extraction with supercritical CO_2.
2) In general, regardless of the encapsulation material and the type of extract, fine, orange-colored powders with microparticles of different sizes have resulted.
3) Of the tested variants, 3 variants presented superior values of phytochemical parameters, antioxidant activity and microencapsulation efficiency. Therefore, variants no.1, 12 (S40) and 12 (S45) were selected for further experiments.

4) The efficiency of microencapsulation for the selected variants varied depending on the biopolymer matrix, between 37% and 89%, which allowed the hypothesis that the selection of the matrix consisting of whey protein isolate and acacia gum (2: 1) allowed a more efficient encapsulation compared to the same matrix, in combination of 5: 1.
5) Variant 1 showed the highest content of biologically active compounds, of 46.75 ± 0.30 mg / g su total carotenoids, 34.97 ± 0.13 mg / g su β-carotene and lycopene of 29.09 ± 0.20 mg / g su. Thus, lycopene in encapsulated powder accounted for approximately 62% of the total carotenoid content. The antioxidant activity of the powder was 1.77 ± 0.05mMol Trolox / g su.
6) The storage of the selected powders induced changes in the content of biologically active compounds, with a tendency to decrease over time the concentration of studied compounds, which was described using the kinetic model of order 1.
7) Experimental variants 12 showed higher values for the half-life of the concentration of biologically active compounds compared to variant 1, which showed a lower sensitivity to storage. It seems that lycopene is the compound that has the highest sensitivity to the storage of powders, having both the constants of the highest concentration reduction rates, respectively lower values of half-lives compared to total carotenoids and β-carotene.
8) V12 microencapsulated variants showed an inhibitory effect on α-amylase of 80%, while the inhibitory effect on α-glucosidase was lower for, with inhibition of only 6%.
9) Variants 12 were tested for antifungal and antimicrobial activity, using Aspergillus niger MIUG M5 and Bacillus subtilis MIUG B1 as indicator microorganisms. The highest antimicrobial activity (with an inhibition ratio of 38.23%) was identified in case of V12 (S40) against Aspergillus niger MIUG M5, while none of the variants had an effect on the culture of Bacillus subtilis MIUG B1 .
10) A proliferative effect of variants 12 was highlighted, the IC50 concentration values being estimated at 100 µg / mL in the case of sample V12 (S40) and 750 µg / mL in the case of sample V12 (S45), for the HT-29 cell line.
11) The results indicated that the microencapsulated powders with S40 and S45 extracts exerted an antiproliferative effect at minimum concentrations of 5 µg / ml and 25 µg / ml in HT-29 cells, respectively, after 24 hours of culture.
12) Structural and morphological analysis highlighted the microencapsulation of phytochemical compounds in tomato skins in the form of large, polygonal scales with dimensions between 163.68–209.36 µm within which spherical vesicles (spherosomes) with diameters of 5 can be observed. , 29–16.77 µm (in P40) or 7.94–23.5 µm (in P45).
13) The heat stability of biologically active compounds in powders was tested in the range of 110-140 ° C, being described by the first order kinetic model.
14) These data support the hypothesis that microencapsulation allows to obtain stable powders / powders, as functional as ingredients and with added value given by the functional properties beneficial to human health.

CHAPTER 7. APPLICATION RESEARCH THROUGH THE DEVELOPMENT OF TECHNOLOGICAL VARIANTS FOR OBTAINING VALUE-ADDED FOODS

7.1. General aspects

The use of dressing in the preparation of salads is a custom still untapped on the Romanian consumer market. In Romania, salad dressing is still a niche category given the fact that the consumption habit is formed on the classic mixture of oil - vinegar and salt.

7.2. Objectives of the study

Technological variant no.1 was selected for testing.

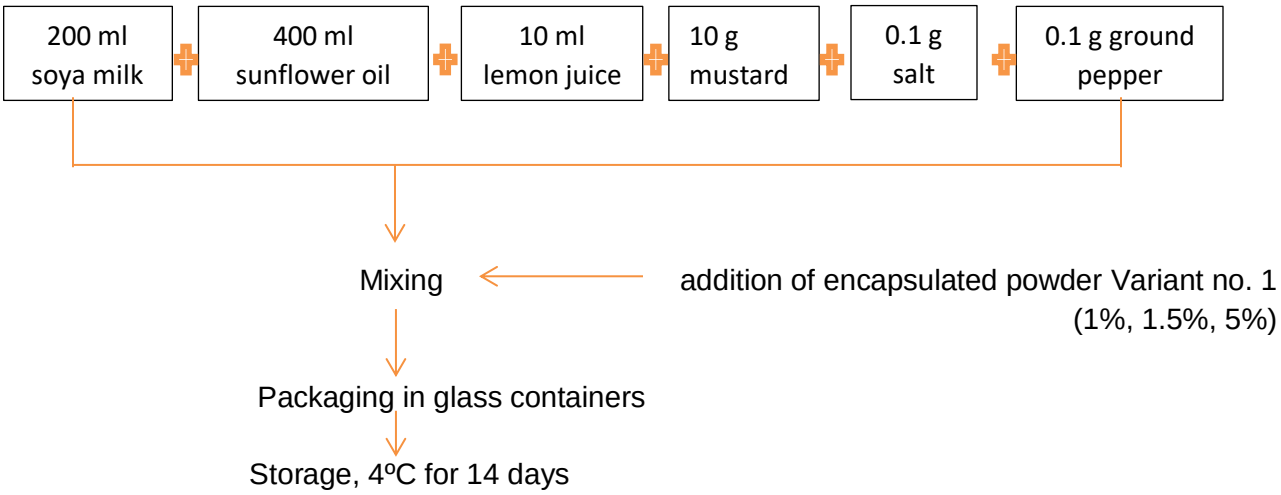
This study has as main objectives: establishing the technological recipe for obtaining the value-added product, the comparative analysis of the physico-chemical and rheological characteristics as well as the analysis of their sensory characteristics.

7.3. Materials and methods

7.3.1. Materials

The commercial products (soy milk, sunflower oil, mustard, lemon, salt, pepper) were purchased from a supermarket in Galati.

7.3.2. Technology of obtaining a dressing type product (vegetable mayonnaise) with the addition of microencapsulated powder variant no.1



Scheme for obtaining vegetable dressing with microencapsulated powder

7.3.3. Determination of rheological characteristics

The rheological behavior of the dressing was determined within 3 hours of preparation using a Rheometer AR2000ex stress control rheometer, TA which allows temperature control through a Peltier cylinder.

7.3.4. Determination of carotenoid content

The carotenoid content was determined spectrophotometrically, respectively over 2 g of plant product (control, 1%, 1.5% and 5%) 10 ml of hexane / acetone mixture (3/1; v / v) were added in order to extract the biologically active compounds. , then the samples were placed in the ultrasonic bath for 40 minutes at a maximum temperature of 40 ° C. The samples were then centrifuged at 9000 rpm for 10 minutes at a temperature of 4ºC after which the absorbance of the spectrophotometric was determined at different wavelengths, respectively 470 nm, 450 nm, 503 nm. The amount of carotenes was expressed in mg / g su.

7.4. RESULTS AND DISCUSSIONS

7.4.1. Phytochemical characterization of value - added foods

In order to develop technologies for obtaining value-added products, a scheme for obtaining dressing-type products was selected, in which variant no.1 microencapsulated powder was added. The table below shows the results obtained for the phytochemical profile.

The amount of total carotenoids, β-carotene, lycopene and antioxidant activity in technological dressing variants with added value

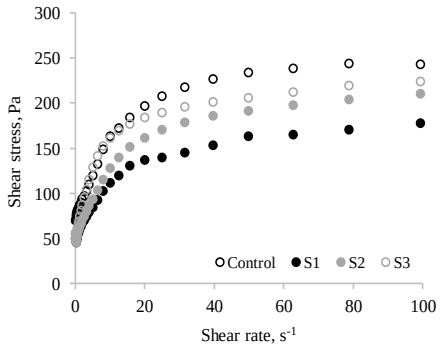
Addition	Total carotenoids, mg/g s.u.	β-carotene, mg/g s.u.	Lycopene, mg/g s.u.	Antioxidant activity mMol E Trolox/g s.u.
0%	0.43±0.009	0.37±0.005	0.19±0.003	20.24±1.60
1%	1.19±0.070	1.08±0.063	0.69±0.042	28.03±0.64
1,5%	1.70±0.016	1.52±0.013	1.00±0.012	29.70±0.82
5%	4.55±0.080	3.84±0.085	2.73±0.056	33.52±0.32

7.4.2. Rheological analysis of value-added foods

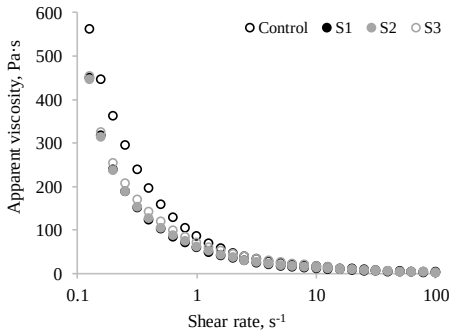
Forced flow conditions were applied to the dressing samples to estimate the impact of the addition of microcapsules on their flow behavior.

All samples showed a thinning behavior of the shear. The results are presented as averages of three repeated measurements, and the standard deviation was less than 6.5%.

a)



b)



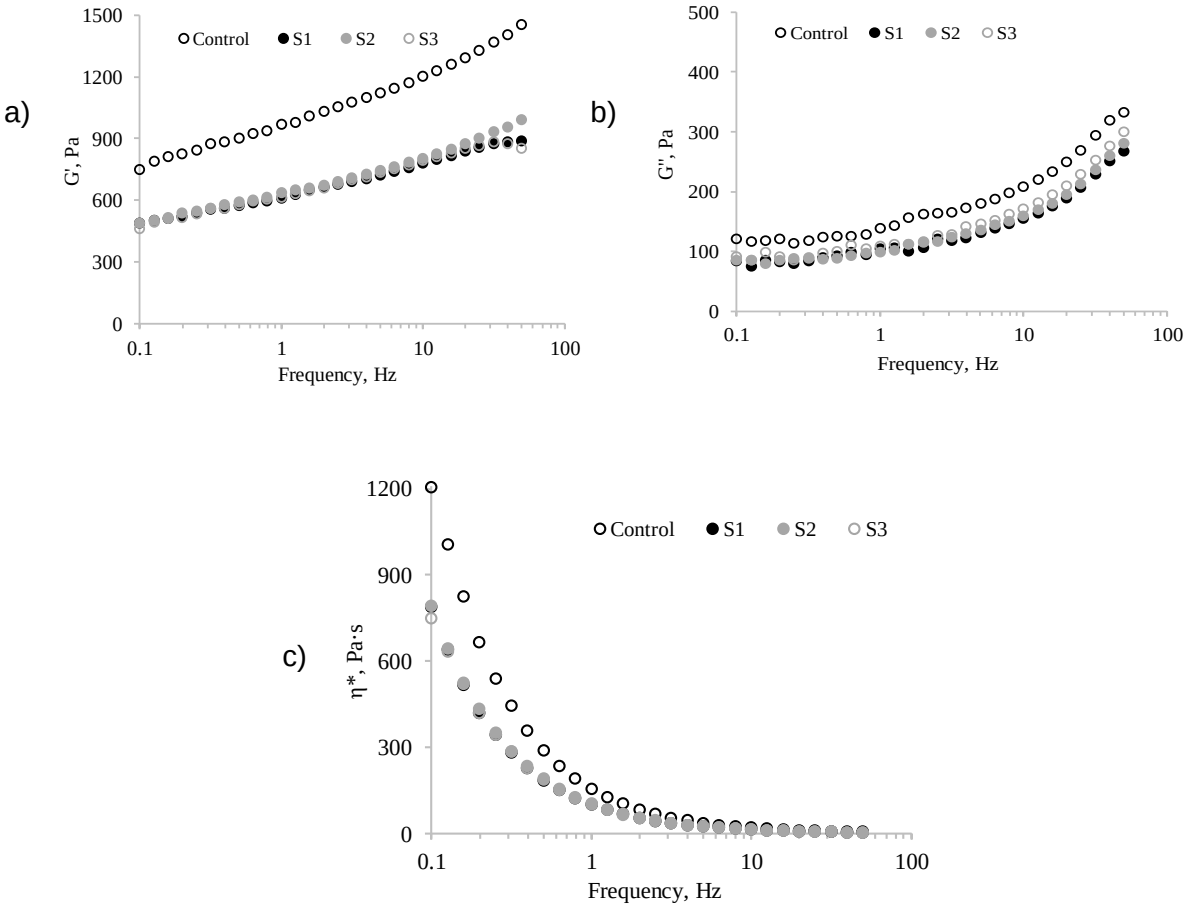
Rheograms (a) and flow curves (b) of dressing rooms with the addition of different amounts of microencapsulated powder (0% - Control, 1% - S1, 1,5% - S2, 5% - S3) at 10 ° C .

Rheological properties at 10 ° C of value-added products (0% - Control, 1% - S1, 1.5% - S2, 5% - S3).

Sample	Continuous flow			Low amplitude dynamic oscillator tests		
	K, Pa·s^n	*n*	η_{50}, Pa·s	γ_c, %	$\gamma_{y,\%}$	*G`*, Pa
Control	92.728	0.221	4.709	2.0	35.34	977.9
S1	63.236	0.229	3.364	1.2	35.01	619.3
S2	66.477	0.263	3.969	1.0	34.77	642.3
S3	76.859	0.267	4.261	1.0	34.36	643.2

Flow parameters: consistency index - K, flow index - n and apparent viscosity at shear rate of 50 s-1 - Ƞ50; low amplitude dynamic oscillation tests: critical tension - γc, tension efficiency - γy and complex coefficient G * at a frequency of 1 Hz

The viscoelastic characteristics of the emulsions were then monitored by performing frequency tests in LVR. Samples with microencapsulated powder showed significantly lower G` and G`` values compared to the control sample:



Evolution of storage modulus (a), loss modulus (b) and complex viscosity (c) of dressing samples with the addition of microencapsulated powder (0% - Control, 1% - S1, 1.5% - S2, 5% - S3) during frequency tests.

All samples showed a solid-like behavior, characterized by "predominant G values over G" over the entire frequency range tested.

7.5. Partial conclusions

In this chapter, technological variant no.1 was used for the development of value-added products, namely dressing products. 3 technological variants of value-added products were obtained, expressed by the content of biologically active compounds and antioxidant activity.

1) It was observed that by the addition of microencapsulated powder, the content of biologically active compounds increases significantly, almost 2.8 times in the sample with 1% addition, 3.95 in the sample with 1.5% addition and about 10 times in the sample with 5% for total carotenoid.
2) The β-carotene content increases by about 2.9 times in the sample with the addition of 1%, 4 times in the sample with 1.5% and 10.3 times in the sample with the addition of 5%. The concentration of lycopene is higher by 3.6%, 5.26% and 14.4% in value-added samples. In terms of antioxidant activity, it increased by approx. 39% in the sample with 1%, 47% in the sample with 1.5% addition and 66% in the sample with 5% addition.
3) Rheology measurements indicated that, regardless of the proportion added, the dressing samples showed a solid behavior.
4) These results support the hypothesis that the bioactive ingredients obtained allow to obtain functional foods and value-added foods, especially in terms of antioxidant activity.

CHAPTER 8. FINAL CONCLUSIONS

The doctoral thesis entitled "***FUNCTIONAL COMPOUNDS BASED ON OLEORESIN AND PROTEINS FOR USE IN FOOD INDUSTRY***" had as main purpose the identification and scientific establishment of strategies to capitalize on the nutritional and functional potential of by-products resulting from industrial processing tomatoes.

Tomato peels was obtained as a by-product in the tomato processing industry, with an abundant flow of solid waste worldwide. A large amount of waste is sent to landfills or as feed. From a quantitative point of view, but especially in terms of phytochemical profile, tomato peels presents an opportunity to capitalize on biologically active compounds, with well-defined functions for the human body and also creates an incentive for industries that facilitate the transition to bioproducts. renewable.

The doctoral thesis aimed at the comparative study of the efficiency of different extraction techniques on the quantitative and qualitative profile of extracts, the study of mechanisms and interaction between the main biologically active compounds in extracts and binding matrices, such as whey proteins, from the perspective of microencapsulation, development variants for obtaining functional composites, with the role of natural ingredients, stable for potential uses in the food industry.

Various extraction techniques and modern investigation methods were tested (extraction with supercritical fluids, gas chromatography, high performance liquid chromatography,

molecular modeling) for advanced phytochemical profiling of extracts, and by statistical analysis of the results optimal extraction conditions were established to obtain functional extracts, enriched in valuable compounds. Another modern, advanced analysis technique aimed at scientifically substantiating the structural and conformational changes of carotenoids in extracts of native tomato peels (Solanum lycopersicum), which involved elucidating the structural changes of carotenoid compounds in tomato peels, from the perspective of industrial optimization , in order to ensure optimal / optimized food functionality.

The thesis makes significant contributions to elucidate the binding mechanisms between bovine β-lactoglobulin and lycopene extracted from tomato peels, using fluorescence intensity quenching techniques, the calcul of binding parameters and thermodynamic parameters and molecular modeling. Within the approaches, the binding mechanisms were scientifically substantiated and the main interaction forces were elucidated. This database with binding parameters can serve as a basis for optimizing microencapsulation conditions.

A series of microencapsulation variants were developed, which used as emulsification techniques, complex coacervation and lyophilization, varying the type of extract and the microencapsulation matrices. The powders were characterized in terms of phytochemical profile and antioxidant and antimicrobial activity. The selected microencapsulated powders showed biological activity, inhibition of some enzymes involved in the metabolic syndrome, had significant antioxidant and antimicrobial activity and did not show cytotoxicity.

In the applied research stage, 3 technological variants of value-added products were obtained, by adding microencapsulated powders, their functionality being expressed by the content of biologically active compounds and antioxidant activity. It was observed that by the addition of microencapsulated powder, the content of biologically active compounds increases significantly, while the rheological properties indicated that, regardless of the added proportion, the dressing samples showed a solid behavior.

CHAPTER 9. PERSONAL CONTRIBUTIONS AND PERSPECTIVES FOR FUTURE STUDIES

The doctoral thesis entitled "***FUNCTIONAL COMPOUNDS BASED ON OLEORESIN AND PROTEINS FOR USE IN FOOD INDUSTRY***" is an original study, with a bottom-top approach, which allowed to go through some stages of fundamental research and applied research, in order to to establish strategies for the recovery and reintegration into the food chain of some functional compounds, with demonstrated beneficial effects on health.

The studies undertaken had an integrated approach, which consisted of:

1) Testing of different extraction techniques from the perspective of optimizing the conditions for recovery of biologically active compounds from tomato peels;
2) Advanced characterization of the obtained complex extracts and phytochemical and functional profiling;
3) Advanced study of the ways of interaction between selected ligands (lycopene) and protein matrices, respectively whey proteins.

4) Development of microencapsulated variants with the role of ingredients, with the main advantage of stabilizing the biological and technological functionality of lipophilic compounds in extracts and the potential maximization of their bioavailability;
5) Reintegration of biologically active compounds from tomato peels in food, which have thus gained added value and specific functionality, especially antioxidant.
6) Scientific substantiation of standard, integrated approaches to recovery and reintegration of biologically active compounds from by-products resulting from industrial processing of tomatoes into functional foods, thus contributing to the application of the principles of emerging bioeconomy in Romania.

From the perspective of further studies, the approach in the doctoral thesis entitled "***FUNCTIONAL COMPOUNDS BASED ON OLEORESINS AND PROTEINS FOR USE IN FOOD INDUSTRY***" can be extrapolated to other by-products of industrial processing of vegetables and fruits (eggplant, onions, pumpkin, berries, cherries, cherries, plums, etc.) for the reintegration of biologically active compounds into foods with a positive impact on the potential to increase the quality of life through food and nutrition.

CHAPTER 10. DISSEMINATION OF RESEARCH RESOULTS

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

A. Articles published in ISI rated journals

1. **Ionica Dima (Gheonea)**, Aprodu, I. Râpeanu, G, **Stănciuc., N. 2018**. Binding mechanisms between lycopene extracted from tomato peels and bovine β-lactoglobulin. *Journal of Luminescence, 203*, 582-589, https://doi.org/10.1016/j.jlumin.2018.07.017.

2. **Ionica Dima (Gheonea),** Aprodu, I., Enachi, E., Horincar, G., Bolea, C.A., Bahrim, G.E., Râpeanu, G., Stănciuc, N. **2020**. Investigations on thermostability of carotenoids from tomato peels in oils using a kinetic approach. *Journal of Food Processing and Preservation,* 44, e14303. https://doi.org/10.1111/jfpp.14303.

3. **Ionica Dima (Gheonea),** Aprodu, I., Cîrciumaru, A., Râpeanu, G., Bahrim, G.E., Stănciuc, N. **2021**. Microencapsulation of lycopene from tomatoes peels by complex coacervation and freeze-drying: Evidences on phytochemical profile, stability and food applications, *Journal of Food Engineering*, 288, 110166, https://doi.org/10.1016/j.jfoodeng.2020.110166.

4. Mihalcea, L., Crăciunescu, O., **Ionica Dima (Gheonea),** Prelipcean, A.-M., Enachi, E.,

Barbu, V., Bahrim, G.E., Râpeanu, G., Oancea, A., Stănciuc, N. **2021**. Supercritical CO_2 extraction and microencapsulation of lycopene-enriched oleoresins from tomato peels: Evidence on antiproliferative and cytocompatibility activities. *Antioxidants*, 10(2), 222. doi: 10.3390/antiox10020222.

B. Works communicated at international scientific events

1. Elena Enachi, **Ionica Dima (Gheonea)**, Carmen Alina Bolea, Georgiana Horincar, Iuliana Aprodu, Gabriela Râpeanu ,Nicoleta Stănciuc, *Tomatoes´s skins as a rich source of natural pigments*, European Biotehnology Congress 2019, Valencia-Spain, 11-13 September 2019, poster presentation
2. **Ionica Dima (Gheonea)**, Râpeanu Gabriela, Iuliana Aprodu, Liliana Mihalcea, Nicoleta Stănciuc, *Carotenoids thermal degradation in tomato waste extract-a kinetic study,* The 9th International Symposium EuroAliment, Dunărea de Jos University of Galati, 5-6 September 2019, Galați, România, poster presentation
3. Gabriela Iordăchescu, Gabriela Ploscuțanu, Liliana Mihalcea, **Ionica Dima (Gheonea)**, Augustin Octavian Mihalache, Octavian Baston, Octavian Barna, Eugenia Mihaela Pricop, Vanessa Guemkam Boudjeka, Exploiter les déchets de tomatoes pour obtenir des produits sains et à haute valeur nutritionnelle, Conference finale SaIN déroulée à l´Université de Médicine et Pharmacie Grigore T. Popa, 6 December 2019, Iași, România, oral presentation
4. **Ionica Dima (Gheonea)**, Liliana Mihalcea,Gabriela Râpeanu, Nicoleta Stănciuc, *Extraction of lycopene from tomato peels with Supercritical Carbon Dioxide and the effect of antioxidant activity,* European Biotehnology Congress 2020, Vienna-Praga, 24-26 September 2020, poster presentation

C. Works communicated at national scientific events

1. **Ionica Dima (Gheonea)**, Iuliana Aprodu, Gabriela Râpeanu, Liliana Mihalcea, Nicoleta Stănciuc, *Lycopene extraction fron tomato peels-characterisation and thermal degradation kinetics*, PhD student conference CSSD-UDJG, Dunărea de Jos University of Galați, Romania, 7nd-8th of June 2018, oral presentation
2. **Ionica Dima (Gheonea)**, Iuliana Aprodu, Liliana Mihalcea, Nicoleta Stănciuc, *Microencapsulation of lycopene from tomato peels extract*, PhD student conference CSSD-UDJG, Dunărea de Jos University of Galați, Romania, 13nd-14th of June 2019, poster presentation
3. **Ionica Dima (Gheonea)**, Gabriela Râpeanu, Nicoleta Stănciuc, *Microencapsulation of lycopene from tomato peels by complex coacervation and freeze-drying:evidences of stability*, PhD student conference CSSD-UDJG, Dunărea de Jos University of Galați, Romania, 18nd-19th of June 2020, poster presentation
4. **Ionica Dima (Gheonea)**, Liliana Mihalcea, Gabriela Râpeanu, Nicoleta Stănciuc, *Comparative study on Supercritical CO2 extraction of lycopene and β-carotene from fresh tomato peels and ultrasound extraction and the effect of antioxidant activity*, PhD

student conference CSSD-UDJG, Dunărea de Jos University of Galați, Romania, 18nd-19th of June 2020, poster presentation

5. **Ionica Dima (Gheonea)**, Râpeanu Gabriela, Aprodu Iuliana, Stănciuc Nicoleta, *Interactions of β-lactoglobulin and tomato lycopene: studies on binding mechanism,* PhD student conference CSSD-UDJG, Dunărea de Jos University of Galați, Romania, 10nd-11th of June
6. **Ionica Dima (Gheonea)**, (Gheonea) Ionica, Râpeanu Gabriela, Aprodu Iuliana, Stănciuc Nicoleta, *The functional potential of lycopen from tomato by-products: A review,* PhD student conference CSSD-UDJG, Dunărea de Jos University of Galați, Romania, 10nd-11th of June 2021, poster presentation

D. Other publications in ISI-rated journals:

1. Gabriel-Dănuţ Mocanu, Oana-Viorela Nistor, Doina Georgeta Andronoiu, Liliana Ceclu, **Ionica Dima Gheonea**, Liliana Mihalcea, Viorica Vasilica Barbu, Oana Emilia Constantin, Livia Pătrașcu, *Effects of drying methods on quality parameters of potato and red beetroot purée with Lactobacillus delbrueckii,* Journal of Food and Nutrition Reseach.

2. Other international conferences

1. Dănut G. Mocanu, **Ionica (Gheonea) Dima**, Liliana Mihalcea, Vasilica V. Barbu, Oana V. Nistor, Doina G. Andronoiu, Oana E. Constantin, Livia Pătrașcu, Elisabeta Botez, *The effects of drying methods on the quality parameters of potatoes and red beetroot purée*, International Conference on Agronomy and Food Science and Technology-2019, 20-21 June, Istanbul, Turcia, poster presentation
2. Liliana Mihalcea, Monica Ioan, Livia Pătrașcu, Dănut G. Mocanu, **Ionica (Gheonea) Dima**, *New Sugar-Free Product on the Basis of Almond Flour and Pumpkin Pulp*, International Conference on Agronomy and Food Science and Technology-2019, 20-21 June, Istanbul, Turcia, poster presentation

3. Other national conferences

1. Dănut G. Mocanu, L.A. (Butnariu) Tănase, Oana V. Nistor, **Ionica (Gheonea) Dima**, A. C. Chirilă, Doina G. Andronoiu, Oana E. Constantin, Vasilica V. Barbu, Livia Pătrașcu, Elisabeta Botez, 5th International Conference on Chemical Engineering-Innovative Materials and Processes for a Sustainable Development, Helth promoters from potato and pumpkin instant purée, ICCE 2020, 28-30 Oct, Iași, România, poster presentation
2. Monica Ioan, Dănut G. Mocanu, Doina G. Andronoiu, **Ionica (Gheonea) Dima**, Livia Pătrașcu, Liliana Mihalcea*, Rheological and Nutritional Properties of Sugar-Free Roulade with Pumpkin Pulp*, Week of Banat´s University of Agricultural Sciences and Veterinary Medicine, King Michael I of Roumania, Timoșoara, 20-22 May 2019, poster presentation

3. Dănut G. Mocanu, Oana V. Nistor, Doina G. Andronoiu, Oana E. Constantin, Vasilica V. Barbu, **Ionica (Gheonea) Dima**, Livia Pătrașcu, Liliana Ceclu, Liliana Mihalcea, Elisabeta Botez, *Quality Characteristics of Fresh and Reconstituted Probiotc Potatoes Purée with Sea Buckthorn Supercritical CO2 Extract*, Week of Banat´s University of Agricultural Sciences and Veterinary Medicine, King Michael I of Roumania, Timoșoara, 20-22 May 2019, poster presentation
4. Liliana Mihalcea, Livia Pătrașcu, **Ionica (Gheonea) Dima**, "*Agglutinated roll with low sugar content*", Innovation and Research Ugal Invent, 16-18 Oct 2019, Galați, România, poster presentation, Paper awarded by the Romanian Inventors Forum with the Euroinvent Medal Award.
5. Dănut G. Mocanu, Oana V. Nistor, Doina G. Andronoiu, Oana E. Constantin, Vasilica V. Barbu, **Ionica (Gheonea) Dima**, Livia Pătrașcu, Liliana Ceclu, Liliana Mihalcea, Elisabeta Botez, *"Quality characteristics of the Probiotic Mashed Potato with Sea Buckthorn Oil",* Innovation and Research Salon Ugal Invent, 16-18 Oct 2019, Galați, România, poster presentation.