„Dunărea de Jos" University of Galaţi

Doctoral school of Fundamental Sciences and Engineering

PhD thesis

RED BEET BY-PRODUCT VALORISATION
(PhD thesis summary)

PhD student,

Silvia (Mistrianu) Lazăr

Scientific coordinator,

Prof.univ.dr.eng. habil. Gabriela RÂPEANU

Seria I.7: Food engineering Nr. 22

GALATI

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Content

Introduction

Vegetables contain considerable amounts of essential nutrients such as vitamins, minerals, fiber, and phytochemical compounds with significant beneficial effects on health (Panghal et al., 2017). Beetroot is one of the important vegetables, being rich in carbohydrates, fats, proteins, and micronutrients with characteristics and properties beneficial to health. Beet processing and consumption of value-added products have steadily increased due to the recognition of beetroot as an important source of natural antioxidants.

Beetroot (also known as beet, garden beet, table beet) is a traditional and popular vegetable in many parts of the world. This is a red root, most often associated with the word "beet". It is very popular in Eastern and Central Europe, where it is the main ingredient of borscht, vinaigrette salad, Russian salad, and sauerkraut with beetroot. Today, beetroot is regularly consumed as part of the normal diet, either fresh or after heat processing or fermentation, and is commonly used in industry as a food coloring known as E162 (Ceclu and Nistor, 2020).

Beetroot (*Beta vulgaris* L.) contains high amounts of bioactive substances, among which are betalains, carotenoids, polyphenols, B vitamins (B1, B2, B3, B6, and B12), folate minerals, fibers, as well as valuable sugars low energy (Kale et al., 2018) and inorganic nitrate (Clifford et al., 2015). All parts of this plant have different medicinal uses: they have an antioxidant, antidepressant, antimicrobial, antifungal, anti-inflammatory, diuretic, expectorant, and carminative role (Jasmitha et al., 2018), hepatoprotective (Olumese et al., 2018) and cardiovascular protector. Other reported benefits (Olumese et al., 2018; Wruss et al., 2015) include inhibition of lipid peroxidation and chemo-preventive effects (Babarykin et al., 2019; Lechner and Stoner, 2019).

Currently, the valorization of agro-food by-products is important for the reduction of generated waste and could be achieved by implementing a circular economic model to protect the environment. Moreover, these by-products can be used as an alternative source of natural additives to improve the quality of food products (Martillanes et al., 2020).

The tendency of consumers to opt for the consumption of healthy, natural products has led to the development of natural, innovative products with improved sensory properties (taste, aroma, color, texture), responding to the demands and needs of consumers. The added value of the obtained products is highlighted by the high intake of natural antioxidants present in the beetroot skins, which have remarkable antioxidant potential and are free of toxicity. In addition, this study aims to replace the additives of chemical synthesis with natural ones, present in beetroot skins, which bring numerous benefits and directly contribute to increasing the quality of life.

The doctoral thesis entitled "**RED BEET BY-PRODUCT VALORISATION**" aimed at the study of the biochemical and functional behavior of the pigments from the red beet skins, mainly

the betalain compounds, to obtain functional composites. These fruits are rich in betalain compounds with multiple benefits for human health due to their antioxidant and antimicrobial properties. The development of new directions for the use of bioactive components from the red beet peel was also pursued, directions aimed at the development of new technologies for the production of various products with added value.

The main ***scientific objectives*** targeted during the doctoral studies are:

• Testing conventional and modern extraction methods and choosing an effective extraction method to obtain betalain extracts rich in bioactive compounds of major interest;

• The determination of the phytochemical profile and the antioxidant activity of the extracts from red beetroot skins, as well as the thermal stability, to determine the optimal conditions for obtaining, processing, and storing products rich in betalain compounds;

• *In vitro* testing of the biological activity of the extract from the red beetroot skin powder, evaluating for this purpose the inhibition potential of the enzymes α-glucosidase, α-amylase, lipase, and lipoxygenase, enzymes involved in the metabolic syndrome and pro-inflammatory processes;

• The development of some technological options for obtaining food products such as mayonnaise, marshmallows, and sauce with added value and their characterization from a phytochemical, physicochemical, sensory point of view, etc.

The doctoral thesis includes:

I. **THE DOCUMENTARY STUDY**, entitled "**THEORETICAL CONSIDERATIONS REGARDING THE CHARACTERIZATION OF BIOLOGICALLY ACTIVE COMPOUNDS FROM RED BEET SKIN**", includes one chapter (**Chapter 1**) divided into three subchapters in which recent data from the specialized literature are presented regarding the characteristics of bioactive compounds (mainly betalains) and the impact they have in the food industry, of course, with an emphasis on their beneficial effects on human health. There is also information on extraction methods, but also theoretical data on the stability and availability of bioactive compounds.

II. **PERSONAL CONTRIBUTIONS** includes the results of investigations carried out throughout the doctoral internship, and is briefly described in four chapters:

Chapter 2, entitled "**COMPARATIVE EVALUATION OF SOME EXTRACTION METHODS APPLIED TO RED BEET PEELS FROM THE PERSPECTIVE OF THE CONTENT IN BIOLOGICALLY ACTIVE COMPOUNDS**", presents information on the results obtained from the different extraction methods of the biologically active compounds (total betalains, total flavonoids, and total polyphenols), but also the phytochemical characterization of betalains from beetroot peel from the perspective of obtaining extracts with superior antioxidant activity.

Chapter 3, entitled "**THE OPTIMIZATION OF THE EXTRACTION OF BETALAINIC PIGMENTS AND TOTAL POLYPHENOLIC COMPOUNDS RESULTED FROM THE PROCESSING OF RED BEET PEELS**", presents information on the results obtained from the optimization and the validation studies of the extraction of betalain compounds from red beetroot peels.

Chapter 4, entitled "**ADVANCED CHARACTERIZATION OF THE OPTIMIZED RED BEET PEELS EXTRACT**", presents the results obtained after testing the impact of heat treatment on betalain compounds and the antioxidant activity at different temperature-time combinations, as well as the results obtained after evaluating the effectiveness of polyphenols from the extract obtained from the beetroot peel powder against the inhibition of enzymes associated with the metabolic syndrome and pro-inflammatory processes.

Chapter 5, "**DEVELOPMENT OF FOOD PRODUCTS WITH ADDED VALUE THROUGH THE ADDITION OF BEET PEEL POWDER**", presents the results obtained from the use of freeze-dried beetroot peels as a source of natural antioxidants and other lipophilic bioactive compounds

to obtain value-added products. In addition, the impact of beetroot peel powder supplementation on the phytochemical composition, sensory characteristics, viscosity, color, and textural properties of value-added products was also investigated.

Chapter 6, **FINAL CONCLUSIONS**, presents the main conclusions resulting from the investigations carried out, which focused on the study of betalains from matrices derived from red beetroot skins.

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*.

The doctoral thesis comprises **148** pages, in which **27** figures and **42** tables are included. The documentary study represents 30% and the experimental part 70%.

Finally, the ***original contributions*** of the doctoral thesis are presented, as well as the dissemination of the results obtained in the research field addressed. Thus, the research results were capitalized by the elaboration of **2 scientific articles** published in ISI-rated journals (***Inventions, Processes***), **3 patent applications**, as well as **10 communications at representative scientific events** for the field of food product engineering, from abroad and from home.

The research activities within the doctoral thesis were carried out with the help of the modern research infrastructure of the *Integrated Research, Expertise, and Technological Transfer Center (BioAliment-TehnIA)* (www.bioaliment.ugal.ro), within the Faculty of Food Science and Engineering , "Dunarea de Jos" University from Galati.

The thesis was carried out under the scientific coordination of **Prof. Dr. Eng. Gabriela RÂPEANU**, as PhD supervisor and of the guidance committee made up of: **Prof. Dr. Eng. Nicoleta STĂNCIUC**, **Prof. Dr. Eng. Iuliana APRODU**, **Associate Prof. Dr. Eng. Luminița GEORGESCU** and **Associate Prof. Dr. Eng. Oana CONSTANTIN**.

Chapter 2. COMPARATIVE EVALUATION OF SOME EXTRACTION METHODS APPLIED TO RED BEET PEELS FROM THE PERSPECTIVE OF THE CONTENT IN BIOLOGICALLY ACTIVE COMPOUNDS

2.1. General aspects

Annual world production of beetroot was recorded at 274 million tonnes in 2018, with the top five producers being France, the United States of America, Russia, Germany, and Ukraine (FAO, 2020). Mereddy et al., (2017) reported that about 85% of beetroot is processed (cut), while almost 30% of the total beetroot produced is wasted due to quality control rejection. Therefore, the use of by-products from the beetroot processing industry for the extraction of plant pigments is a good option to obtain value-added products (Zin et al., 2020b). Pigments can be extracted from beetroot simply by maceration with water, however, the protein and carbohydrate content in beetroot limits the use of water as an extraction solvent (Sawicki and Wiczkowski, 2018). Therefore, ethanol or methanol was used to increase the extraction efficiency (Nestora et al., 2016). Various extraction techniques, including conventional techniques (Pandey et al., 2018) and new emerging techniques (López et al., 2009) have been used to achieve higher extraction yields of the pigments of interest. According to Galanakis, (2012), the isolation of bioactive compounds of interest from food waste usually follows five universal recovery steps, such as i) macroscopic pretreatment, ii) separation of macro and micromolecules, ii) extraction, iv) isolation and purification, and v) product formation.

The extraction of polyphenols from plant sources and other bioactive compounds is influenced by the portion of the plant matrix used, particle size and sample preparation, storage conditions, extraction solvent and method used for the extraction, as well as the chemical nature of the compound of interest (Adadi et al., 2018; Nirmal et al., 2015). In addition, the solubility and stability of the compounds are affected by the type of solvent, pH, temperature, and the presence of other chemical constituents (Galanakis, 2015). Another important parameter that should be considered during the extraction of betalains is the inactivation of the enzymes polyphenol oxidase (PPO) and peroxidase (POD), which are responsible for the degradation of betalains (Marszałek et al., 2017). Although, betalains are water-soluble nitrogen pigments, ethanol or methanol, or slightly acidic conditions are often used to obtain higher extraction yields (Cardoso-Ugarte et al., 2014). The extraction of betalains from beetroot is a solid-liquid extraction process involving maceration or grinding of the root (Sivakumar et al., 2009). The applied external mechanical force helps to release the pigment bound to the cell membrane into the solvent (Deng et al., 2015). In general, the beetroot is cleaned, cut into small pieces, and blanched before the actual extraction process is implemented. Conventional extraction techniques involve simple solvent mixing or maceration (Halwani et al., 2018) or boiling and juice extraction (Sawicki and Wiczkowski, 2018) while emerging technologies focus more on higher yield, lower consumption of solvents and energy, fewer processing steps, and time efficiency (Cardoso-Ugarte et al., 2014).

2.2. Study objectives

The main objectives of this study were: to evaluate four extraction methods (conventional solvent extraction, ultrasound-assisted solvent extraction, enzyme-assisted extraction, and microwave-assisted extraction) in order to maximize the content of biologically active compounds in beetroot peel, such as the content of betalains, total polyphenols and antioxidant activity;

comparing the results of the content of betalains (betacyanins and betaxanthins), total polyphenols and antioxidant activity of extracts obtained from beetroot peel using spectrophotometric evaluation techniques.

2.3. Materials and methods

The study focused on: the initial preparation of the beetroot skins from which the biologically active compounds were extracted using conventional solvent extraction: ultrasound-assisted ethanol extraction; microwave-assisted ethanol extraction; and enzymatic-assisted extraction. In order to phytochemically characterize the beetroot peel extracts, the content of total betalains (BT) was determined, and the results were expressed as mg total betalains/100 g d.w.; the total polyphenol content (CTP) was also determined, where the results were expressed as mg catechin equivalents/100 g d.w.; and the antioxidant activity (AA) was also determined, and the results were expressed as mM Trolox/g d.w.

2.5. Results and discussion

2.5.1. Determination of compounds with biological value from the obtained extracts

Betalains are the main class of compounds with high antioxidant activity in beetroot peel. High antioxidant activity was associated with increased concentrations (more than 20-fold) of the total phenolic compounds, which may have synergistic effects with betalains.

Four conventional and modern extraction methods were used for the extraction of betalains, namely: conventional solvent extraction, ultrasound-assisted ethanolic extraction, microwave-assisted ethanolic extraction, and enzymatic extraction.

For the quantification of betalains, a spectrophotometric method was used based on the measurement of absorbance at two different wavelengths, namely 480 nm for betaxanthins and 538 nm for betacyanins. Betalain content was expressed in mg/g d.w. For the efficient extraction of phenolic compounds, in the case of conventional solvent extraction, ultrasound-assisted extraction, and microwave-assisted extraction, two different solvent concentrations were used, namely, 20% ethanol and 50% ethanol acidified with citric acid in two different concentrations 0.1% and 1%.

For the conventional extraction, the extraction processes took place both at the temperature of 20°C and at 70°C, for 15 and 50 minutes, and for the ultrasound-assisted extraction, respectively 15 and 50 minutes, at temperatures of 20°C and 45°C. In the case of microwave-assisted extraction, the extraction of beetroot peel was carried out at a microwave power of 315W and 525W, for 10 and 15 seconds.

For the extraction with the addition of enzymes, three different enzymes were used (cellulase, pectolytic enzymes, and xylanase), the extraction time being 15, 30, and 60 minutes.

All these extraction variants were tested to see which extraction method and which combination of parameters extracted the highest content of bioactive compounds from beetroot peel.

Table 2.1. presents the content of total betalains obtained after conventional extraction by varying the extraction parameters and **Table 2.2.** shows the total content of betacyanins and betaxanthins following conventional extraction by varying the extraction parameters.

Regarding the extraction of betalain compounds by the conventional method with 20% ethanol concentration, it is noted that the highest content of compounds of 118.5 ± 76.00 mg/100 g d.w. was obtained in the case of conventional solvent extraction in the presence of 1% citric acid solution, after 15 minutes of extraction at a temperature of 20°C. Thus, from the total content of betalains obtained, the amount of betaxanthins is 69.6 ± 4.00 mg/100 g d.w. and the amount of betacyanins is 49.00 ± 00.2 mg/100 g d.w.

Table 2.1. Total betalain content obtained following conventional solvent extraction

Total Betalains, mg/100 g d.w.								
Solvent	**Citric acid 0.1 %**				**Citric acid 1 %**			
Temperature	20°C		70°C		20°C		70°C	
Time	15 min	50 min	15 min	50 min	15 min	50 min	15 min	50 min
20% Ethanol	99.4±4.00^{aA}	118.4±8.00^{aB}	85.1±2.00 ^{aA}	71.5±1.00 ^{aB}	118.5±6.00 ^{aA}	64.0±4.00 ^{aB}	115.2±2.00 ^{aA}	89.9±3.00 ^{aB}
50% Ethanol	94.3±4.00^{bA}	107.3±2.00^{bB}	93.4±4.00 ^{bA}	70.7±3.00 ^{aB}	74.1±0.6 ^{bA}	115.3±7.00 ^{bB}	85.0±1.00 ^{bA}	80.0±2.00 ^{bB}

Means in the same row that do not have a letter in common (a, b, c) are significantly different at p<0.05. Means in the same row that do not have a capital letter in common (A, B) are significantly different at p <0.05 using ANOVA and Tukey's test.

Table 2.2. The total content of betacyanins and betaxanthins following conventional solvent extraction

Betacyanins and betaxanthins, mg/100 g d.w								
Solvent	**Citric acid 0.1 %**							
Temperature	20°C				70°C			
Time	15 min		50 min		15 min		50 min	
Betacyanins and betaxanthins /	Bx	Bc	Bx	Bc	Bx	Bc	Bx	Bc
20% Ethanol	57.5±0.10^{aA}	41.9±2.00 ^{aB}	71.5±5.00 ^{aA}	47.0±2.00 ^{aB}	50.0±0.2 ^{aA}	35.2±0.5 ^{aB}	40.9±1.00 ^{aA}	30.7±1.00 ^{aB}
50% Ethanol	52.5±3.00 ^{bA}	41.9±1.00 ^{aB}	61.6±1.00 ^{bA}	45.7±0.7 ^{aB}	56.3±3.0 ^{bA}	37.1±1.0 ^{aB}	42.3±2.00 ^{aA}	28.5±1.00 ^{aB}
Solvent	**Citric acid 1 %**							
Temperature	20°C				70°C			
Time	15 min		50 min		15 min		50 min	
Betacyanins and betaxanthins /	Bx	Bc	Bx	Bc	Bx	Bc	Bx	Bc
20% Ethanol	69.6±4.00 ^{aA}	49.0±0.2 ^{aB}	37.7±2.0 ^{aA}	26.3±1.0 ^{aB}	67.7±1.0 ^{aA}	47.6±0.7 ^{aB}	53.7±2.0 ^{aA}	36.2±1.0 ^{aB}
50% Ethanol	45.1±0.5 ^{bA}	29.1±0.2 ^{bB}	69.5±0.4 ^{bA}	45.9±3.0 ^{bB}	52.3±1.0 ^{bA}	33.2±0.6 ^{bB}	48.5±1.5 ^{bA}	31.6±0.9 ^{bB}

Means in the same row that do not have a letter in common (a, b, c) are significantly different at p<0.05. Means in the same row that do not have a capital letter in common (A, B) are significantly different at p <0.05 using ANOVA and Tukey's test.

Table 2.3. The content of total betalains obtained from ultrasound-assisted ethanol extraction

Total Betalains, mg/100 g d.w.								
Solvent	**Citric acid 0.1 %**				**Citric acid 1 %**			
Temperature	20° C		45°C		20°C		45°C	
Time	15 min	50 min	15 min	50 min	15 min	50 min	15 min	50 min
20% Ethanol	88.7±1.00^{aA}	98.0±7.00^{aB}	66.0±9.00^{aA}	49.1±5.00^{aB}	93.2±3.00^{aA}	78.3±5.00^{aB}	52.8±4.00^{aA}	77.1±24.00^{aB}
50% Ethanol	82.2±8.00^{bA}	104.7±3.00^{bB}	66.1±1.00^{aA}	54.3±2.00^{bB}	76.3±4.00^{bA}	87.0±4.00^{bB}	35.0±9.00^{bA}	51.7±8.00^{bB}

Means in the same row that do not have a letter in common (a, b, c) are significantly different at p<0.05. Means in the same row that do not have a capital letter in common (A, B) are significantly different at p <0.05 using ANOVA and Tukey's test.

Table 2.4. The total content of betacyanins and betaxanthins obtained from ultrasound-assisted ethanol extraction

Betacyanins and betaxanthins, mg/100 g d.w.								
Solvent	**Citric acid 0.1 %**							
Temperature	20°C				45°C			
Time	15 min		50 min		15 min		50 min	
Betacyanins and betaxanthins /	Bx	Bc	Bx	Bc	Bx	Bc	Bx	Bc
20% Ethanol	52.0±0.70^{aA}	36.7±1.00^{aB}	57.9±4.00 ^{aA}	40.1±3.00 ^{aB}	38.2±6.00 ^{aA}	27.8±3.00 ^{aB}	26.2±5.00 ^{aA}	23.0±0.90 ^{aB}
50% Ethanol	49.9±5.00^{bA}	32.3±3.00^{aB}	63.7±2.00 ^{bA}	41.1±1.00 ^{aB}	40.0±1.00 ^{aA}	27.2±0.80 ^{aB}	31.5±1.00 ^{bA}	22.8±1.00 ^{aB}
Solvent	**Citric acid 1 %**							
Temperature	20°C				45°C			
Time	15 min		50 min		15 min		50 min	
Betacyanins and betaxanthins /	Bx	Bc	Bx	Bc	Bx	Bc	Bx	Bc
20% Ethanol	56.4±2.00^{aA}	36.8±1.00^{aB}	47.1±3.00^{aA}	31.2±2.00^{aB}	31.4±2.00^{aA}	21.4±4.00^{aB}	44.9±16.00^{aA}	32.3±7.00^{aB}
50% Ethanol	46.5±2.00^{bA}	29.8±2.00^{bB}	53.6±2.00^{bA}	33.5±1.00^{aB}	20.6±6.00^{bA}	14.4±2.00^{bB}	31.4±5.00^{bA}	20.4±2.00^{bB}

Means in the same row that do not have a letter in common (a, b, c) are significantly different at p<0.05. Means in the same row that do not have a capital letter in common (A, B) are significantly different at p <0.05 using ANOVA and Tukey's test.

The extraction with 50% ethanol, after which the highest betalain content of 115.3 ± 0.60 m/100 g d.w. was obtained, took place at the temperature of 20°C, for 20 minutes in the presence of 1% citric acid.

Although the combination of the two parameters contributed to the extraction of considerable amounts of betalains, the combination of parameters such as a 20% ethanol and 1% citric acid at a temperature of 20°C for 20 minutes allowed the extraction of higher amounts of betalains of 118.5 ± 76.0 mg/100 g d.w. by conventional solvent extraction. Also, parameters such as 20% ethanol, 0.1% citric acid at a temperature of 20°C for 50 minutes led to a total amount of betalains of 118.4 ± 8.00 mg/100 g d.w. of which an amount of betaxanthins of 71.50 ± 5.00 mg/g d.w. and betacyanins of 47.00 ± 2.00 mg/100 g d.w.

The least suitable combination of parameters that extracted a smaller amount of betalain compounds turned out to be the one with 20% ethanol acidified with 1% citric acid, after 50 minutes of extraction, at a temperature of 20°C, which led to a total betalain content of only 64.00 ± 4.00 mg/100 g d.w. The results obtained in this study are in agreement with other studies reported by other authors.

The data in **Table 2.3**. and **2.4.** shows the variation of the content of total betalains (BT) as well as of the total content of betacyanins and betaxanthins by ultrasound-assisted ethanol extraction, depending on the studied parameters.

Ultrasound-assisted ethanol extraction allowed the obtaining of a maximum betalain content of 104.70 ± 3.00 mg/100 g d.w. with the help of 50% ethanol acidified with 0.1% citric acid, after 15 minutes of extraction at a temperature of 20°C. Of the total betalains obtained, betaxanthins represented 63.70 ± 2.00 mg/g d.w. and betacyanins 41.10 ± 1.00 mg/100 g d.w.

Extraction with 20% ethanol acidified with 0.1% citric acid resulted in a significantly different amount ($p < 0.05$) of betalains of 98.00 ± 7.00 after 50 minutes of extraction at a temperature of 20°C. Extraction with 50% ethanol and 1% citric acid led to the extraction of a small amount of betalains of only 35.00 ± 9.00 mg /100 g d.w., but this time after only 15 minutes of extraction at a temperature of 45 °C.

It can be concluded that the extraction temperature and time had a significant effect on the extracted betalain content. A temperature of up to 80°C for 30 minutes, as applied, allowed to obtain a higher betalain content, while for the extract obtained by using the temperature of 100°C, for 20 minutes led to a 0.63-fold decrease in betalain content for betacyanins and 0.77-fold for betaxanthins, respectively. It can be concluded that temperature and extraction time had a significant effect on the betalain content.

Microwave-assisted extraction is one of the most used modern extraction methods due to the reduced extraction time and low solvent consumption. This technique is characterized by breaking the cell walls due to the heating of the solvent with the help of microwaves. In **Tables 2.5.** and **2.6.** the data on the content of total betalains and, respectively, the total content of betacyanins and betaxanthins obtained following ethanol extraction assisted by microwaves by varying the parameters are presented.

This microwave-assisted ethanol extraction method resulted in the recovery of lower amounts of betalains compared to the other two types of extraction (conventional and ultrasound-assisted extraction) described above, by varying the same extraction parameters.

Thus, the combination of 20% ethanol and 0.1% citric acid allowed to obtain the highest amount of betalains of 87.70 ± 1.00 mg/100 g d.w., after an extraction time of 15 seconds at a power of 315W. Extraction with 50% ethanol yielded a betalain concentration of only 78.60 ± 8.00 mg/100 g d.w. in combination with 0.1% citric acid after 15 seconds of microwave treatment at a power of 315W.

Following microwave treatment, the solvents used probably reached temperatures that degraded the betalains. Thus, the lowest concentration of betalains recovered by the microwave method was observed in the extraction with 20% ethanol and 0.1% citric acid at a value of 53.20 ± 3.00 mg/g d.w. for 10 seconds at a power of 525W.

Table 2.5. Total betalain content obtained from microwave-assisted ethanol extraction

Total Betalains, mg/100 g d.w.								
Solvent	**Citric acid 0.1 %**				**Citric acid 1 %**			
Time	10 seconds		15 seconds		10 seconds		15 seconds	
Microwave power	315 W	525 W	315 W	525 W	315 W	525 W	315 W	525 W
20% Ethanol	74.8 ± 7.00^{aA}	53.2 ± 3.00^{aB}	87.7 ± 1.00^{aA}	56.6 ± 2.00^{aB}	71.4 ± 6.00^{aA}	64.7 ± 2.00^{aB}	64.6 ± 5.00^{aA}	79.4 ± 1.00^{aB}
50% Ethanol	77.0 ± 5.00^{bA}	55.2 ± 1.00^{aB}	78.6 ± 8.00^{bA}	60.2 ± 1.00^{bB}	76.5 ± 6.00^{bA}	65.2 ± 2.00^{aB}	55.6 ± 2.00^{bA}	64.9 ± 5.00^{bB}

Means in the same row that do not have a letter in common (a, b, c) are significantly different at p<0.05. Means in the same row that do not have a capital letter in common (A, B) are significantly different at p <0.05 using ANOVA and Tukey's test.

Table 2.6. The total content of betacyanins and betaxanthins obtained from microwave-assisted ethanol extraction

Betacyanins and betaxanthins, mg/100 g d.w.								
Solvent	**Citric acid 0.1 %**							
Time	10 seconds		10 seconds		15 seconds		15 seconds	
Microwave power	315 W		525 W		315 W		525 W	
Betacyanins and betaxanthins	Bx	Bc	Bx	Bc	Bx	Bc	Bx	Bc
20% Ethanol	42.2 ± 2.00^{aA}	32.6 ± 4.00^{aB}	40.7 ± 3.00^{aA}	12.5 ± 0.30^{aB}	52.4 ± 1.00^{aA}	35.3 ± 0.90^{aB}	43.6 ± 1.00^{aA}	13.0 ± 1.00^{aB}
50% Ethanol	43.6 ± 1.00^{aA}	33.4 ± 3.00^{aB}	43.3 ± 1.00^{aA}	11.9 ± 0.20^{aB}	46.2 ± 6.00^{bA}	32.5 ± 2.0^{bB}	47.7 ± 1.00^{bA}	12.5 ± 0.30^{aB}
Solvent	**Citric acid 1 %**							
Time	10 seconds		10 seconds		15 seconds		15 seconds	
Microwave power	315 W		525 W		315 W		525 W	
Betacyanins and betaxanthins	Bx	Bc	Bx	Bc	Bx	Bc	Bx	Bc
20% Ethanol	41.0 ± 2.00^{aA}	30.5 ± 4.00^{aB}	51.1 ± 1.00^{aA}	13.7 ± 0.90^{aB}	50.6 ± 5.00^{aA}	14.1 ± 0.80^{aB}	63.9 ± 1.00^{aA}	15.5 ± 0.30^{aB}
50% Ethanol	46.3 ± 0.30^{bA}	30.2 ± 2.00^{aB}	51.5 ± 1.00^{aA}	13.7 ± 1.00^{aB}	43.6 ± 2.00^{bA}	12.0 ± 0.20^{aB}	51.7 ± 4.00^{bA}	13.2 ± 1.00^{aB}

Means in the same row that do not have a letter in common (a, b, c) are significantly different at p<0.05. Means in the same row that do not have a capital letter in common (A, B) are significantly different at p <0.05 using ANOVA and Tukey's test.

Enzymatic additive extraction is known to help reduce solvent and energy consumption while helping to recover a greater amount of biologically active compounds. In this study, three enzyme preparations such as cellulase, pectolytic enzymes, and xylanase were used, and the results of the total betalain content obtained by the extraction with the addition of enzyme preparations are shown in **Table 2.7**.

The enzyme with pectolytic activity (Zymorouge) was able to extract betalains from cell walls after 15 minutes of hydrolysis, obtaining a maximum amount of 118.00 ± 3.00 mg/100 g d.w.

Regarding the content of betalains after cellulase treatment, a betalain concentration of 91.00 ± 2.00 mg /100 g d.w. is noted. after 15 min of extraction. However, in the case of the extraction of betalains from beetroot peel, it is observed that the lowest concentration of betalain compounds was extracted after xylanase treatment, namely only 49.00 ± 4.00 mg/100 g d.w. after 30 minutes of hydrolysis. Thus, increasing the extraction time, we can state that the betalains started to degrade up to 49.00 ± 5.00 mg /100 g d.w. after 1 hour of hydrolysis.

2.5.2. The total content of polyphenols in the extracts obtained

The quantification of polyphenols was carried out using the Folin Ciocâlteu test, which is based on the redox reaction between phenolic compounds and a mixture of tungsten and molybdenum in an alkaline environment.

Table 2.7. The content of total betalains obtained after extraction with the addition of enzyme preparations

BT content, mg CE/100 g d.w.									
Enzymes	Pectolytic enzymes			Cellulase			Xylanase		
Time	15 min	30 min	60 min	15 min	30 min	60 min	15 min	30 min	60 min
BT	118.00±3.00^{a}	58.00±3.00^{a}	74.00±2.00^{a}	91.00±2.00^{b}	58.00±1.00^{a}	63.00±2.00^{b}	74.00±6.00^{c}	49.00±4.00^{b}	49.00±5.00^{c}

Means in the same row that do not share a letter (a, b, c) are significantly different at p<0.05 between samples obtained with different enzyme preparations and the same extraction time using ANOVA and Tukey's test

Table 2.8. Total polyphenol content obtained by conventional solvent extraction

CTP, mg GAE/g d.w.								
Solvent	Citric acid 0.1 %				Citric acid 1 %			
Temperature	20°C		70°C		20°C		70°C	
Time	15 min	50 min	15 min	50 min	15 min	50 min	15 min	50 min
20% Ethanol	225.36±1.97^{a} A	214.04±2.60^{a} B	209.94±2.14^{a} A	186.89±4.48^{a} B	237.79±6.83^{a} A	186.82±10.16^{a} B	300.25±14.01^{a} A	231.97±2.26^{a} B
50% Ethanol	210.32±9.42^{b} A	210.06±0.95^{a} A	199.65±8.92^{b} A	171.46±5.27^{b} B	201.73±6.25^{b} A	246.85±4.20^{bB}	228.66±9.11^{bA}	213.40±2.62^{b} B

Means in the same row that do not have a letter in common (a, b, c) are significantly different at p<0.05. Means in the same row that do not have a capital letter in common (A, B) are significantly different at p <0.05 using ANOVA and Tukey's test

Table 2.9. Total polyphenol content obtained following ultrasound-assisted extraction

CTP, mg GAE/g d.w.								
Solvent	Citric acid 0.1 %				Citric acid 1 %			
Temperature	20°C		45°C		20°C		45°C	
Time	15 min	50 min	15 min	50 min	15 min	50 min	15 min	50 min
20% Ethanol	196.94±9.77^{a} A	199.21±10.38^{a} B	167.28±14.55^{a} A	156.59±5.95^{a} B	216.98±12.95^{a} A	193.67±4.17^{a} B	107.28±2.71^{a} A	228.18±4.37^{a} B
50%Ethanol	196.70±7.39^{a} A	199.22±10.53^{a} B	193.42±15.11^{b} A	162.25±4.99^{b} B	184.25±15.06^{b} A	198.64±8.65^{b} B	110.77±6.33^{b} A	179.24±5.00^{b} B

Means in the same row that do not have a letter in common (a, b, c) are significantly different at p<0.05. Means in the same row that do not have a capital letter in common (A, B) are significantly different at p <0.05 using ANOVA and Tukey's test

This reaction leads to the creation of a blue complex that is quantified at a wavelength of 765 nm. The results were expressed as mg gallic acid equivalent (EAG)/g lyophilized peel. In **table 2.8**. the total polyphenol content obtained by conventional solvent extraction is shown.

In the case of conventional extraction, the highest total polyphenol content of 300.25 ± 14.01 mg EAG/g d.w. it was obtained by extraction with 20% ethanol concentration and 1% citric acid, after 15 minutes of extraction at a temperature of 70°C.

However, a rather high content of total polyphenols of 246.85 ± 4.20 mg EAG/g d.w. also presented the extract obtained with 50% ethanol with 0.1% citric acid, after 50 minutes of extraction at a temperature of 20°C.

The lowest value of the content of total polyphenols was obtained by using 50% ethanol with 0.1% citric acid, after 50 minutes of extraction, at the temperature of 70°C, which led to obtaining only 171.46 ± 5.27 mg EAG/g d.w.

The extraction yield of total polyphenols obtained following ultrasound-assisted extraction is presented in **Table 2.9**.

The highest content of total polyphenols obtained after ultrasound-assisted extraction was observed for the extraction version with 20% ethanol acidified with 1% citric acid, after 50 minutes of treatment at a temperature of 45°C, respectively 228, 18 ± 4.37 mg EAG/g d.w.

The most unsuccessful combination of extraction parameters proved to be that of 20% ethanol acidified with 1% citric acid, after 15 minutes of extraction at 45°C, which led to obtaining only 107 .28 ± 2.71 mg EAG /g d.w.

In the case of microwave-assisted extraction (**table 2.10**), it was observed that the 20% ethanol acidified with 1% citric acid had the highest extraction yield of total polyphenols, being 257.26 ± 12.88 mg EAG/g d.w. Significant differences ($p<0.05$) were recorded for the extraction with 50% ethanol and 1% citric acid, the content of total polyphenols being 214.48 ± 1.36 mg EAG/g d.w.

In contrast, using 20% ethanol acidified with 1% citric acid extracted the highest amount of total polyphenols of 257.26 ± 12.88 mg EAG/g d.w. after 15 sec. of microwave treatment at a power of 525 W.

Enzyme-assisted extraction (enzymes with cellulase enzymatic activity) led to a higher content of total polyphenols compared to the yield of total polyphenols obtained in the case of microwave-assisted extraction. Thus, cellulase treatment extracted a total polyphenol content of 94.32 ± 7.65 mg EAG/g d.w. after 1 hour of hydrolysis. The enzymes with pectolytic activity (Zymorouge) extracted a CTP of 93.45 ± 0.74 mg EAG/g d.w. after 30 minutes of hydrolysis (**table 2.11.**), however, after treatment with xylanase the lowest concentration of polyphenols of only 80.67 ± 1.26 mg EAG/g d.w. was obtained. after 15 minutes of hydrolysis.

2.5.3. Antioxidant activity of extracts obtained by different extraction methods

Antioxidants play an important role in food preservation by inhibiting oxidation processes and contribute to health promotion being offered by many dietary supplements, nutraceuticals, and functional food ingredients. Antioxidant activity can be monitored by a variety of techniques with different mechanisms, the most commonly used being the DPPH free radical scavenging method which relies on the electron donation of antioxidants to neutralize the DPPH radical.

In **table 2.12**. the results of the antioxidant activity of the extracts obtained with the help of conventional solvent extraction, expressed as mMol Trolox/g d.w., are centralized. Antioxidant activity values ranged from 13.61±2.41 mM Trolox/g d.w. at 47.65 ± 0.21 mMol Trolox/g d.w. The highest antioxidant activity value of 47.65 ± 0.21 mMol Trolox/g d.w. was obtained for the extract obtained with 50% ethanol acidified with 1% citric acid, after 15 minutes of extraction at a temperature of 70°C.

Table 2.10. Total polyphenol content obtained from microwave-assisted extraction

CTP, mg EAG/g s.u.								
Solvent	**0.1 % Citric acid**				**1 % Citric acid**			
Time	10 seconds		15 seconds		10 seconds		15 seconds	
Microwave power	315 W	525 W	315 W	525 W	315 W	525 W	315 W	525 W
20 % Ethanol	$195.85\pm2.52^{a}_{A}$	181.06 ± 1.57^{aB}	$207.20\pm7.68^{a}_{A}$	179.16 ± 5.49^{aB}	$202.98\pm5.48^{a}_{A}$	188.68 ± 7.40^{aB}	188.39 ± 3.18^{aA}	$257.26\pm12.88^{a}_{B}$
50 % Ethanol	$191.33\pm5.94^{b}_{A}$	$188.12\pm18.07^{b}_{B}$	$206.37\pm1.53^{a}_{A}$	$199.00\pm30.22^{b}_{B}$	$214.48\pm1.36^{b}_{A}$	$178.54\pm13.43^{b}_{B}$	$192.70\pm14.49^{b}_{A}$	204.80 ± 6.07^{bB}

Means in the same row that do not have a letter in common (a, b, c) are significantly different at p<0.05. Means in the same row that do not have a capital letter in common (A, B) are significantly different at p <0.05 using ANOVA and Tukey's test

Table 2.11. The total content of polyphenols obtained after extraction with the addition of enzyme preparations

CTP, mg EC/g s.u.									
Enzymes	**Pectolytic enzymes**			**Cellulase**			**Xylanase**		
Time	15 min	30 min	60 min	15 min	30 min	60 min	15 min	30 min	60 min
CTP	92.27 ± 11.05^{a}	93.45 ± 0.74^{a}	88.68 ± 2.63^{a}	83.95 ± 1.50^{b}	93.73 ± 1.97^{a}	94.32 ± 7.65^{b}	80.67 ± 1.26^{c}	88.11 ± 0.82^{b}	85.36 ± 1.22^{c}

Means in the same row that do not have a letter in common (a, b, c) are significantly different at p<0.05. Means in the same row that do not have a capital letter in common (A, B) are significantly different at p <0.05 using ANOVA and Tukey's test

Table 2.12. Antioxidant activity of extracts obtained by the conventional method with solvents

Antioxidant activity, mM Trolox/mg d.w.								
Solvent	**0.1 % Citric acid**				**1 % Citric acid**			
Temperature	20°C		70°C		20°C		70°C	
Time	15 min	50 min	15 min	50 min	15 min	50 min	15 min	50 min
20% Ethanol	19.75 ± 0.59^{aA}	13.61 ± 2.41^{aB}	34.08 ± 5.18^{aA}	31.87 ± 0.83^{aB}	22.94 ± 0.94^{aA}	17.45 ± 1.77^{aB}	18.78 ± 3.27^{aA}	42.51 ± 2.02^{aB}
50% Ethanol	17.12 ± 0.94^{bA}	26.54 ± 3.96^{bB}	26.06 ± 5.26^{bA}	38.42 ± 0.80^{bB}	21.52 ± 0.61^{aA}	19.78 ± 2.48^{aB}	47.65 ± 0.21^{bA}	37.44 ± 0.51^{bB}

Means in the same row that do not have a letter in common (a, b, c) are significantly different at p<0.05. Means in the same row that do not have a capital letter in common (A, B) are significantly different at p <0.05 using ANOVA and Tukey's test

The antioxidant activity of the analyzed extracts increased with the increase in temperature during the extraction, with all experimental variants showing satisfactory values of the antioxidant activity.

The lowest antioxidant potential was obtained for the 20% ethanol extract with 0.1% citric acid after 15 minutes of extraction at 20°C, which resulted in a value of only 13.61 ± 2.41 mMol Trolox /g d.w.

The results regarding the antioxidant activity of beetroot peel extracts obtained by ultrasound-assisted extraction are presented in **Table 2.13**.

Through ultrasound-assisted extraction, antioxidant activity values between 33.42 ± 1.60 and 3.38 ± 1.80 mMol Trolox/g d.w. were obtained. The lowest value of the antioxidant activity was obtained using an ethanol concentration of 20% and the highest antioxidant activity was obtained using an ethanol concentration of 50%, in different combinations of parameters. Thus, the highest antioxidant potential was presented by the 50% ethanol extract acidified with 1% citric acid at a temperature of 20°C, and the extraction time was 15 minutes. The combination of 20% ethanol acidified with 0.1% citric acid at the temperature of 45°C, and the extraction time of 50 minutes led to obtaining a low antioxidant activity with a value of only 3.38 ± 1.80 mMol Trolox/g.

The antioxidant activity of the extracts determined by microwave-assisted extraction varied according to the extraction parameters, according to the data presented in **Table 2.14**.

Looking as a whole, it can be seen that the extracts with 20% ethanol concentration had the best antioxidant potential, presenting the highest values of antioxidant activities between the value of 20.21 ± 1.01 and the value of 30.54 ± 0.75 mMol Trolox/g d.w.

However, the highest value of the antioxidant activity was obtained for the extraction with 50% ethanol concentration acidified with 1% citric acid for 10 seconds, having a value of 30.82 ± 0.33 mMol Trolox/g d.w. At the same time, it is observed that for all the extraction variants analyzed, time led to a slight reduction in the antioxidant activity.

Table 2.15. shows the antioxidant activity of the extracts obtained by extraction with the addition of enzyme preparations.

It is observed that also in the case of antioxidant activity, the enzymes with pectolytic activity (Zymorouge) gave the best yield for the extraction of biologically active compounds with antioxidant activity on the DPPH radical.

Table 2.13. Antioxidant activity of extracts obtained by ultrasound-assisted extraction

Antioxidant activity, mM Trolox/g d.w.								
Solvent	**0.1 % Citric acid**				**1 % Citric acid**			
Temperature	20°C		45°C		20°C		45°C	
Time	15 min	50 min	15 min	50 min	15 min	50 min	15 min	50 min
20 % Ethanol	19.88 ± 0.42^{aA}	26.60 ± 1.49^{aB}	5.86 ± 0.87^{aA}	3.38 ± 1.80^{aB}	20.43 ± 1.15^{aA}	15.61 ± 0.27^{aB}	10.71 ± 2.26^{aA}	6.92 ± 0.41^{aB}
50 % Ethanol	14.43 ± 0.72^{bA}	9.43 ± 1.42^{bb}	15.89 ± 0.77^{bA}	28.29 ± 3.79^{bB}	33.42 ± 1.60^{bA}	26.43 ± 0.34^{bB}	31.37 ± 0.34^{bA}	30.64 ± 0.77^{bB}

Means in the same row that do not have a letter in common (a, b, c) are significantly different at p<0.05. Means in the same row that do not have a capital letter in common (A, B) are significantly different at p <0.05 using ANOVA and Tukey's test

Table 2.14. Antioxidant activity of extracts obtained by microwave-assisted extraction

Antioxidant activity, mM Trolox/g d.w.								
Solvent	**0.1 % Citric acid**				**1 % Citric acid**			
Timp	10 secunde		15 secunde		10 secunde		15 secunde	
Putere microunde	315 W	525 W	315 W	525 W	315 W	525 W	315 W	525 W
Etanol 20 %	20.27 ± 1.19^{aA}	26.00 ± 2.48^{aB}	18.74 ± 6.13^{aA}	24.62 ± 1.76^{aB}	26.48 ± 2.15^{aA}	30.54 ± 0.75^{aB}	22.27 ± 1.68^{aA}	20.21 ± 1.01^{aA}
Etanol 50 %	20.59 ± 2.76^{aA}	26.88 ± 0.74^{aB}	15.37 ± 1.14^{bA}	26.52 ± 1.66^{aB}	26.16 ± 3.10^{aA}	30.82 ± 0.33^{aB}	26.63 ± 1.43^{bA}	26.73 ± 2.10^{bA}

Means in the same row that do not have a letter in common (a, b, c) are significantly different at p<0.05. Means in the same row that do not have a capital letter in common (A, B) are significantly different at p <0.05 using ANOVA and Tukey's test

Table 2.15. Antioxidant activity of extracts obtained by extraction with the addition of enzyme preparations

Antioxidant activity, mM Trolox/g d.w.									
Enzymes	**Pectolytic enzymes**			**Cellulase**			**Xylanase**		
Time	15 min	30 min	60 min	15 min	30 min	60 min	15 min	30 min	60 min
AA	13.74 ± 0.05^{a}	19.11 ± 0.27^{a}	11.98 ± 0.42^{a}	14.17 ± 0.86^{b}	18.40 ± 0.45^{b}	12.57 ± 0.37^{b}	14.75 ± 1.57^{b}	18.97 ± 0.06^{b}	12.30 ± 1.68^{b}

Means in the same row that do not have a letter in common (a, b, c) are significantly different at p<0.05. Means in the same row that do not have a capital letter in common (A, B) are significantly different at p <0.05 using ANOVA and Tukey's test

After only 30 minutes of extraction, it resulted in an extract with high antioxidant activity of 19.11 ± 0.27 mMol Trolox/g d.w. It was observed, however, that with the increase of the extraction time, up to 60 minutes, the antioxidant activity decreased to the value of 11.98 ± 0.42 mMol Trolox/g s.u due to the degradation of the biologically active compounds with antioxidant activity on the DPPH radical.

A decrease in antioxidant activity over time was observed after xylanase treatment, with low antioxidant activity values of 12.30 ± 1.68 mMol Trolox/g d.w.

In **figures 2.1. - 2.3.** an analysis of the content of biologically active compounds from the extracts obtained from beetroot peel was carried out by the three extraction methods analyzed, respectively, the conventional extraction method with solvents, the ultrasound assisted ethanol extraction method, and the microwave assisted ethanol extraction method.

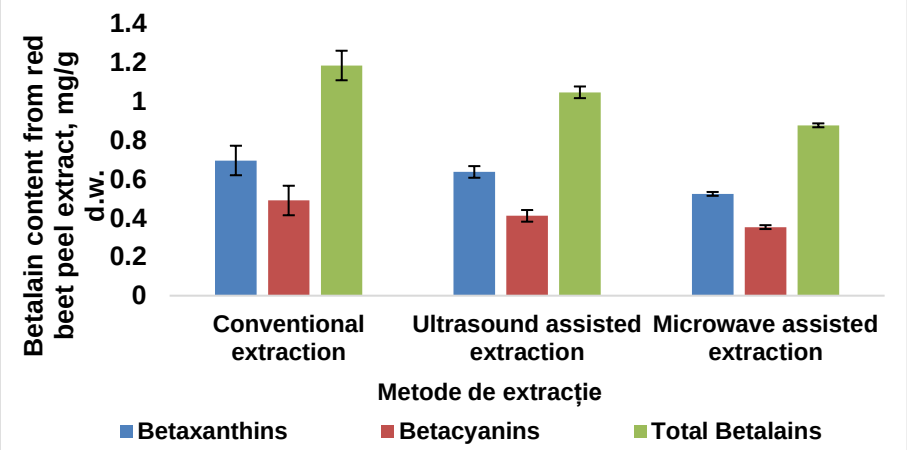

Figure 2.1. The total content of betalains (betaxanthins and betacyanins) in beetroot peel extracts obtained by different extraction methods

As can be seen in **figure 2.1**, the highest values of the content of total betalains were obtained by conventional extraction with solvents (20% ethanol) being approximately 118.5 ± 6.00 mg/100g d.w. Similar values were also obtained in the case of ultrasound-assisted ethanol

extraction, where the total betalain content was 104.7 ± 3.00 mg/100g d.w. The microwave-assisted ethanol extraction method led to the recovery of lower amounts of betalains than the other two types of extractions. Thus, the combination of 20% ethanol acidified with 0.1% citric acid allowed obtaining amounts of betalains of 87.7 ± 1.00 mg/100g d.w., after a time of 15 seconds at a power of 315 W.

Therefore, high ultrasound power or frequency could have degraded the betalain pigments, thus resulting in a lower extraction yield. Therefore, process optimization is an important step to achieve the maximum extraction efficiency of the compounds of interest.

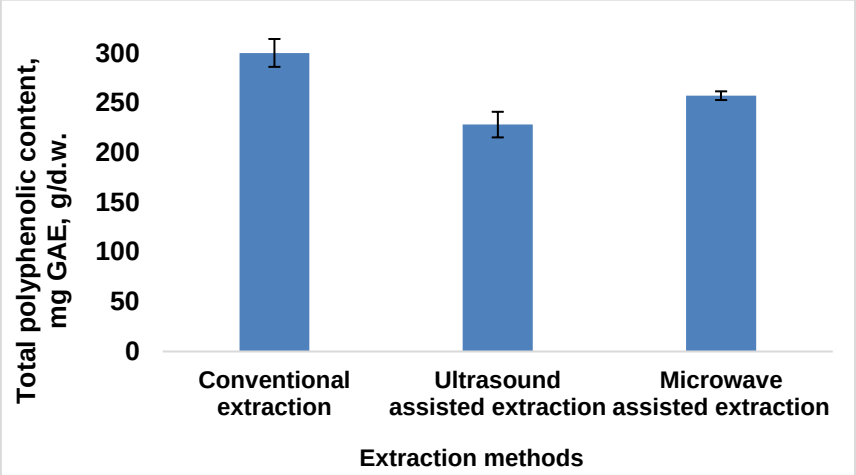

Figure 2.2. Total polyphenol content of beetroot peel extracts obtained by different extraction methods

Figure 2.2. shows the total polyphenol content following conventional extraction compared to the total polyphenol content obtained following ethanol ultrasound assisted extraction and respectively the total polyphenol content obtained following microwave assisted ethanol extraction. Thus, as can be seen, the highest values of total polyphenol content were obtained by conventional extraction 300.25 ± 14.01 mg EAG/g d.w. with 20% concentration ethanol and 1% citric acid as extraction parameters, after 15 minutes of extraction at a temperature of 70°C. Similar values were also obtained by the microwave-assisted ethanol extraction method, where the content of total polyphenols was 257.26 ± 12.88 mg EAG/g d.w.

The ethanol ultrasound assisted extraction method led to the recovery of a smaller amount of polyphenolic compounds compared to the other two types of extraction presented, respectively of 228.18 ± 4.37 mg EAG/g d.w.

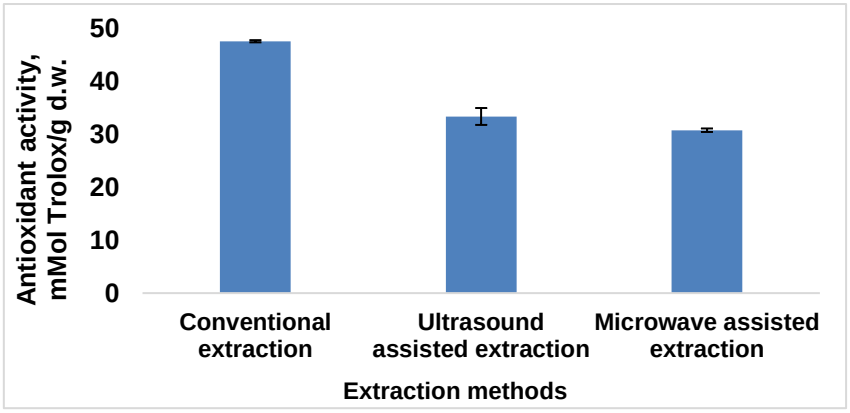

Figure 2.3. Antioxidant activity of beetroot peel extracts obtained by different extraction methods

According to the results presented in **figure 2.3**, the highest values of antioxidant activity were obtained by conventional extraction, being 47.65 ± 0.21 mMol Trolox/g d.w. using parameters such as ethanol concentration of 50% and citric acid 1%, after 15 minutes of extraction at a temperature of 70°C. Regarding the ultrasound assisted ethanol extraction method, an antioxidant activity of 33.42 ± 1.60 mMol Trolox/g d.w. was obtained, and by the microwave assisted ethanol extraction method, antioxidant activity values of 30.82 ± 0 were obtained .33 mMol Trolox/g d.w. having as extraction parameters ethanol concentration of 50%, and citric acid 1% for 10 seconds.

Regarding the extraction with the addition of enzyme preparations, three different enzyme preparations were used (cellulase, pectolytic enzymes, and xylanase), the extraction time being 15, 30, and 60 minutes. The enzyme extraction with pectolytic activity (Zymorouge) was able to extract betalains from cell walls after 15 minutes of hydrolysis, obtaining a maximum amount of 118.00 ± 3.00 mg/g d.w.

However, in the case of the extraction of betalains from beetroot peel, it is observed that the lowest concentration of betalains was extracted following xylanase treatment, of only 49.00 ± 4.00 mg/g d.w. after 30 minutes of hydrolysis. Increasing the extraction time, the betalains started to degrade up to 49.00 ± 5.00 mg /g d.w. after 1 hour of hydrolysis.

The extraction in the presence of the enzymes based on cellulase led to obtaining a higher content of polyphenolic compounds compared to that obtained in the case of microwave assisted ethanol extraction. Thus, cellulase treatment extracted a total polyphenol content of 94.32 ± 7.65 mg EAG/g d.w. after 1 hour of hydrolysis. The enzyme preparation with pectolytic activity extracted a similar amount of 93.45 ± 0.74 mg EAG/g d.w. after 30 minutes of hydrolysis (see **table 2.15.**). However, in the case of the extraction of polyphenols from beetroot peel, it is observed that the lowest concentration of polyphenols was extracted with xylanase, of 80.67 ± 1.26 mg EAG/g d.w. after 15 minutes of hydrolysis.

It is observed that also in the case of antioxidant activity, the enzyme extraction with pectolytic activity (Zymorouge) led to the best antioxidant potential. After only 30 minutes of extraction, it led to obtaining an extract with antioxidant activity of 19.11 ± 0.27 mMol Trolox/g d.w. It was observed, however, that with the increase in the extraction time, up to 60 minutes, the antioxidant activity decreased to the value of 11.98 ± 0.42 mMol Trolox/g d.w.

2.6. Partial conclusions

This study aimed to choose the extraction method and the optimal parameters that lend themselves best to the beetroot peel extract, to increase the extraction yield of betalains and polyphenolic compounds, in correlation with obtaining an improved antioxidant activity. Four different extraction methods were used for this purpose, namely the conventional solvent extraction method, the ultrasound-assisted ethanol extraction method, the microwave-assisted ethanol extraction method, and the extraction method with the addition of enzymes. In all these methods, the concentration of ethanol (20%, 50%), citric acid (0.1%, 1%), as well as the extraction time and temperature were varied.

The highest values of total betalain content were obtained by conventional extraction 118.5 ± 6.00 mg/g d.w. using 20% ethanol and 1% citric acid for extraction at a temperature of 20°C for 15 minutes.

Regarding the content of total polyphenols, the highest yield of 300.25 ± 14.01 mg EAG/g d.w. it was obtained by conventional extraction with 20% concentration ethanol and 1% citric acid as extraction parameters, after 15 minutes of extraction at a temperature of 70°C.

The highest values of antioxidant activity were obtained by conventional extraction of 47.65 ± 0.21 mMol Trolox/g d.w. what was obtained for the extracts with 50% ethanol concentration and 1% citric acid, after 15 minutes of extraction at a temperature of 70°C.

With the help of the enzymes with pectolytic activity (Zymorouge), an amount of betalains was extracted from the cell walls, after 15 minutes of hydrolysis, of 118.00 ± 3.00 mg/g d.w.

Cellulase addition extraction extracted a CTP of 94.32 ± 7.65 mg EAG/g d.w. after 1 hour of hydrolysis. In the case of antioxidant activity, the enzyme preparation with pectolytic activity (Zymorouge) gave the best antioxidant potential of 19.11 ± 0.27 mMol Trolox/g s.u after only 30 min of extraction.

Chapter 3. THE OPTIMIZATION OF THE EXTRACTION OF BETALAINIC PIGMENTS AND TOTAL POLYPHENOLIC COMPOUNDS RESULTED FROM THE PROCESSING OF RED BEET PEELS

3.1. General aspects

Beetroot (Beta vulgaris L.) is a plant belonging to the family Chenopodiaceae that includes about 105 genera with 1400 species (Chawla et al., 2015), widely cultivated in Europe, America and Asia (Wruss et al., 2015). Similarly, beetroot is probably the most cultivated root vegetable in south-eastern Romania. The edible part of the beet used in the food sector is the tuberous root. The leaves are also used but are generally considered raw materials for obtaining fodder. Beetroot is mainly available in the cold season, but it is also adapted to high temperatures. The optimum growth temperature of the plant varies between 15 and 19 °C, with lower temperatures influencing the accumulation of betalain pigments in larger quantities.

Initially, beetroot was cultivated for medicinal uses, and later from the beginning of the third century, it was predominantly used for human consumption (Neha et al., 2018). The best-known existing species are sugar beet (Beta vulgaris saccharifera), fodder beet (Beta vulgaris crassa), leaf beet (*Beta vulgaris* cicla), and garden beet (*Beta vulgaris* rubra).

Beetroot is rich in flavonoids, vitamins (niacin, biotin, and pyridoxine), minerals (potassium, sodium, phosphorus, calcium, magnesium, copper, iron, zinc, etc.) (Wootton et al., 2011). Beetroot is also one of the richest sources of betalains, which are the water-soluble betalamic acid-derived plant pigments responsible for a deep red (betacyanins) or yellow (betaxanthins) color (Vorobiev et al., 2013).

The intensity of the beetroot color depends on the ratio of betacyanins to betaxanthins. More than 80% of all beet pigments are betacyanin compounds (Liu et al., 2008). Betalains from *Beta vulgaris* rubra show high coloring capacity, as well as remarkable antioxidant capacity, being able to protect in vivo from disorders caused by oxidative stress.

The functions of betalains refer to reducing homocysteine concentration, which regulates vascular homeostasis, maintaining platelet function, thrombotic activity, vascular tone, and blood vessel stability by releasing compounds with vasodilator and vasoconstrictor properties.

Some effects attributed to these compounds include antioxidant capacity (Albano et al., 2015; Ravichandran et al., 2013) antiproliferative (Kumar et al., 2014), cardioprotective (Hobbs et al., 2013), anti-inflammatory (Vidal et al. ., 2014) as well as antimicrobial effects (Faridah et al., 2015).

Betalains present in beetroot are used to color various food products, for example, frozen yogurt, wine, jams, and yogurt (Kusznierewicz et al., 2021). For this reason, extraction is an essential step in the process of separating biologically active compounds. Currently, there are many extraction methods, but the most widely used method is conventional solvent extraction. It is widely used due to its efficiency, short extraction time, and low economic costs, despite

disadvantages such as the use of large amounts of extraction solvents (Azmir et al., 2013). Betalains are usually extracted from plant matrices by conventional methods as well as by Soxhlet extraction (RamLi et al., 2014; Hilou et al., 2013).

3.2. Study objectives

The main objective of this study was to optimize conventional solvent extraction conditions for betalain compounds and polyphenolic compounds content in beetroot peel extract using the Central Composite Design screening matrix.

In this study, the influence of four independent variables such as ethanol concentration, citric acid concentration, extraction temperature, and extraction time was tested. A factorial experimental model with three central points (CCD "Central Composite Design") was used, which allowed the design of 19 experimental variants, to optimize the extraction conditions of the evaluated responses, namely the content of phenolic compounds, as well as the content betalain compounds of the extract obtained from beetroot peel.

3.5. Results and discussion

To determine the optimal parameters for the optimization of the extraction process, the Central Composite Design (CCD) projection matrix and the response surface analysis method were used, in which BT and CTP contents were measured as responses. Table 3.2. shows the full CCD projection matrix used to optimize the main studied variables and their corresponding values.

The extraction of phytochemical compounds from plant materials is influenced by the extraction parameters used. Moreover, the different polarities of the compounds extracted by applying the experimental model could have an unpredictable impact on the extraction conditions. Therefore, extractions were performed with extractants having different polarities, controlled by adjusting the ratio of water and ethanol. Thus, adding water to the ethanol solution can increase the extraction yield of betalain content (Fu et al., 2020), and the extracts can be easily used in biological systems. Optimizing the extraction conditions of total betalains led to the addition of citric acid to the extraction mixture to acidify the medium, mainly because betalains are stable at pH in the range of 3.0–7.0. (Castro-Enriqez et al., 2020).

Table 3.2. CCD projection matrix with the actual values of the main variables studied

	Factor 1	**Factor 2**	**Factor 3**	**Factor 4**	**Answer 1**	**Answer 2**
Nr.	A: Citric acid	B: Ethanol	C: Temperature	D: Time	Betalains	CTP
	%	%	°C	min	mg/g d.w.	mg GAE/g d.w.
1	0.80	35.00	40.00	3.07	0.80	202.01
2	0.80	35.00	40.00	32.50	0.98	230.20
3	1.98	35.00	40.00	32.50	1.03	238.04
4	0.10	50.00	20.00	50.00	0.88	212.66
5	0.10	50.00	60.00	50.00	0.70	192.14
6	1.50	20.00	20.00	50.00	0.85	200.11
7	1.50	20.00	60.00	50.00	0.62	180.32
8	0.80	35.00	6.36	32.50	0.65	189.41
9	0.80	35.00	40.00	32.50	1.02	228.52
10	1.50	50.00	60.00	15.00	0.76	196.48

11	0.10	20.00	20.00	15.00	0.78	205.17
12	0.80	35.00	73.64	32.50	0.29	164.35
13	0.03	35.00	40.00	32.50	0.67	199.65
14	0.80	60.23	40.00	32.50	1.44	274.21
15	0.80	35.00	40.00	61.93	1.14	187.04
16	1.50	50.00	20.00	15.00	0.99	222.31
17	0.80	35.00	40.00	32.50	1.10	226.71
18	0.80	9.77	40.00	32.50	0.65	181.08
19	0.10	20.00	60.00	15.00	0.36	177.04

3.5.1. Influence of extraction parameters on total betalains (BT) content

The regression equations obtained after ANOVA analysis of variance described the BT content of the extract obtained from beet peel, according to the extraction factors analyzed (**Table 3.2**).

The regression equations obtained from the ANOVA analysis of variance described the BT content of the beet peel extract obtained, depending on the factors of the extraction medium (**Table 3.3**).

The regression model obtained for BT indicated a correlation coefficient of R^2 = 0.96, suggesting that only 0.04 of the variation in BT could not be described by the current model. The F-value of no correlation of 1.12 indicates that the non-correlation is not significant relative to the pure error. p-values less than 0.0500 indicate that the model terms are significant, and in this case, A, B, C, D, AB, AD, A^2, C^2 are the significant model terms. The non-essential terms of the model were excluded and thus a simplification of the model was achieved.

As a result, the model equation indicating the relationship between the betalain content (R1) and the variables expressed in coded units is represented in **equation 2.1**. The predicted R^2 value (Pred R^2 - 0.8796) is comparable to the adjusted R^2 value (Adj R^2 - 0, 9452):

$$R1\ (BT)= +1{,}01+0{,}08A+0{,}23B-0{,}12C+0{,}1011D+0{,}08AB+0{,}14AD-0{,}06A^2-0{,}19C^2 \quad (\mathbf{2.1})$$

The b coefficients in the regression equation indicated that temperature had a significant negative effect on the extraction of total betalains. Also, significant negative effects were determined by the double effect of temperature (C^2), while the concentration of citric acid (A^2) had a smaller influence. Moreover, ethanol concentration (B) and extraction time (D) had a significant positive influence on BT extraction. The interaction between temperature and extraction time (AB) moderately affected the extraction, while citric acid concentration and ethanol concentration (AD) had a better effect.

The CCD projection matrix revealed that a low temperature of 6.3°C is insufficient for the total extraction of betalains, and also temperatures higher than 73.54°C have a negative influence on betalains that can lead to their degradation (Chhikara et al., 2019).

Figure 3.1 (**A, a**) represents the correlation between ethanol and citric acid concentration on the extraction yield. The betalain content increased as the concentration of the ethanol solution increased reaching 40% and 1.30% for the citric acid concentration. The extraction of betalanins was influenced by the correlative effect of citric acid concentration and extraction time (**Figure 3.1B, b**). The extraction of betalain compounds was negatively influenced both at low concentrations of citric acid (0.10%) and at high concentrations exceeding 1.50%.

Similarly, extraction time negatively affects the process at values greater than 50 min and less than 15 min. It can be concluded that by increasing the extraction time and decreasing the concentration of citric acid in the extraction medium, the extraction yield of betalain compounds decreases significantly.

In the analysis of deviation from the set point, a steep or curved slope for a specific factor shows that the response is sensitive to that factor, while a relatively flat line demonstrates insensitivity to changes in that factor. The main factor affecting BT extraction is ethanol (**Figure 3.2.a, curve B**) followed by extraction time (**Figure 3.2.a, curve D**).

The response that influences the extraction the least is citric acid (**Figure 3.2.a, curve A**). Since betalains are sensitive to high temperatures and long processing, a possible "green" method was sought to keep these compounds in the product.

The experimental study revealed variations in betalain content ranging from 0.29 to 1.44 mg/g d.w. (**Table 3.2.**).

Table 3.3. ANOVA for the reduced quadratic model of BT and CTP

	Total Betalains (TB) Content					Total Polyphenol Content (TPC)				
Source	**Sum of Squares**	**df**	**Mean Square**	**F-Value**	***p*-Value**	**Sum of Squares**	**df**	**Mean Square**	**F-Value**	***p*-Value**
Model	1.30	8	0.1625	39.84	<0.0001 [a]	1.22	9	0.1356	80.87	<0.0001
A-Citric acid	0.0898	1	0.0898	22.02	0.0009	0.0765	1	0.0765	45.61	<0.0001
B-Ethanol	0.3120	1	0.3120	76.50	<0.0001	0.4336	1	0.4336	258.56	<0.0001
C-Temperature	0.2026	1	0.2026	49.67	<0.0001	0.1340	1	0.1340	79.93	<0.0001
D-Time	0.0578	1	0.0578	14.17	0.0037	0.0125	1	0.0125	7.44	0.0233
AB	0.0213	1	0.0213	5.23	0.0453	0.1383	1	0.1383	82.49	<0.0001
AD	0.0687	1	0.0687	16.85	0.0021	0.0345	1	0.0345	20.58	0.0014
A^2	0.0567	1	0.0567	13.90	0.0039	0.0097	1	0.0097	5.77	0.0397
C^2	0.5475	1	0.5475	134.20	<0.0001	0.4186	1	0.4186	249.63	<0.0001
D^2	-	-	-	-	-	0.1666	1	0.1666	99.38	<0.0001
Residual	0.0408	10	0.0041			0.0151	9	0.0017		
Lack of Fit	0.0333	8	0.0042	1.12	0.5545 [b]	0.0145	7	0.0021	7.58	0.1215
Pure Error	0.0075	2	0.0037			0.0005	2	0.0003		
Cor Total	1.34	18				1.24	18			

SS—Sum of Squares, MS—Mean Square; [a] Significant; [b] Not significant.

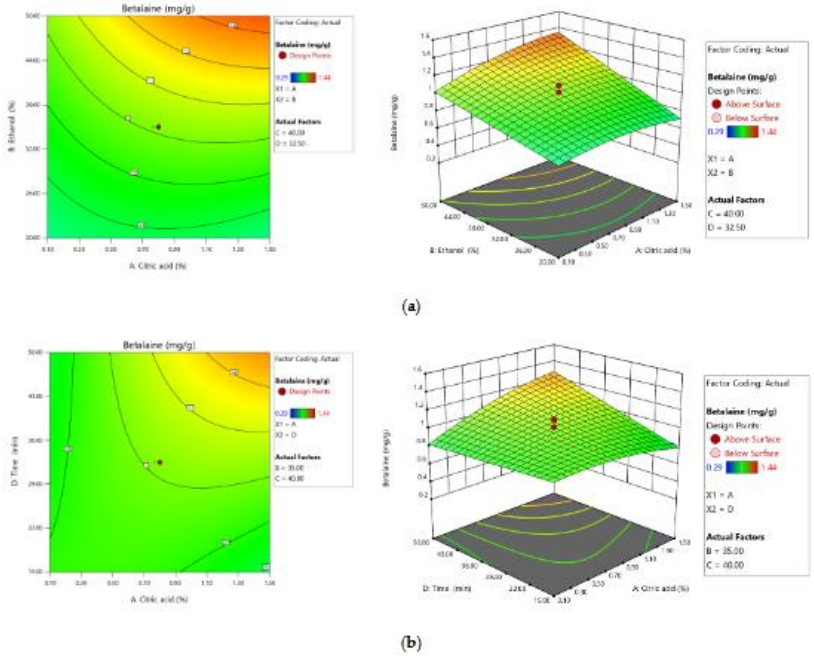

Figure 3.1. Second-order contour plots (left) and response surface plots (right) analyzing the effect of ethanol and citric acid concentration (A, a) and extraction time and citric acid concentration (B, b) on total yield of total betalain content (BT) extraction

The diverse distribution of betalains in different parts of the red beet indicated a potential value of the petiole from the red-colored leaves, which show the highest level of isobetalain.

In addition, the high content of glutamine-betaxanthin in beetroot pulp makes it a good source of pigments and also represents a potential opportunity to exploit at an industrial level for the production of natural yellow dyes. The distribution of betalains obtained was from 5.33 to 31.04 mg/g d.w. for bark, from 0.35 to 8.65 mg/g d.w. for pulp, and from 0.85 to 11.10 mg/g d.w. for petiole.

3.5.2. The influence of extraction parameters on the total content of polyphenolic compounds (CTP)

The model F value of 80.87 indicates that the model is significant. The regression coefficient of determination R2 = 0.98 suggests that only 0.02 of the variation in CTP cannot be described by the current model. Subsequently, from **Table 3.3**, it was observed that the lack of adjustment of the F value of 7.58 indicates that, the non-correlation was insignificant, which showed that the model is also significant. p-values less than 0.0500 indicate that the model terms are significant, and in this case, the significant model terms are A, B, C, D, AD, BD, A^2, C^2, D^2.

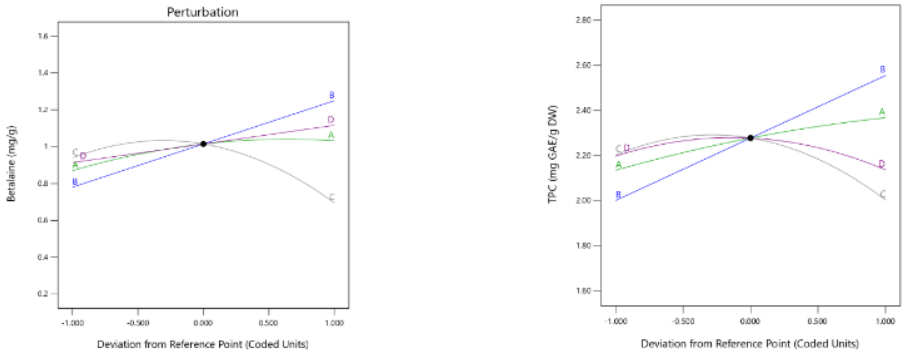

Figure 3.2. Response perturbation plots depicting the effect of each independent variable on BT (a) and CTP (b) extraction

As a result, the model equation indicating the correlation between the content of polyphenols (R^2) and the variables expressed in coded units is represented in **equation 2.2**. Insignificant terms of the model were ignored and thus a simplification of the model was achieved. Furthermore, the predicted R^2 value (Pred R^2 - 0.8875) is comparable to the adjusted R^2 value (Adj R^2 - 0.9756).

$$R^2\,(CTP) = + 2{,}28 + 0{,}11A + 0{,}27B - 0{,}99C - 0{,}30D + 0{,}20AD + 0{,}10BD - 0{,}26A^2 - 0{,}17C^2 - 0{,}11D^2 \quad (\mathbf{2.2})$$

The coefficients b in the regression equation indicated that temperature and time had a minor negative effect on the extraction of polyphenolic compounds. On the other hand, extraction time positively influences CTP extraction when associated with ethanol and citric acid concentration.

Second-order contour plots were designed to predict the relationship between independent and dependent variables (**Figure 3.2.**) and to illustrate the synergistic effects of independent factors on CTP extraction. Response surface plots depict the correlative effect of selected factors on CTP extraction. The coordinates of the center point in the contour plot correspond to the optimal concentration of the four extraction process factors to achieve maximum extraction process efficiency.

The effects of varying extraction time and citric acid concentration on the extraction of phenolic compounds are shown in **Figure 3.3.** (**A, a**). The concentration of polyphenols is lower

as the extraction time and citric acid concentration increase simultaneously. **Figure 3.3.** (**B, b**) confirmed that the extraction was not influenced by the variation of the extraction time, but was influenced by the ethanol concentration.

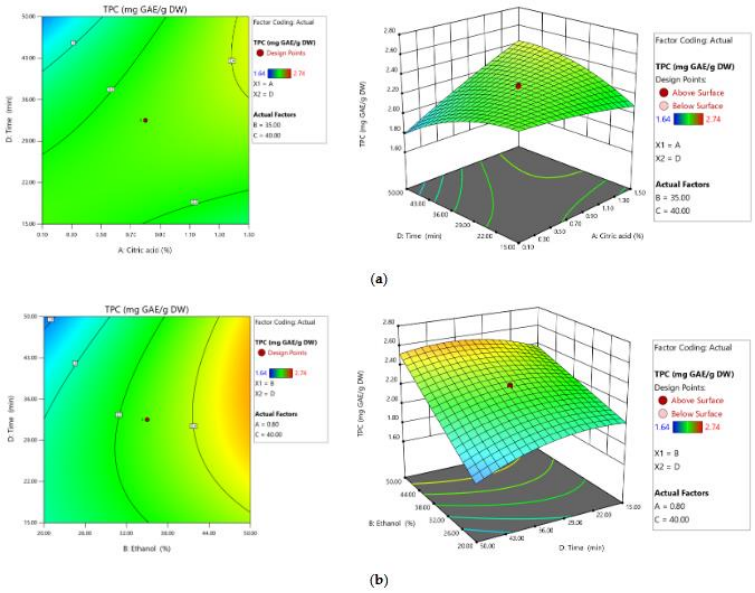

Figure 3.3. Second-order contour plots (left) and response surface plots (right) analyzing the effect of extraction time and citric acid concentration (A, a) as well as extraction time and ethanol (B, b) on yield extraction of the content of polyphenols (CTP)

According to the response perturbation plot depicting the effect of each independent variable, CTP extraction is strongly influenced by ethanol concentration and, to a lesser extent, by temperature and time (**Figure 3.2.b, curves B, C, and D**). Thus, a maximum polyphenol content of 2.74 mg EAG/g d.w. was determined. at 40°C, it has a maximum ethanol concentration of 60.23%.

3.5.3. Optimization and validation of extraction parameters

The developed model identified the optimal conditions based on maximizing the desirability of the responses to validate the model equation. A desirability value of 0.956 suggested that all selected conditions are in a suitable combination (**Figure 3.4., Table 3.4.**).

The optimal conditions for the maximum recovery of total betalains and total polyphenols were citric acid concentration of 1.5%, ethanol concentration of 50%, extraction temperature of 52.52°C, and extraction time of 49.9 min.

Table 3.4. Validation of the mathematical model

Dependent variables	Predicted value	Confidence interval 95%	Experimental value
BT (mg/g d.w.)	1,15	1,06-1,25	1,20
CTP (mg EAG/g d.w.)	244	2,39-2,49	239

The model estimated the maximum concentration of betalains and total polyphenols of 1.15 mg /g d.w. and 244 mg EAG/g d.w., respectively, and the experimental results indicated responses close to those predicted by the model, namely 1.20 mg/g d.w. and 239 mg EAG/g d.w. (**Table 3.4.**).

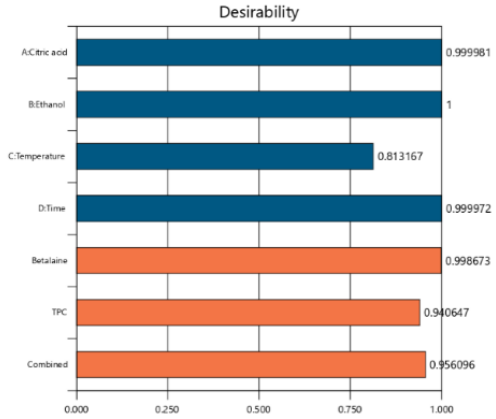

Figure 3.4. Specific graphs for identifying individual and cumulative desirability

3.6. Partial conclusions

The use of conventional solvent extraction proved to be the most effective method of extracting biologically active compounds from beetroot peel extract, thus, this extraction method was subjected to mathematical and statistical modeling experiments with the Design Expert program using Central Composite Design.

The Central Composite Design (CCD) projection matrix and response surface analysis technique were used to investigate the effect of extraction parameters in order to optimize the extraction parameters of total betalains and total polyphenols from beetroot peel. A quadratic model was determined for all parameters used. Several variables were applied by varying the extraction parameters (ethanol and citric acid concentration, temperature, and time). The maximum and minimum variables investigated in the experimental plan are citric acid concentration (0.10-1.5%), ethanol concentration (10-50%), extraction temperature (20-60°C), and extraction time (15 -50 minutes).

Mathematical modeling experiments indicated variations in total betalain content from 0.29 to 1.44 mg/g d.w., and polyphenol yield varied from 164 to 274 mg/g d.w. The optimized conditions for the maximum extraction of betalains and polyphenols were: citric acid concentration of 1.5%, ethanol concentration of 50%, temperature of 52.52°C, and extraction time of 49.9 min. It can thus be seen that the extraction process can be improved by adjusting the operating variables to maximize the model responses.

This study was carried out with the main aim of maximizing the extraction of bioactive compounds from beetroot peel for future uses with the aim of obtaining new value-added food products. Due to the significant concentration of valuable bioactive constituents in beetroot peel, the use of these compounds could expand in various branches of the food industry, as well as in the pharmaceutical and nutraceutical industries.

Chapter 4. ADVANCED CHARACTERIZATION OF THE OPTIMIZED RED BEET PEELS EXTRACT

4.1. General aspects

Beetroot (Beta vulgaris L. ssp. vulgaris) is a traditional vegetable distributed throughout the world, cultivated commercially for salads, juice, and natural pigments. The red beet color is due to the presence of betalains in the cell vacuoles. Beetroot is an excellent source of these nitrogen pigments, consisting mainly of two red-purple betacyanins (betanin and isobetanin) as well as yellow betaxanthins (in minor amounts). Betanin is also known for its non-toxic properties, and beetroot has been the main subject of scientific experiments of great interest because the

red pigment can be used in the pharmaceutical and food industries. As a nutrient-dense vegetable, beetroot is ranked among the ten most powerful vegetables in terms of antioxidant activity. In addition to carotenoids, chlorophylls, and anthocyanins, betalains are among the most widely used plant pigments that give yellow, orange, red, and purple colors to various flowers, herbs, fruits, and vegetables. Unlike anthocyanins, which practically participate in the coloring of all members of angiosperms, betalains are synthesized only in the suborder Chenopodiniae of the Caryophyllales and in some genera of Basidiomycetes. Interestingly, betalains and anthocyanins, the two red color pigments, have never been detected in the same plant simultaneously (Slatnar et al., 2015).

The food sector and food by-products are among the key areas highlighted by the European Commission. The European Commission estimates that, in the EU alone, 90 million tonnes of food, or 180 kg per person is disposed of as waste every year. As an example between 22 kg/(year/inhabitant) in France and Bulgaria and over 600 kg/(year/inhabitant) in Holland and Ireland. In Romania, the level is 62 kg/(year/inhabitant), comparable to that in Turkey, Hungary, Slovenia, and Portugal (Eurostat, 2007). However, very few consumers realize that many of these by-products are still fit for human consumption.

Food waste is produced in every phase of the production and supply chain, as well as in the consumption phase (FAO, 2016).

There are many studies related to the stability of betalains, to optimize the processing conditions of the extracts so that betalains degradation can be avoided, such as high hydrostatic processing, fermentation, and ultrasound treatment (Celli and Brooks, 2017), while other authors also investigated the stabilizing effect of ascorbic acid, glucose oxidase that can form complexes with betalain (Martins et al., 2017), to give betalain compounds more stability in the food matrix.

Heat treatment is an important process in the food industry, but it also has its disadvantages that have an impact on the bioavailability and bioactivities of the compounds present in beetroot. In the food industry, thermal processing is applied to extend the stability of fruit-based products (Dewanto et al., 2002). Thermal processing involves treatments at temperatures between 50°C and 150°C, depending on the pH of the product and the desired shelf life, involving preheating before the actual thermal treatment to ensure quality and food safety criteria (Abu-Ghannam and Jaiwal, 2015).

Metabolic syndrome represents a multitude of conditions, including hypertension, elevated blood glucose, obesity, and abnormal lipid levels, which ultimately lead to the development of heart disease, stroke, and diabetes (Carlström et al., 2010). However, most people with metabolic syndrome have insulin resistance, elevated blood glucose, disturbed lipid profile, and are obese (Hadipour et al., 2020). Due to their valuable physiological properties, plant extracts are of great interest for food and pharmaceutical applications. Studies show that beetroot reduces obesity. Neutrophils of obese women have been reported to have high levels of reactive oxygen species and beetroot juice at a concentration of 0.1–10% over 24 hours reduces oxidative stress in the said population (Hadipour et al., 2020). Consumption of nitrate from beetroot juice (140 mL) for 14 days was shown to reduce cholesterol, blood pressure, and LDL cholesterol in 55 people with hypertension (Kerley et al., 2017). It has been estimated that by the year 2020, approximately 366 million people will be affected by diabetes (Wild et al., 2004). Since diabetic patients have higher oxidative factors than non-diabetic patients, it seems that beetroot may provide a beneficial effect in this type of disease (Lee et al., 2009). The use of beetroot extract (2 g/kg extract for 28 days) has been shown to increase both the number and the secretory volume of insulin-producing cells in diabetic mammals. One possible mechanism for the blood glucose-lowering effect is the consumption of red beetroot juice (10%), which inhibited the absorption and digestion of glucose

in the intestine when used in 30 people for 6 weeks. In addition, the extract decreased insulin and C-peptide concentrations due to increased cortisol concentration (Olumese and Oboh, 2016).

Betalains as antioxidant and anti-inflammatory agents play an important role in NO release and blood pressure lowering. The modulation of oxidative and inflammatory factors can also be attributed to the regulatory function in the metabolic syndrome, such as the blood pressure lowering effect and more importantly in the reduction of hyperlipidemia. In addition to betalanin, flavonoids and fiber in beetroot are attributed to the blood sugar-lowering effect (Hadipour et al., 2020).

4.2. Study objectives

The aim of this study is the advanced characterization of the beetroot peel extract using high-performance chromatographic techniques; evaluation of the impact of heat treatment on biologically active compounds (betalains, total polyphenols, and antioxidant activity) from the extract obtained from beetroot peel in the temperature range 20–170°C, with a holding time between 0–20 minutes; Also the evaluation of some biological properties of the extract obtained from the beetroot peel powder by estimating the in vitro inhibition potential of some enzymes associated with metabolic syndrome and pro-inflammatory processes (α-glucosidase, α-amylase, lipase and lipoxygenase (LOX)), is another main objective in this study.

4.3. Materials and methods

4.5. Results and discussion

4.5.1. Chromatographic profile of betalain compounds in beetroot peel extract

Figure 4.3 shows the chromatogram of the optimized beet peel extract which shows a distinct peak at a retention time of 1.87 ± 0.2 min.

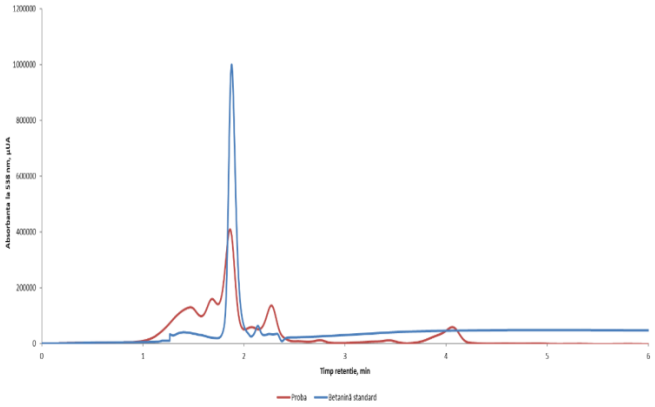

Figure 4.3. Chromatographic profile of the optimized beetroot peel extract

Comparing the retention time obtained in the case of the extract, with the chromatogram generated for the betanin standard (1.84 ± 0.01 min), a correlation can be observed between it.

Sawicki et al., (2016) reported the presence of eighteen betacyanins of which twelve betaxanthins were identified in thirteen Polish beetroot varieties. In comparison, only three betalains (betanin, isobetanin, and vulgaxanthin I) have been identified in beetroot cultivars grown in the USA and Finland (Kujala et al., 2002; Lee et al., 2014). Differences in betalain content and type may be due to varietal diversity, local growth, and climatic conditions as well as post-harvest conditions (Wiczkowski et al., 2014).

4.5.2. The influence of heat treatment on the content of total betalains in beetroot peel powder

Betalains are the main class of compounds with high antioxidant activity present in beetroot skin. High antioxidant activity was associated with increased concentrations of total polyphenol content, which may have synergistic effects with betalains.

For the quantification of betalains, a spectrophotometric method was used based on the measurement of absorbance at two different wavelengths, namely 480 nm for betaxanthins and 538 nm for betacyanins. Betalain content was expressed in mg/100 g d.w.

In **Figure 4.3.** the stability results of the total betalain content of beetroot peel powder extracts subjected to thermal treatment at different temperatures are centralized.

The results showed a decrease in total betalain content by 80% after heating the extract at a temperature of 170°C for 15 minutes. The total betalain content was reduced by 59% after 15 minutes of treatment at 110°C, while after the same treatment time at 110°C the decrease was greater at 75% as shown in **Figure 4.4**.

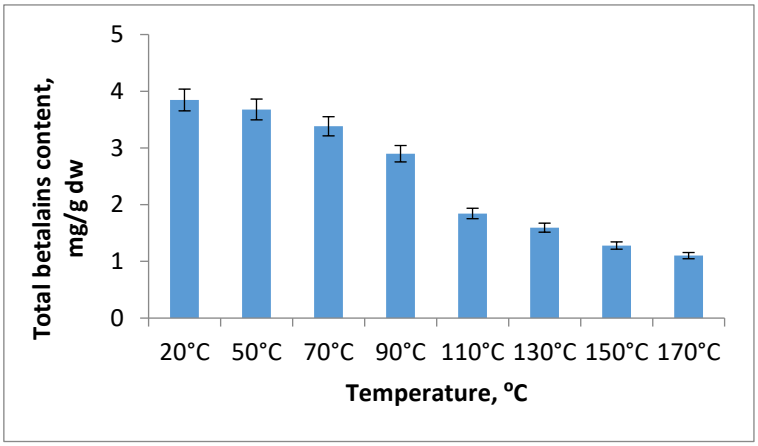

Figure 4.4. Stability of BT content in beetroot peel extract at different temperatures

The results are consistent with the study by Wong and Siow (2015), who reported the stability of betalains from red-fleshed dragon fruit juice in the studied temperature range of 65°–80°C with a heating time between 10 and 30 minutes. Also, the content of betalains decreases with the increase in temperature up to the temperature of 95°C.

Although betacyanins are structurally more stable, which may explain their greater stability at the beginning of heat treatment, they can also undergo degradation reactions as a result of heating.

4.5.3. The influence of heat treatment on the total polyphenol content of beetroot peel powder

Phenolic compounds are a large class of secondary metabolites (8000 polyphenols) of plants and have an important role in the quality of plant-based foods. Beetroot is a rich source of polyphenolic compounds and flavonoids.

The quantity and quality of the content of phenolic compounds are also influenced by the way of industrial processing. In addition, storage conditions are critical for maintaining optimal levels of phenolic compounds and antioxidant activity.

Quantification of polyphenols was carried out using the Folin Ciocâlteu test, and the results were expressed as mg gallic acid equivalent (EAG)/g lyophilized peel.

In **Figure 4.5.** the results of the total polyphenol content of beetroot peel extracts obtained following thermal treatment at different temperatures are centralized.

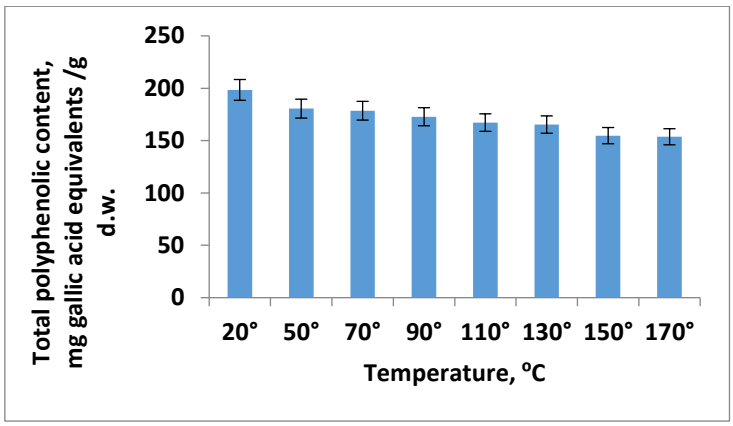

Figure 4.5. Stability of the total content of polyphenolic compounds CTP in the beetroot peel extract at different temperatures (20°, 50°, 70°, 90°, 110°, 130°, 150°, 170ºC)

The content of total polyphenols decreased by 47% following heat treatment in the temperature range 20°C-170°C for 15 minutes. In this way, it can be stated that a thermal treatment in the temperature range of 20°C to 170°C for 15 minutes can facilitate the extraction of bioactive compounds from beetroot peel, but a longer duration of the thermal treatment in combination with high-temperature values can exert a degrading effect on these compounds.

4.5.4. Influence of heat treatment on the antioxidant activity of beetroot peel powder

Betalains are antiradical agents and have a significant ability to scavenge reactive oxygen species, which is highly dependent on their molecular structure and their ability to donate hydrogen to reactive species.

The results of the heat treatment on the antioxidant activity of the beetroot peel powder are illustrated in **Figure 4.6** which shows the influence of the heat treatment on the stability of the antioxidant activity of the beetroot peel extract at different temperatures between 20°C and 170°C.

The antioxidant activity (AA) shows a downward trend similar to that of the polyphenolic content of the beetroot peel extract. AA decreases by 42% at the temperature of 110°C, then at 130°C it decreases by 21% after 15 minutes of treatment, and then suddenly decreases by 35% at 170°C as shown in **Figure 4.6**.

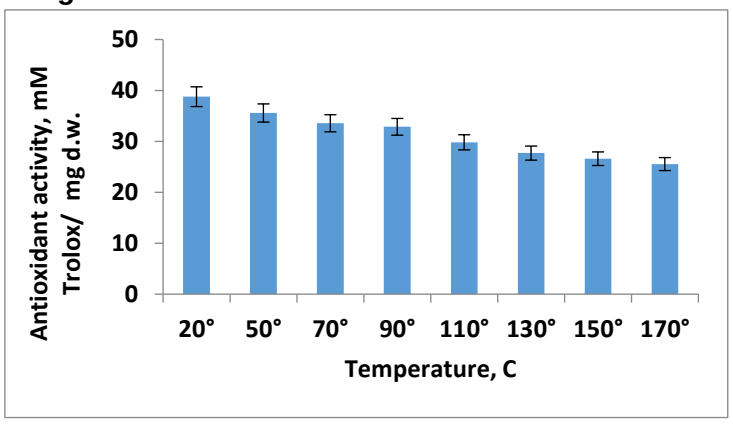

Figure 4.6. Stability of antioxidant activity from beetroot peel extract at different temperatures (20°, 50°, 70°, 90°, 110°, 130°, 150°, 170ºC)

The results are in **Table 4.1**. showed a decrease in total betalain content by 59% after 20 minutes of heat treatment at a temperature of 110°C. The total betalain content decreased by 80% after heating the extract at a temperature of 170°C for 20 minutes. The decrease in the

content of total polyphenols was 47% after 20 minutes of heat treatment at a temperature of 130°C.

Table 4.1. Total betalain content, total polyphenols, and antioxidant activity of beetroot peel extracts following heat treatment at different temperatures

Temperature, °C	Holding time, minute	BT mg/g d.w.	CTP mg GAE/g d.w.	AA mM Trolox/g d.w.
20	0	3.846±0.157[a]	165.84±0.126	25.54±1.343
	20	3.679±0.135	165.33±0.126	25.59±1.343
50	0	3.382±0.090	167.64±0.095	26.59±0.995
	20	3.283±0.136	167.19±0.053	26.54±0.979
70	0	2.898±0.109	168.10±0.102	27.70±1.199
	20	2.863±0.129	167.55±0.572	29.81±2.306
90	0	1.834±0.102	172.79±0.871	33.52±0.281
	20	1.845±0.108	176.13±0.969	33.55±0.397
110	0	1.595±0.345	167.10±0.793	35.28±0.887
	20	1.584±0.386	180.55±0.741	36.27±0.252
130	0	1.279±0.101	154.74±0.633	28.65±1.235
	20	1.101±0.085	153.66±0.609	35.28±0.454
150	0	0.940±0.039	178.54±0.708	35.57±0.945
	20	0.458±0.034	177.93±0.831	32.86±2.230
170	0	0.866±0.022	198.41±0.962	38.77±0.635
	20	0.728±0.021	197.51±0.763	38.53±0.435

[a] – *standard deviation*

Following thermal stability studies, the total content of betalains in the temperature range of 20-90°C decreased linearly from 3.846 to 2.898 mg/g d.w., while in the range of 90-150°C the decrease in content was gradual. The decrease in the content of betalains at the temperature of 170°C may be due to an accumulation of enzymatic browning compounds of the type obtained by the Maillard reaction, and it can be highlighted that the content of betalains decreased sharply at high temperatures of the heat treatment (Uolla et al., 2017).

As can be seen in **Table 4.1**., the content of total polyphenols increased at the temperature of 90°C, followed by a sharper increase at the temperature of 170°C.

Regarding the antioxidant activity, a downward trend similar to that of the polyphenolic content is observed. Thus, with the increase in thermal processing temperature, a gradual decrease in the antioxidant activity was noted, decreasing by 21% at 130°C and then increasing by 35% at the temperature of 170°C.

4.5.5. Evaluation of the inhibition potential of beetroot peel extract on some enzymes involved in the metabolic syndrome and pro-inflammatory processes

The term metabolic syndrome is used to describe a group of metabolic abnormalities associated with an increased risk of coronary heart disease, cardiovascular disease, stroke, and type 2 diabetes. Beetroot peel extract has been analyzed as a potential enzyme inhibitor involved in metabolic syndromes and pro-inflammatory processes. The inhibitory activity of the extract obtained from the beetroot peel was evaluated on α-glucosidase and α-amylase, lipase, and lipoxygenase enzymes.

α-Amylase is present in the saliva of humans and other mammals, where the chemical process of digestion begins. α-Amylases are glycosidic hydrolases and act on α-1,4-glycosidic bonds. A therapeutic approach that can reduce hyperglycemia is to stop the production and/or absorption of glucose by inhibiting the enzymes responsible. Therefore, the search for alternative agents with potent antioxidant properties that could also decrease postprandial hyperglycemia, thus providing a way to control hyperglycemia and other diabetic complications resulting from oxidative stress, is of utmost importance (Ibrahim et al., 2014).

α-Glucosidase is found on the surface of the small intestine. This enzyme by hydrolyzing carbohydrates releases glucose molecules into the blood (Saleem et al., 2022).

Lipases are the enzymes that digest fats, including triacylglycerol and phospholipids. Human lipases include pre-duodenal (lingual and gastric) and extra-duodenal (pancreatic, hepatic, lipoprotein, and endothelial) lipases. Inhibition of pancreatic lipase is one of the most studied mechanisms for determining the potential efficacy of natural products as antiobesity agents. Orlistat is one of two drugs clinically approved for the treatment of obesity that has been shown to act by inhibiting obesity (Birari and Bhutani 2007).

Lipoxygenases are widespread enzymes that catalyze the oxidation of polyunsaturated fatty acids (linoleic, linolenic, and arachidonic acid) to produce hydroperoxides. Lipoxygenase reactions can be desirable, but they can also react in undesirable ways. Most byproducts of lipoxygenase reactions are aromatic compounds that can affect food properties, especially during long-term storage (Loncaric et al., 2021).

In this study, the in vitro inhibitory activity of beetroot peel extract on four tested enzymes namely α-glucosidase, α-amylase, lipase, and lipoxygenase was evaluated. The inhibitory potential was tested using three different extract concentrations of 0.50 µg/mL, 1.00 µg/mL, and 5.00 µg/mL, and the results are shown in **Table 4.2.** and **Table 4.3**.

Table 4.2. The percentage of enzyme inhibition of the extract obtained from the beetroot peel and the standard inhibitors on the enzymes α-amylase, α-glucosidase, lipase, and lipoxygenase at different concentrations

Sample	Sample concentration, µg/mL	α-Amylase inhibition, %	α-Glucosidase inhibition, %	Lipase inhibition, %	Lipoxygenase inhibition, %
Extract	5	53.93 ± 0.27^{aA}	66.79 ± 0.14^{aA}	93.10 ± 0.52^{aB}	45.17 ± 1.33^{aA}
	1	51.46 ± 1.22^{bA}	64.63 ± 0.13^{bA}	92.50 ± 0.26^{abA}	42.25 ± 1.77^{bA}
	0.5	49.92 ± 1.33^{cA}	63.74 ± 0.68^{cA}	92.20 ± 0.69^{bA}	40.20 ± 0.66^{cA}
Acarbose	5	8.09 ± 0.33^{dB}	2.92 ± 0.13^{dB}	-	-
	1	6.92 ± 0.38^{eB}	1.92 ± 0.08^{eB}	-	-
	0.5	5.12 ± 0.19^{fB}	1.11 ± 0.11^{fB}	-	-
Orlistat	5	-	-	94.14 ± 1.41^{cA}	-
	1	-	-	92.59 ± 0.93^{cdA}	-
	0.5	-	-	90.74 ± 0.93^{dB}	-
Quercetin	5	-	-	-	66.15 ± 0.90^{dB}
	1	-	-	-	65.10 ± 0.52^{deB}
	0.5	-	-	-	63.54 ± 0.52^{eB}

Values in a column that do not share the same lowercase letter for the three concentrations are significantly different ($p<0.05$). Values in a column that do not share the same capital letter of extract and standard inhibitor for the same

concentration are significantly different (p<0.05). Measurements are expressed as mean ± standard deviation of three results.

Beetroot peel extract showed a high potential to inhibit the tested enzymes, at relatively low concentrations. The tested extract exerted an inhibitory effect on α-amylase, the IC50 value being 4.22 ± 0.40 μg/mL extract. This suggests that by increasing the bioavailability of the bioactive compounds (betalains) in the beetroot peel extract in the human body, they could be involved in reducing glucose metabolism, α-amylase being an enzyme that catalyzes the hydrolysis of starch into simple sugars.

The optimized beetroot peel extract showed inhibitory effects against the activity of the analyzed enzymes with an inhibition percentage of 53.93 ± 0.27% on α-amylase, 66.79 ± 0.14% on α-glucosidase, 93.10 ± 0.52% on pancreatic lipase as well as 45.17 ± 0.33% on lipoxygenase activity in a concentration-dependent manner. From the results obtained, it was found that the extract from the beetroot peel at all concentrations used (0.5 μg/mL, 1 μg/mL, 5 μg/mL) presented a more pronounced inhibitory potential on pancreatic lipase with an inhibition percentage varying from 92.20±0.69 to 93.10±0.52%. Beetroot peel extract also showed inhibitory activity, indicated in IC50 (μg/mL) values of 4.22 ± 0.40 μg/mL for α-amylase, 3.24 ± 0.27 μg/ mL for α-glucosidase, 1.05 ± 0.23 μg/mL for lipase and of 5.24 ± 0.59 μg/mL for lipoxygenase compared to standard inhibitors acarbose (IC50 2.69 ± 0.08; IC50 1.78±0.06), orlistat (IC50 3.35±0.23) and quercetin (IC50 2.40±0.10).

Table 4.3. Enzyme inhibition results (IC50 values; μg/mL) by beetroot peel extract on α-amylase, α-glucosidase, lipase, and lipoxygenase

Sample	**IC50 (μg/mL)**			
	α-Amylase	α-Glucosidase	Lipase	Lipoxygenase
Extract	4.22±0.40^{a}	3.24±0.27^{a}	1.05±0.23^{b}	5.24±0.59^{a}
Acarbose	2.69±0.08^{b}	1.78±0.06^{b}	-	-
Orlistat	-	-	3.35±0.23^{a}	-
Quercetin	-	-	-	2.40±0.10^{b}

Values in a column that do not share the same letter are significantly different (p<0.05). Measurements are expressed as mean ± standard deviation of three results

4.6. Partial conclusions

The chromatogram of the optimized beetroot peel extract shows a distinct peak at a retention time of 1.87 ± 0.2 min correlated with the betanin standard.

The thermal stability of biologically active compounds such as total betalains, CTP, and antioxidant activity from the extract obtained from beetroot peel was studied in the temperature range 20-170°C for 20 minutes.

Following thermal stability studies, the total content of betalains in the temperature range of 20-90°C decreased linearly from 3.846 to 2.898 mg/g d.w., while in the range of 90-150°C the decrease in content was gradual. The decrease in betalain content at the temperature of 170°C may be due to an accumulation of enzymatic browning compounds of the type obtained by the Maillard reaction.

The content of total polyphenols decreased by 47% following heat treatment, thus it can be concluded that heat treatment in the temperature range of 20°-170°C for 15 minutes, can facilitate the extraction of bioactive compounds from beetroot peel, but a longer duration of heat

treatment in combination with high-temperature values can exert a degrading effect on these compounds.

Following thermal stability studies, a maintenance of the antioxidant activity is observed in the temperature range of 20-70ºC, followed by a decrease of 25.54% at the temperature of 170ºC, this phenomenon can be explained by a possible release of other types of biologically active compounds (flavonoids) from the matrix analyzed following heat treatment. The thermal degradation of the biologically active compounds from the beetroot peel extract intensifies with increasing processing temperatures. By extending the duration of the processing treatment at different temperatures, it is confirmed that the change in the content of phytochemical compounds and antioxidant activity is dependent on temperature. Therefore, the targeted use of biologically active compounds from beetroot peel powder in heat-treated value-added products must take into account their thermal stability.

The study also had in mind the evaluation of the anti-diabetic, anti-obesity, and anti-inflammatory potential in vitro of the beetroot peel extract by determining its activity as a potential inhibitor of α-glucosidase, α-amylase, lipase, and lipoxygenase, enzymes associated with the metabolic syndrome and processes pro-inflammatory.

The results demonstrated that this optimized bioactive extract acts as an inhibitor of α-glucosidase, α-amylase, lipase, and lipoxygenase, which suggests that the extract obtained from beet peel has the potential to contribute effectively to the control of postprandial glycemia, as well as for stress cellular oxidative stress related to diabetes, as well as on diseases related to hyperlipidemia.

The experimental studies in this chapter provide important scientific information of fundamental and applied value regarding the industrial thermal processing of beetroot peel extract to ensure the stability of functional and technological characteristics.

Chapter 5. DEVELOPMENT OF FOOD PRODUCTS WITH ADDED VALUE THROUGH THE ADDITION OF BEET PEEL POWDER

5.1 General aspects

The processing of red beet results in an important amount of vegetable by-products, important sources of flavor compounds, dyes, and natural antioxidants that can replace chemically synthesized additives in the composition of food products, contributing to increasing the quality of life and ensuring the circular economy worldwide. In addition, the use of bioactive compounds from beetroot skins as flavoring substances, colorings, and natural antioxidants provides antioxidant protection to food products and ensures the improvement of sensory characteristics (taste, aroma, color), thus contributing to increasing the attractiveness and diversity of food products among consumers.

Sensory characteristics of food, especially color, have a major impact on consumer perception, selection, acceptance, and consumption. That is why natural food colors are considered of great importance. Currently, the demand for natural food colors is constantly increasing in the market, mainly due to the awareness of consumers of the multiple benefits that they can bring, in contrast to synthetic colors. All these consumer demands open up opportunities for future applications in the food market.

5.2. Study objectives

The specific objectives of the research carried out in this chapter aimed at the development of three products with added value (mayonnaise, marshmallows, casserole) that exploit the functional potential of the biologically active compounds of the lyophilized red beetroot

powder, as well as the evaluation of the phytochemical characteristics, the sensory characteristics, the viscosity, the color and the textural properties of the obtained value-added products as well as the determination of the storage stability of their bioactive potential.

5.3. Materials and methods

5.4.2. Obtaining mayonnaise with the addition of beetroot peel powder

A mayonnaise recipe was developed by combining the following ingredients in the following weight ratios (w/w): sunflower oil (80%), egg powder (8%), water (7%), vinegar (2 %), lemon salt (3%), salt (0.3%) and different proportions (S1-1.5%, S2-3%, S3-5% and S4-7%) of beetroot powder tomato hydrated with water (1:1).

To begin with, a coarse emulsion was made in water by dissolving egg yolk powder, lemon salt, salt, and vinegar. The mayonnaise was prepared by gradually adding the oil to the aqueous mixture and rapidly blending the components with a Morphy Richards 1.5 hand blender (Argos, Milton Keynes, UK) for 10–15 min. Beetroot peel powder was further added to the mayonnaise at four different concentrations of 1.5%, 3%, 5%, and 7%, continuing mixing until the samples became uniform purple and were coded S1, S2, S3, and S4, respectively. Mayonnaise – the control sample was produced in the same way as the experimental mayonnaise, but without the addition of beetroot peel powder. The mayonnaise samples obtained were stored at 4°C until the measurements were made.

5.4.3. Making marshmallows with the addition of beetroot peel powder

The added value marshmallows were obtained from the following ingredients: 27 g egg white powder (equivalent to one egg), warm water (40ºC), 50 g powdered sugar, and beetroot peel powder (B1 - 4%, B2 - 7% and B3 - 10%). The process described is simple, involving the mixing of the ingredients shown above, with the powder from the beetroot peels being added as an ingredient.

The process of obtaining value-added marshmallows has the following stages:

1. The egg white powder was dissolved in slightly heated water (40ºC), in a ratio of 1:2., mixing gently for homogenization. Then, the mixture was homogenized with the mixer at low speed for 2-3 minutes until foam appeared.
2. Later, sugar was added in small amounts, gradually.
3. After the total amount of sugar has been homogenized, and the egg white has become glossy and firm, check if the sugar has been completely dissolved in the prepared composition.
4. After the composition was homogeneous, the beetroot peel powder previously hydrated with warm water was added. Homogenization was continued to even out the color of the product.
5. The marshmallows were formed on a tray lined with baking paper.
6. After that they were placed in the oven for 60-90 min, at a temperature of 90 ºC, or until they became firm, but without changing their color so that they could be easily removed from the baking paper.
7. Then, the marshmallows were left to dry for about 4 hours.
8. Finally, the marshmallows were packed in plastic containers, hermetically sealed, and stored in a dry and cool place.

The resulting marshmallows presented a consistency specific to the traditional product, a red color, specific to beetroot, a sweet, pleasant taste, and a homogeneous texture, specific to the conventional product. For comparison, a control sample (B) was also made, which followed the same technology, but in which no beetroot peel powder was added.

5.4.4. Obtaining the pan with the addition of beetroot peel powder

The added value sauce is obtained from the following ingredients % (w/w): sugar (52%), honey (26%), egg white (16%), lemon juice (2%), salt (0, 2%), and beetroot peel powder previously hydrated with water in a 1:1 ratio (A1 - 2%, A2 - 4% and A3 - 6%), water (the rest, up to 100%).

The process of obtaining the added value pan includes the following stages (Figure 5.3.):

- Initially, with the help of a mixer, the egg whites together with the sugar are homogenized at high speed for 12-15 minutes until the composition has become foamy and the sugar has completely melted.

- Later, lemon juice was added and homogenized for another 5 minutes, until a dense, glossy, firm foam was obtained.

- The foam obtained was transferred to a bain-marie vessel, and the fluid honey was added in a thin thread, homogenizing for 60 minutes, until the foam lost its volume and the consistency became sticky.

- The product was then removed from the bain-marie and tempered for 10 minutes.

- The beetroot peel powder (relative to the amount of product obtained) previously hydrated was incorporated, in such a way that the obtained composition is uniform in terms of color and texture.

- The composition was then poured between two sheets of wafers and kept under refrigerated conditions (4-5°C), to carry out experimental studies to characterize the obtained products.

The value-added beetroot had a moderate consistency, a remarkable purple-red color typical of beetroot, a sweet, pleasant taste, and a fine, homogeneous texture typical of the conventional product. For comparison, a control sample was also made, which followed the same technology, but in which no beetroot peel powder was added.

The technological processes were carried out within the Integrated Center for Research, Expertise and Technology Transfer for the Food Industry at the Faculty of Food Science and Engineering, Lower Danube University in Galati (https://erris.gov.ro/FOOD-BIOTECHNOLOGY).

5.5. Results and discussion

Incorporation of compounds with natural antioxidant activity into food products has a great potential to improve the oxidative stability of food products and thus these value-added products can attract a wider group of consumers because the bioactive compounds in their composition could bring, health-promoting benefits of their health (Ghorbani Gorji et al., 2016).

5.5.1. Characterization of beetroot peel powder

The results obtained for the phytochemical characterization of the beetroot peel extract are presented in **Table 5.1**. Analyzing the data presented in **Table 5.1.** it can be observed that the ethanolic extract from the beetroot peel showed a high content of biologically active compounds. A content of 2.31 ± 0.12 mg betalain/g beetroot skin and a much higher concentration of total polyphenols of 281.11 ± 5.09 mg EAG/g skin is noted. High concentrations of bioactive compounds resulted in high antioxidant activity.

Table 5.1. Phytochemical characteristics of beetroot peel powder extract

Characteristics	Beetroot Peel Powder Extract
Total Betalains (mg/g d.w.)	2.31 ± 0.12[a]
Total Polyphenols (mg GAE/g d.w.)	281.11 ± 5.09

Antioxidant activity (mM Trolox/g d.w.)	47.65 ± 0.21

[a]-*standard deviation*

The obtained results are in agreement with the data reported in other studies. However, the phytochemical composition of beetroot peel extracts may vary depending on genetic and agronomic factors, different extraction conditions (e.g. type of solvent, temperature, pH, light intensity), and applied methods for quantifying bioactive compounds.

5.5.2. Characterization of the bioactive potential of value-added mayonnaise samples and their storage stability

Phytochemical characterization was performed to highlight the added value of mayonnaise samples. The phytochemical composition and antioxidant activity of mayonnaise with added beetroot powder are shown in **Table 5.2.** as well as storage stability for 28 days at 4 °C. As expected, the content of bioactive compounds increased as a greater amount of beetroot peel powder was added to the mayonnaise samples.

Betalains were not identified in the sample without beetroot peel powder, and the polyphenol content was lower at only 24.6 ± 0.06 mg EAG/100 g. The addition of beetroot peel powder (1%, 5%, 3%, 5%, and 7%) in the mayonnaise composition caused a significant increase ($p < 0.05$) in the levels of bioactive compounds analyzed. Thus, betalains varied from 1.32 ± 0.01 to 5.61 ± 0.16 mg/100 g, and polyphenol content had values from 197.10 ± 1.91 to 325.9 ± 5 .61 mg EAG/100 g for mayonnaise supplemented with beetroot peel powder. In addition, all samples (S1, S2, S3, and S4) had significantly higher levels of bioactive compounds ($p < 0.05$) compared to the control sample. Also, mayonnaise samples supplemented with beetroot peel powder showed a higher antioxidant activity of 52.09 ± 2.91 mM Trolox/100 g in the case of sample S4 compared to the control sample due to increased levels of bioactive compounds in the powder from beetroot peel 1.81 ± 0.01mM Trolox/100 g.

Table 5.2. Phytochemical characteristics and antioxidant activity of value-added mayonnaise samples

Phytochemical characteristics		**Mayonnaise samples**				
		S0	**S1**	**S2**	**S3**	**S4**
Total Betalains, (mg/100 g)	0 days	-	1.32 ± 0.01^{aD}	2.48 ± 0.06^{aC}	4.19 ± 0.09^{aB}	5.61 ± 0.16^{aA}
	14 days	-	1.10 ± 0.04^{abD}	2.20 ± 0.02^{bE}	3.69 ± 0.24^{aB}	4.86 ± 0.09^{bA}
	28 days	-	0.82 ± 0.13^{bD}	1.81 ± 0.02^{cC}	2.84 ± 0.11^{bB}	4.12 ± 0.06^{cA}
Total Polyphenols, (mg/100 g)	0 days	24.60 ± 0.06^{aE}	197.10 ± 1.91^{aD}	271.4 ± 11.06^{aC}	307.4 ± 4.06^{aB}	325.9 ± 5.61^{aA}
	14 days	20.85 ± 0.64^{bE}	188.10 ± 3.96^{aD}	251.65 ± 1.34^{bC}	278.95 ± 3.75^{bB}	310.50 ± 1.27^{bA}
	28 days	18.15 ± 1.06^{bE}	152.15 ± 2.19^{bD}	227.50 ± 1.98^{cC}	253.85 ± 7.71^{cB}	285.05 ± 0.78^{cA}
Antioxidant activity (mM Trolox/100 g)	0 days	1.81 ± 0.01^{aE}	29.5 ± 0.11^{aD}	37.07 ± 0.90^{aC}	45.60 ± 0.61^{aB}	52.09 ± 2.91^{aA}
	14 days	1.77 ± 0.03^{aD}	27.20 ± 0.42^{aC}	34.65 ± 1.77^{abB}	39.25 ± 1.20^{bB}	48.75 ± 1.63^{abA}
	28 days	1.60 ± 0.10^{aE}	21.15 ± 0.6^{bD}	30.35 ± 0.78^{bC}	34.80 ± 1.27^{cB}	46.05 ± 0.21^{bA}

Means from the same row (uppercase letters) and the same column (lowercase letters) for each sample analyzed that do not share a letter are significantly different (p < 0.05).

After storage for 28 days at a temperature of 4 ºC, a slight decrease in the content of total betalains and total polyphenols, as well as antioxidant activity, is observed in all analyzed mayonnaise variants.

The oxidative stability of the bioactive compound of the mayonnaise sample revealed the degradation of polyphenols (p > 0.05) at the end of the storage period for all analyzed samples. Similar results were identified for antioxidant activity, with a slight decrease for sample S4.

5.5.3. Physico-chemical characterization of mayonnaise samples with added value

Value-added mayonnaise samples were obtained by adding different amounts of beetroot peel powder (1%, 5%, 3%, 5%, and 7%) and their physicochemical composition was evaluated (**Figure 5.4., Table 5.3.**).

Figure 5.4. Images of mayonnaise samples without beetroot peel powder, control (S0); mayonnaise with 1.5% beetroot peel powder (S1); mayonnaise with 3% beetroot peel powder (S2); mayonnaise with 5% beetroot peel powder (S3); mayonnaise with 7% beetroot peel powder (S4)

The physicochemical composition of mayonnaise (without beetroot peel powder) was 72.05 ± 0.01 g/100 g for lipids, 2.65 ± 0.01 g/100 g for carbohydrates, and 5.4 ± 0.01 g/100 g, for protein content. The composition of mayonnaise with beetroot peel powder revealed a significant difference (p < 0.05) between the analyzed samples.

The results showed that the moisture content was lower as beetroot powder was added and the ash content was higher as the beetroot powder mayonnaise enrichment was achieved. Moreover, the carbohydrate content was increased by the addition of beetroot peel powder.

Table 5.3. Physicochemical characteristics of mayonnaise samples with beetroot peel powder

Physicochemical characteristics	S0	S1	S2	S3	S4
Proteins, g/100 g	5.4 ± 001^{a}	5.2 ± 0.01^{b}	5.1 ± 0.03^{c}	5.02 ± 0.01^{d}	4.91 ± 0.01^{e}
Lipids, g/100 g	72.05 ± 0.01^{a}	71.6 ± 0.14^{ab}	71.3 ± 0.14^{b}	71.7 ± 0.14^{ab}	71.5 ± 0.14^{b}
Carbohydrates, g/100 g	2.65 ± 0,01^{e}	3.11 ± 0.03^{d}	3.26 ± 0.01^{c}	3.52 ± 0.01^{b}	3.7 ± 0.01^{a}
Humidity, g/100 g	18.04 ± 0.01^{a}	17.97 ± 0.01^{b}	17.92 ± 0.01^{b}	17.15 ± 0.01^{c}	17.01 ± 0.01^{d}
Ash, g/100 g	1.91 ± 0.01^{e}	2.12 ± 0.01^{d}	2.42 ± 0.01^{c}	2.61 ± 0.01^{b}	2.88 ± 0.01^{a}

This means that the same rows that do not share a letter are significantly different (p < 0.05).

5.5.4. Color parameters of value-added mayonnaise samples

Mayonnaise samples were analyzed for CIELAB colorimetric parameters using a portable colorimeter. The results were expressed as L*, a* and b*. Color parameter values including L* (brightness), a* (tendency to red for a* "+" or green for a* "-"), b* (tendency to yellow for b* "+" or blue for b* "-") and total color change ΔE were analyzed (**Table 5.4.**).

Table 5.4. CIELAB color parameters of value-added mayonnaise samples

CIELAB color parameters		Mayonnaise samples				
		S0	**S1**	**S2**	**S3**	**S4**
L*	0 days	67.14 ± 0.00^{aA}	26.63 ± 0.03^{bB}	24.44 ± 0.03^{cC}	21.72 ± 1.73^{cD}	20.06 ± 0.30^{cE}
	14 days	68.76 ± 0.91^{abA}	28.24 ± 0.18^{bB}	26.27 ± 0.49^{bB}	23.66 ± 0.36^{bC}	22.75 ± 0.11^{bC}
	28 days	70.88 ± 1.09^{bA}	31.90 ± 1.10^{aB}	29.03 ± 0.15^{aBC}	26.68 ± 0.05^{aCD}	24.71 ± 0.54^{aD}
a*	0 days	−1.19 ± 0.02^{bE}	13.82 ± 0.22^{aD}	16.20 ± 0.08^{aC}	18.20 ± 0.02^{aB}	23.14 ± 0.02^{aD}
	14 days	1.10 ± 0.01^{aE}	12.69 ± 0.40^{bD}	15.34 ± 0.23^{bC}	17.16 ± 0.19^{abB}	20.86 ± 0.30^{bA}
	28 days	1.44 ± 0.33^{aE}	12.02 ± 0.13^{bD}	14.94 ± 0.17^{bC}	16.24 ± 0.30^{bB}	20.19 ± 0.17^{bA}
b*	0 days	32.92 ± 0.16^{bA}	5.11 ± 0.09^{cB}	3.06 ± 0.07^{bC}	−1.2 ± 0.01^{bD}	−2.01 ± 0.03^{bE}
	14 days	33.82 ± 0.13^{bA}	6.72 ± 0.28^{bB}	5.84 ± 0.10^{aC}	1.42 ± 0.06^{aD}	−1.83 ± 0.11^{bE}
	28 days	36.24 ± 0.40^{aA}	7.92 ± 0.06^{aB}	6.45 ± 0.30^{aC}	1.53 ± 0.02^{aD}	0.97 ± 0.08^{aD}
ΔE	0 days	-	51.38 ± 0.05^{aD}	54.95 ± 0.02^{aC}	60.04 ± 0.16^{aB}	63.49 ± 0.01^{aA}
	14 days	-	50.12 ± 0.46^{bD}	52.37 ± 0.38^{bC}	57.37 ± 0.30^{bB}	61.04 ± 0.12^{bA}
	28 days	-	49.33 ± 0.15^{cD}	52.42 ± 0.24^{bC}	57.43 ± 0.13^{bB}	60.38 ± 0.51^{cA}

L—luminosity; a*—green to red; b*—blue to yellow. Means from the same row (uppercase letters) and the same column (lowercase letters) for each sample analyzed that do not share a letter are significantly different ($p < 0.05$).*

The value of the parameter b* suggests a color closer to yellow. The addition of beetroot peel powder to mayonnaise led to significant color changes. The red color of beetroot has been attributed to the presence of essential amounts of betalains that occur in two forms, i.e. betacyanin (red-purple pigment) and betaxanthin (yellow-orange pigment) (Chhikara et al., 2019).

Estimated values of total color change ΔE indicated significant differences in all analyzed mayonnaise samples. The total color difference value ΔE in mayonnaise samples increased with the added powder concentration, and ΔE was higher (63.49 ± 0.01) for mayonnaise with 7% beetroot peel powder.

The brightness of the samples decreased considerably ($p<0.05$) with the amount of beetroot peel powder added to the analyzed mayonnaise samples. As a result of increasing the amount of beetroot peel powder added to the mayonnaise samples, which provides significant amounts of beetroot pigments, the a* parameter values changed from greenish to red shades (a* of 23.14 for S4). In contrast, the yellow color decreased in intensity, and the value of the b* parameter reached -2.01 for sample S4.

An increase in the brightness and intensity of the yellow color was observed during storage in all samples, while the red shade decreased. At the end of storage, mayonnaise sample S2 containing 3% beetroot peel powder had the highest values of the L* parameter, and the lowest values of the a* parameter were found in the S1 sample, except for the control sample.

5.5.5. Textural properties of value-added mayonnaise samples

The textural properties of mayonnaise with the addition of beetroot peel powder were evaluated using the texture profile analysis (TPA) method. The textural parameters analyzed were: firmness (expressed in N and defined as the maximum resistance of the sample during the first penetration cycle), adhesiveness (expressed in mJ and defined as the energy required to remove the sample from the test tool), cohesion (the dimensionless size, defined as the strength of the internal bonds that give the consistency of the product) and masticability (expressed in mJ

and defined as the energy required to chew food up to the phase preceding swallowing). The results were processed using TexturePro CT V1.5 software and are shown in **Table 5.5**.

Table 5.5. Texture analysis of value-added mayonnaise samples

Textural parameters	S0	S1	S2	S3	S4
Firmness, N	0.74 ± 0.02^{b}	1.16 ± 0.01^{a}	1.83 ± 0.13^{a}	1.74 ± 0.10^{a}	1.54 ± 0.02^{a}
Adhesiveness, mJ	1.82 ± 0.27^{c}	5.32 ± 0.38^{ab}	5.68 ± 0.45^{a}	4.63 ± 0.14^{ab}	3.93 ± 0.00^{b}
Cohesiveness	0.81 ± 0.01^{a}	0.77 ± 0.03^{ab}	0.79 ± 0.01^{ab}	0.71 ± 0.02^{ab}	0.61 ± 0.07^{b}
Chewiness, mJ	5.56 ± 0.18^{b}	10.99 ± 0.65^{ab}	13.41 ± 1.67^{a}	11.61 ± 1.56^{ab}	8.24 ± 1.67^{ab}

This means that the same rows that do not share a letter are significantly different ($p < 0.05$).

All mayonnaise samples showed low values of firmness and cohesiveness and high values of adhesiveness and chewiness, comparable to those reported in the literature (Rojas et al., 2019; Di Mattia et al., 2015). The results show that the addition of beetroot peel powder in the mayonnaise samples resulted in higher firmness values compared to the control sample. In addition, the addition of beetroot peel powder to the mayonnaise composition improved adhesion and chewiness, giving the product a delicate and soft texture.

5.5.6. The effect of the addition of beetroot peel powder on mayonnaise viscosity

The variation of dynamic viscosity as a function of shear rate indicates a thixotropic rheological behavior for all analyzed mayonnaise samples. This type of behavior is characterized by a decrease in dynamic viscosity as the shear rate increases (**Figure 5.6.**). However, the results show that by adding beetroot peel powder to the mayonnaise samples, the viscosity of the mayonnaise is significantly improved, helping to achieve a creamy consistency. This behavior may be due to the pectin content of the beetroot peel powder, which acts as a thickening agent.

Instead, as shown in the data presented in **Table 5.6**., the consistency index K (Pa□sn) recorded increasing values with the increase in the concentration of beetroot peel powder up to 3% (9.97 Pa□sn for 1.5 % beetroot peel powder and 14.35 Pa□sn for 3% beetroot peel powder). Higher added concentrations of beetroot peel powder (5% and 7%) caused a reduction in the consistency index. This could be due to the increased amount of solid particles, which disrupt the structure.

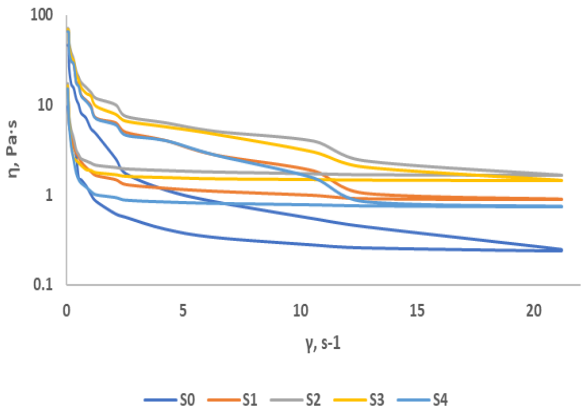

Figure 5.5. Hysteresis loop for mayonnaise samples: S0—mayonnaise without beetroot peel powder, (control); S1—mayonnaise with 1.5% beetroot peel powder; S2—mayonnaise with 3% beetroot peel powder; S3—mayonnaise with 5% beetroot peel powder; S4—mayonnaise with 7% beetroot peel powder

Table 5.6. Power-law model fitting parameters for mayonnaise samples

Parameters	S0	S1	S2	S3	S4
K (Pa·s^n)	4.91	9.97	14.35	12.81	9.46
n	0.07	0.24	0.36	0.33	0.21
R^2	0.93	0.85	0.91	0.91	0.73

5.5.7. Oxidative stability of value-added mayonnaise samples

The oxidative stability of mayonnaise samples with the addition of beetroot peel powder was analyzed in **Table 5.7**. The value of the acidity index was observed to increase during storage Except for sample S4, there was no significant difference ($p < 0.05$) between the samples analyzed on the 28th day of storage. The lower value of the acidity index was recorded for samples S3 and S4, samples with a high concentration of beetroot peel powder, namely 5% and 7%, respectively, beetroot peel powder which inhibited the increase of the acidity index.

Table 5.7. Oxidative stability of value-added mayonnaise

Evaluated indices		S0	S1	S2	S3	S4
Acidity index, mg KOH/g	0 days	0.96 ± 0.01^{cA}	0.85 ± 0.01^{cB}	0.85 ± 0.01^{cB}	0.85 ± 0.01^{cB}	0.85 ± 0.01^{bB}
	14 days	1.21 ± 0.01^{aA}	0.96 ± 0.01^{bB}	0.91 ± 0.01^{bC}	0.89 ± 0.01^{bCD}	0.86 ± 0.01^{bD}
	28 days	1.44 ± 0.02^{bA}	1.12 ± 0.01^{aB}	1.06 ± 0.01^{aC}	0.99 ± 0.01^{aD}	0.94 ± 0.01^{aD}
Peroxide index, meq/kg	0 days	1.81 ± 0.01^{cA}	1.76 ± 0.01^{cB}	1.76 ± 0.01^{cB}	1.76 ± 0.01^{bB}	1.76 ± 0.01^{bB}
	14 days	3.03 ± 0.08^{bA}	2.06 ± 0.01^{bB}	1.91 ± 0.01^{bBC}	1.85 ± 0.05^{bC}	1.80 ± 0.03^{bC}
	28 days	6.94 ± 0.02^{aA}	4.17 ± 0.08^{aB}	3.83 ± 0.05^{aC}	2.62 ± 0.04^{aD}	2.01 ± 0.04^{aD}

Means on the same row (uppercase letters) and the same column (lowercase letters) for each sample analyzed that do not share a letter are significantly different ($p < 0.05$).

Peroxide index values showed a significant difference ($p < 0.05$) for all mayonnaise samples stored at refrigerated temperature for 14 and 28 days, except for samples S3 and S4. The gradual increase in the peroxide index values during storage indicated the initiation of the rancidity process of the mayonnaise samples. Peroxide index values of less than 10 indicated that both powder-added and non-powdered mayonnaise samples are considered safe under refrigerated conditions (Pradhananga et al., 2016).

5.5.8. Sensory evaluation of value-added mayonnaise samples

Sensory characteristics of mayonnaise samples enriched with different concentrations of beetroot peel powder are listed in **Table 5.8**. Among the sensory characteristics, color is the first sign of a product's quality that captures consumers' attention. The color of the mayonnaise was considerably influenced by the concentration of beetroot peel powder ($p < 0.05$). Thus, sample S4 with 7% beetroot powder received the highest color score, followed by S3 with 5% beetroot powder. Enrichment of mayonnaise with beetroot peel powder created an attractive red-purple color due to the increased concentration of beetroot betalain pigments.

Table 5.8. Consumer acceptability scores of value-added mayonnaise samples

Sensory characteristics	Control	S1	S2	S3	S4

Color	7.2 ± 1.39^{c}	8.25 ± 0.71^{b}	8.65 ± 0.48^{ab}	8.80 ± 0.41^{ab}	8.95 ± 0.22^{a}
Aroma	6.45 ± 0.99^{b}	7.05 ± 0.94^{ab}	7.45 ± 0.99^{a}	7.80 ± 0.69^{a}	7.75 ± 0.96^{a}
Taste	6.8 ± 0.89^{c}	7.3 ± 0.86^{bc}	7.8 ± 0.61^{ab}	8.45 ± 0.51^{a}	7.85 ± 0.98^{ab}
Consistency	7.7 ± 0.73^{a}	7.85 ± 0.67^{a}	8.05 ± 0.99^{a}	8.05 ± 0.99^{a}	7.45 ± 0.94^{a}
Texture	8.5 ± 0.68^{a}	8.65 ± 0.58^{a}	8.65 ± 0.58^{a}	8.80 ± 0.41^{a}	8.95 ± 0.22^{a}
Smell	7.15 ± 1.38^{a}	7.30 ± 1.08^{a}	7.2 ± 1.10^{a}	7.25 ± 1.11^{a}	7.25 ± 1.11^{a}
Aftertaste	7.4 ± 0.82^{a}	7.35 ± 1.03^{a}	7.65 ± 1.18^{a}	7.75 ± 1.25^{a}	7.40 ± 1.35^{a}
Spreadability	8.65 ± 0.48^{a}	8.65 ± 0.48^{a}	8.50 ± 0.51^{a}	8.50 ± 0.51^{a}	8.35 ± 0.58^{a}
Acceptability	8.4 ± 0.68^{a}	8.5 ± 0.60^{ab}	8.80 ± 0.41^{ab}	8.80 ± 0.41^{ab}	8.85 ± 0.36^{a}

This means that the same rows that do not share a letter are significantly different ($p < 0.05$).

The amounts of beetroot peel powder added to the mayonnaise samples did not affect the smell, taste, and spreadability. However, the taste and texture of the products were affected by the addition of a higher percentage of beetroot peel powder (7%). In addition, it is worth noting that the addition of higher concentrations of beetroot peel powder in mayonnaise led to a slightly increased consistency score. There were no significant variations in overall acceptability between samples S1, S2 and S3. However, the panelists appreciated the S4 sample with 7% beetroot peel powder due to its attractive color.

5.5.9 Characterization of the bioactive potential of the value-added marshmallow samples and the storage stability of the samples

To highlight the added value of marshmallows, the phytochemical characterization was carried out and the antioxidant activity was determined. The stability of the compounds was also monitored during the storage of marshmallows at room temperature, packed in hermetically sealed plastic containers for 21 days. The results are presented in **Table 5.9**.

From **Table 5.9**. it is observed that the three variants of marshmallows with the addition of beet peel powder showed high concentrations of betalains and polyphenols, also reflected in the antioxidant activity values. It is also observed that with the increase in the concentration of added beetroot peel powder, the concentration of biologically active compounds and, implicitly, the antioxidant activity also increases, as expected. At the same time, however, during the 21 days of storage, the concentrations of betalains and total polyphenols decreased significantly ($p<0.05$) for all the technological variants obtained from the marshmallows.

However, the results are presented in **Table 5.9**. confirms the added value of marshmallows with the addition of beetroot peel powder, by increasing the total content of betalains and polyphenols, which leads to a product with high antioxidant activity. These results demonstrate that beetroot peel powder can be used as a natural substitute for chemical antioxidants.

Table 5.9. Phytochemical characteristics and antioxidant activity of marshmallows with the addition of beetroot powder (B- marshmallows without the addition of beetroot powder, B1, B2, and B3 - marshmallows with the addition of 4, 7, and 10% (w/w) beetroot peel powder)

Phytochemical characterization	**Marshmallows**				
	Time, days	**B**	**B1 (4%)**	**B2 (7%)**	**B3 (10%)**
Total Betalains,	0	-	4.10 ± 0.02^{aA}	6.62 ± 0.05^{aB}	9.93 ± 0.38^{aC}

mg /100g d.w	7	-	3.52 ± 0.04^{bA}	5.19 ± 0.11^{bB}	8.38 ± 0.22^{bC}
	14	-	2.85 ± 0.04^{cA}	4.58 ± 0.06^{cB}	7.24 ± 0.11^{cC}
	21	-	1.60 ± 0.09^{dA}	3.32 ± 0.18^{dB}	5.67 ± 0.15^{dC}
Total Polyphenols, mg EAG/100 g d.w	0	38.36 ± 0.29^{aA}	42.14 ± 1.16^{aA}	52.80 ± 1.23^{aB}	65.90 ± 0.68^{aC}
	7	32.59 ± 0.48^{bA}	32.26 ± 1.13^{bA}	40.92 ± 1.44^{bB}	57.09 ± 0.16^{bC}
	14	28.87 ± 0.19^{cA}	30.31 ± 0.85^{bcA}	36.87 ± 0.29^{cB}	43.90 ± 0.55^{cC}
	21	26.01 ± 0.51^{dA}	28.26 ± 0.41^{cA}	31.69 ± 0.32^{dB}	31.18 ± 1.11^{dB}
Antioxidant activity DPPH, mM Trolox/100g d.w.	0	4.19 ± 0.02^{aA}	17.15 ± 0.55^{aB}	27.59 ± 0.74^{aC}	39.06 ± 0.52^{aD}
	7	3.90 ± 0.12^{aA}	14.29 ± 0.98^{bB}	26.05 ± 1.04^{aC}	35.01 ± 0.68^{bD}
	14	2.38 ± 0.18^{bA}	12.49 ± 0.38^{bB}	24.65 ± 0.74^{aC}	29.87 ± 0.87^{cD}
	21	2.14 ± 0.07^{bA}	11.94 ± 0.19^{bB}	22.33 ± 2.35^{aC}	27.06 ± 0.38^{dC}

The time variation of compound concentration is highlighted by small letters on the column. Differences in compound concentrations between samples are highlighted by uppercase letters per row. Values that share a lower/uppercase letter are not significantly different (p>0.05)

Since beetroot is considered safe for human consumption, one might wonder if consuming powdered betalains is just as safe. Therefore, betalains derived from beetroot possess a huge potential to be used as a natural colorant as well as functional ingredients in the formulation of new food products.

After storage for 21 days at 4ºC of marshmallows with the addition of beetroot peel powder, a slight decrease in the content of total betalains and total polyphenols, as well as in antioxidant activity, is observed in all variants of marshmallows analyzed.

5.5.10. Physico-chemical characterization of value-added marshmallow samples

The value-added marshmallows were analyzed from a physico-chemical point of view, the results being presented in **Table 5.10**.

From **Table 5.10**. it is observed that the addition of beet peel powder resulted in a slight decrease in protein content by up to 4%.

At the same time, a slight increase in the concentration of carbohydrates is observed with the increase in the percentage of added powder. Thus, there is an increase of up to 3% in the carbohydrate content of sample B3 compared to the control sample.

Table 5.10. Physico-chemical characteristics of value-added marshmallows (B - marshmallows without the addition of beetroot powder, B1, B2, and B3 - mayonnaise with the addition of 4%, 7%, and 10% (w/w) beetroot powder Beet)

Physico-chemical characteristics	**Marshmallows**			
	B	**B1 (4%)**	**B2 (7%)**	**B3 (10%)**
Proteins, g/100 g	4.81 ± 0.09^{a}	4.63 ± 0.02^{a}	4.61 ± 0.03^{a}	4.60 ± 0.01^{a}
Sugars, g/100 g	84.62 ± 1.52^{a}	85.12 ± 3.14^{a}	86.30 ± 1.09^{a}	87.04 ± 1.88^{a}
Humidity, g/100 g	9.69 ± 0.01^{a}	9.23 ± 0.18^{a}	7.91 ± 0.36^{b}	7.10 ± 0.49^{b}
Ash, g/100 g	0.88 ± 0.02^{a}	1.02 ± 0.01^{b}	1.18 ± 0.01^{c}	1.26 ± 0.02^{d}
Energy value, %:	6.66 ± 0.01^{a}	367.97 ± 0.02^{a}	372.73 ± 0.01^{ab}	375.72 ± 0.03^{c}
Kcal/kJ	1534.10 ± 0.01^{a}	1539.58 ± 0.02^{a}	1559.50 ± 0.01^{ab}	1572.01 ± 0.03^{c}

This means that the same rows that do not share a letter are significantly different (p < 0.05).

This increase is also reflected in the energy value (**Table 5.10.**).

5.5.11. Color parameters of value-added marshmallow samples

The marshmallows were analyzed for CIELAB colorimetric parameters using a portable colorimeter with illuminator C (Chroma Meter, model CR-410, Konica Minolta, Osaka, Japan). The results were expressed as values of the parameters L* (brightness), a* (tendency to red for an a* "+" or green for an a* "-), and b* (tendency to yellow for b* "+" or blue for b* "-"). The possible color change was also checked after the 21 days of storage. The results are presented in **Table 5.11**.

Table 5.11. Colorimetric parameters of the marshmallows: B- marshmallows without the addition of beetroot powder, B1, B2, and B3 - marshmallows with the addition of 4, 7, and 10% (w/w) beetroot powder

Marshmallow samples	Time, days	L*	a*	b*
B	0	104.42 ± 0.58^{Aa}	7.72 ± 0.12^{aA}	1.90 ± 0.04^{aA}
	21	104.48 ± 0.33^{aA}	7.43 ± 0.07^{aA}	1.69 ± 0.02^{aB}
B1 (4%)	0	60.86 ± 0.77^{bB}	26.70 ± 0.49^{bB}	2.87 ± 0.05^{bC}
	21	70.52 ± 0.19^{bC}	27.11 ± 0.72^{bB}	3.80 ± 0.12^{bD}
B2 (7%)	0	45.93 ± 0.83^{cD}	29.92 ± 0.17^{cC}	4.48 ± 0.04^{cE}
	21	50.95 ± 1.01^{cE}	32.24 ± 0.42^{cD}	5.37 ± 0.54^{cE}
B3 (10%)	0	39.67 ± 0.60^{dF}	30.91 ± 0.23^{cE}	4.29 ± 0.09^{cF}
	21	39.89 ± 0.78^{dF}	30.68 ± 1.07^{cE}	5.61 ± 0.09^{cG}

Color variation over time is highlighted by small letters on the column. The color differences between the samples are highlighted by capital letters in a row. Values that share a lower/uppercase letter are not significantly different (p>0.05)

According to the results presented in **Table 5.11**., value-added marshmallows are characterized by shades of red. It is observed that the intensity of the color of the marshmallows is directly proportional to the percentage of powder added. It is also observed that as the percentage of added powder increases, the brightness of the marshmallows decreases. Following storage, after 21 days an increase in the values of the color parameters is observed for all three samples of value-added marshmallows.

These results demonstrate that beetroot peel powder has high coloring power and can be used as a natural substitute for chemical dyes.

5.5.12. Textural properties of value-added marshmallow samples

For the textural analysis of marshmallows, the Texture Profile Analysis (TPA) method was used, applied with the help of a Bookfield CT3 textural analyzer. The samples were subjected to a double penetration with a metal cylinder with a diameter of 4 mm, up to a depth of 6 mm, with a speed of 0.5 mm/s. The sensitivity threshold was 0.067 N. The textural parameters – firmness, adhesion, cohesiveness, elasticity, and chewiness – were determined using the program TexturePro CT V1.5 (Brookfield Engineering Labs. Inc.). Four determinations were made for each sample and the results are presented as an average in **Table 5.12**.

Table 5.12. Textural parameters of the marshmallows: B- marshmallows without the addition of beetroot powder, B1, B2, and B3 - marshmallows with the addition of 4, 7 and 10% (w/w) beetroot powder

Parameters	B	B1 (4%)	B2 (7%)	B3 (10%)
Firmness, N	10.44 ± 0.77^{a}	10.50 ± 0.99^{a}	10.57 ± 0.90^{a}	10.92 ± 0.14^{a}
Adhesion, mJ	0.30 ± 0.03^{a}	0.28 ± 0.01^{a}	0.28 ± 0.01^{a}	0.31 ± 0.02^{a}
Cohesiveness, -	0.05 ± 0.01^{a}	0.06 ± 0.01^{a}	0.06 ± 0.01^{a}	0.06 ± 0.01^{a}
Elasticity, mm	2.12 ± 0.15^{ab}	2.18 ± 0.03^{ab}	2.06 ± 0.11^{a}	2.30 ± 0.07^{b}
Chewiness, mJ	1.72 ± 0.10^{a}	1.83 ± 0.01^{ab}	1.83 ± 0.17^{ab}	2.00 ± 0.16^{b}

Differences between the analyzed samples were highlighted by lowercase letters per row. Mean values that share a letter are not significantly different (p>0.05)

In **Table 5.12**. it can be noted that the minimum value of firmness, 10.44 N, was recorded for the control sample. For the other samples, the firmness values were increasing, simultaneously with the increase in the added powder content. However, analyzing these results from a statistical point of view, it can be noted that these values do not show significant differences.

The obtained results prove that beet peel powder can be used to obtain marshmallows with improved nutritional value, without significantly influencing the texture.

5.5.13. Sensory evaluation of value-added marshmallow samples

Analyzing the results of the sensory evaluation of value-added marshmallows (**Figure 5.8.**), it is noted that the variants of marshmallows with the addition of beetroot peel powder were evaluated as having a balanced color (**Figure 5.7.**), pleasant, corresponding to red beet, unlike the control version which was the least appreciated. All the samples proposed for analysis were positively appreciated by the team of tasters, who appreciated the value-added marshmallows as having an easily perceptible beet taste and aroma.

The most appreciated marshmallow variant was B1, the one containing 4% powder. It is observed that as the percentage of beetroot peel powder increased, the tasters appreciated their texture, sound, and aftertaste less.

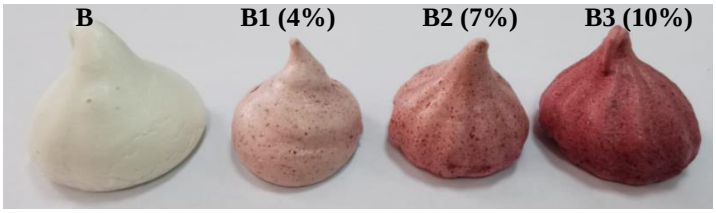

Figure 5.6. Marshmallows with added beetroot powder: B- marshmallows without added beetroot powder, B1, B2, and B3 - marshmallows with 4, 7, and 10% added beetroot powder

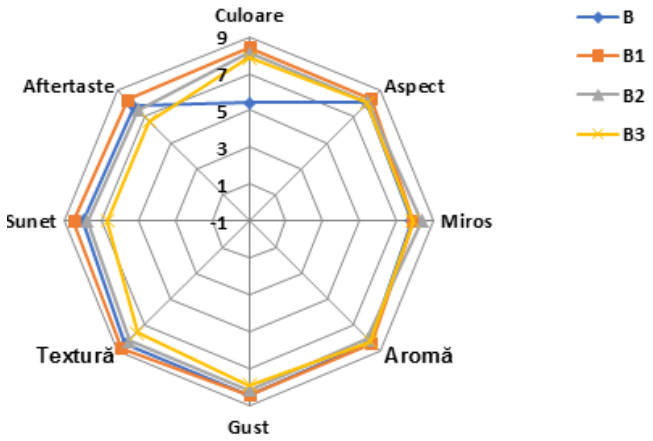

Figure 5.7. The comparative diagram of the sensory attributes specific to the types of marshmallows: B- marshmallows without the addition of beetroot powder, B1, B2, and B3 - marshmallows with the addition of 4, 7, and 10% beetroot powder

5.5.14. Characterization of the bioactive potential of the value-added pan and the storage stability of the samples

In **Table 5.13.** the phytochemical profile of value-added pan samples obtained by incorporating increasing concentrations (2%, 4%, and 6%) of beetroot peel powder is presented. The results are presented in **Table 5.13.** highlights the added value of the pan samples with the addition of beetroot peel powder, by increasing the total content of betalains and polyphenols, which lead to obtaining a product with high antioxidant activity.

Table 5.13. Phytochemical characteristics and antioxidant activity of beetroot powder added (H-beetroot powder-free, H1, H2, and H3 - beetroot powder with 2; 4 and 6% (w/w) beetroot peel powder)

Phytochemical characterization	Pan			
	H	H1 (2%)	H2 (4%)	H3 (6%)
Total betalain content, mg /100g d.w	-	1.78^{c}	2.86^{b}	3.77^{a}
Total polyphenolic content, mg GAE/100 g d.w.	32.92^{c}	38.63^{c}	53.44^{b}	69.48^{a}
Antioxidant activity DPPH, mM Trolox/100g d.w.	2.68^{d}	25.20^{c}	54.94^{b}	73.89^{a}

**Different letters (a-b) in a row for the same analyzed parameter show significant differences between means ($p < 0.05$).*

In **Table 5.14.** the results obtained following the evaluation of the stability of the bioactive compounds in the added value pan during 21 days of storage are presented. The results indicate that during the 21 days of storage, the content of bioactive compounds in the value-added sauce shows a slight decrease and implicitly a slightly lower antioxidant potential. However, the pan samples supplemented with increasing concentrations of beetroot peel powder present a rich profile of betalains and polyphenols compared to the conventional product (**Table 5.14.**).

Regarding the antioxidant potential, the samples of the pan with the addition of powder (4% and 6%) show a higher antioxidant potential than that of the control sample. Therefore, supplementing the pan with concentrations of more than 2% beetroot peel powder contributes to

its enrichment with bioactive compounds that lead to obtaining a product with high antioxidant activity.

5.5.16. Color parameters of value-added pan samples

The pan samples with added beetroot peel powder in different concentrations were analyzed for CIELAB colorimetric parameters using a portable colorimeter with illuminator C (Chroma Meter, model CR-410, Konica Minolta, Osaka, Japan),. The results were expressed as L* (brightness), a* (tendency to red for an a* "+" or green for an a* "-), and b* (tendency to yellow for b* "+" or blue for b* "-") (**Table 5.15.**).

The results are presented in **Table 5.15.** highlights the fact that incorporating beetroot powder into the composition of the pan is characterized by red-violet shades, the intensity of the color being directly proportional to the percentage of powder added (2, 4, and 6%). This consideration confirms that the beetroot peel powder has a high coloring power and can be used as a natural dye in the composition of the pan, increasing the attractiveness of the product and the interest of consumers towards this product (**Figure 5.5.**).

Table 5.14. Evaluation of the phytochemical stability of the added value pan: H - pan without the addition of beetroot peel powder, H1, H2, and H3 - the pan samples with the addition of 2; 4 and 6% (w/w) beetroot powder over the 21-day storage period

Sample	Bioactive compounds/Antioxidant activity	0 days	7 days	14 days	21 days
H	Total betalains (mg /100g g d.w.)	Nd*	Nd*	Nd*	Nd*
	Total polyphenols (mg EAG/100 g d.w.)	32.95 ± 4.19^{a}	30.00 ± 1.99^{a}	26.76 ± 1.82^{ab}	21.71 ± 1.20^{b}
	Antioxidant activity (mM Trolox/100 g d.w.)	2.68 ± 0.36^{a}	2.67 ± 0.09^{a}	2.28 ± 0.02^{ab}	2.02 ± 0.05^{b}
H1	Total betalains (mg /100g g d.w.)	1.78 ± 0.08^{a}	1.49 ± 0.03^{b}	1.17 ± 0.06^{c}	1.02 ± 0.09^{c}
	Total polyphenols (mg EAG/100 g d.w.)	38.63 ± 1.26^{a}	33.36 ± 1.81^{b}	30.73 ± 0.93^{b}	25.46 ± 0.90^{c}
	Antioxidant activity (mM Trolox/100g d.w.)	25.20 ± 0.81^{a}	24.51 ± 0.78^{a}	19.40 ± 1.02^{b}	16.21 ± 0.25^{c}
H2	Total betalains (mg /100g g d.w.)	2.86 ± 0.03^{a}	2.56 ± 0.04^{b}	2.19 ± 0.16^{c}	1.99 ± 0.06^{c}
	Total polyphenols (mg EAG/100 g d.w.)	53.44 ± 1.33^{a}	50.87 ± 0.62^{a}	46.45 ± 1.67^{b}	40.48 ± 0.76^{c}
	Antioxidant activity (mM Trolox/100g d.w.)	54.94 ± 2.67^{a}	50.55 ± 0.71^{b}	46.42 ± 0.97^{c}	41.47 ± 1.17^{d}
H3	Total betalains (mg /100g g d.w.)	3.77 ± 0.09^{a}	3.52 ± 0.09^{b}	3.25 ± 0.11^{c}	2.83 ± 0.08^{d}
	Total polyphenols (mg EAG/100 g d.w.)	69.48 ± 2.88^{a}	66.42 ± 1.82^{ab}	61.55 ± 1.34^{b}	53.65 ± 0.89^{c}
	Antioxidant activity (mM Trolox/100g d.w.)	73.89 ± 3.65^{a}	66.86 ± 1.59^{b}	59.91 ± 2.37^{c}	53.33 ± 1.92^{d}

** Nd - undetectable*

**Different letters (a-b) in a row for the same analyzed parameter show significant differences between means ($p < 0.05$).*

Table 5.15. The colorimetric parameters of the pan samples: H- pan without the addition of beetroot peel powder, H1, H2, and H3 - pan formulas with the addition of 2; 4, and 6% (w/w) beetroot peel powder.

Pan samples	L*	a*	b*
H	104.79 ± 0.45^{a}	7.08 ± 0.06^{c}	5.57 ± 0.48^{b}
H1	75.06 ± 0.35^{b}	36.90 ± 2.59^{b}	5.51 ± 1.16^{b}
H2	53.11 ± 0.50^{c}	42.50 ± 1.33^{ab}	6.38 ± 0.90^{ab}
H3	41.47 ± 1.10^{d}	45.22 ± 3.57^{a}	8.24 ± 1.03^{a}

**Different letters (a-b) on the column for the same analyzed parameter show significant differences between means ($p < 0.05$).*

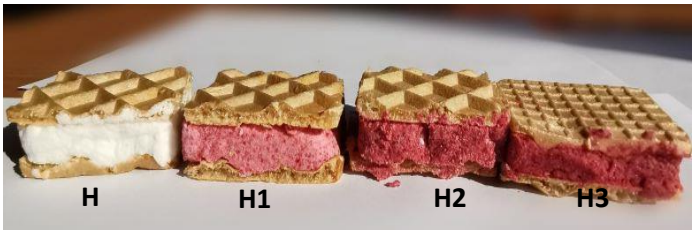

Figure 5.8. Pan with added beetroot powder, value-added product: H - pan without added beetroot powder, H1, H2, and H3 - pan formulas with 2 added; 4 and 6% (w/w) beet peel powder.

5.5.16. Textural properties of value-added pan samples

The texture of the pan was analyzed instrumentally by the Textural Profile Analysis method. This method consists of a double penetration, which simulates mastication. The results of the instrumental texture analysis are presented in **Table 5.16**.

The addition of beetroot powder increased the firmness and adhesion of the pan samples. If the differences between the control sample and the sample with 2% added powder are not significant, when the amount of added powder increases, they become more obvious, the sample with 6% added recording a firmness value almost three times higher compared to the control sample.

Table 5.16. The textural parameters of the pan samples: H - pan without the addition of beetroot peel powder, H1, H2, and H3 - pan with the addition of 2; 4 and 6% (w/w) beetroot peel powder

Analyzed parameter	H	H1	H2	H3
Firmness, N	0.54±0.05^{a}	0.56±0.01^{a}	1.06±0.12^{b}	1.51±0.03^{c}
Adhesion, mJ	1.00±0.03^{a}	1.11±0.18^{a}	1.42±0.02^{a}	1.55±0.03^{a}
Cohesiveness	0.57±0.03^{a}	0.45±0.01^{a}	0.43±0.01^{a}	0.38±0.005^{a}
Elasticity, mm	3.65±0.005^{a}	3.23±0.05^{a}	2.32±0.15^{a}	1.28±0.01^{a}
Gumminess, N	0.29±0.005^{a}	0.26±0.04^{a}	0.23±0.02^{a}	0.17±0.05^{a}
Chewability, mJ	1.06±0.06^{a}	0.70±0.02^{ab}	0.52±0.01^{ab}	0.41±0.01^{c}

Differences between the analyzed samples were highlighted by lowercase letters per row. Mean values that share a letter are not significantly different (p>0.05)

The evolution of firmness is due to the increase in the density and consistency of the paste when the powder is added. Firmness and adhesion positively influence product shape retention during storage. At the same time, the particles in the beetroot peel powder lead to the fragmentation of the protein matrix and the weakening of the internal bonds, a fact demonstrated by the decrease in cohesiveness with the increase in the amount of added powder. This makes the samples more easily disintegrated in the oral cavity during mastication. In **table 5.13.** it can be noted that the energy required to disintegrate the sample (chewability) decreases from 1.06±0.06 mJ for the control sample to 0.41±0.01 mJ for the sample with the highest percentage of added powder. The beetroot peel powder added to the pan also influences the elasticity of the samples. If the control sample can recover 3.65±0.005 mm of deformation, for the other samples this capacity decreases proportionally with the powder percentage, reaching 6% to recover less than half (1.28±0.01 mm). This behavior denotes the irreversible destructuring of the protein matrix during testing.

In conclusion, it can be stated that the addition of beetroot peel powder has a positive effect on the texture of the pan by improving firmness and facilitating mastication.

5.5.17. Sensory evaluation of value-added pan samples

Analyzing the results of the sensory evaluation of the pan with added value (**Figure 5.9.**), it is noted that the pan variants with the addition of beetroot peel powder were evaluated as having a balanced, pleasant color corresponding to beetroot, in contrast to the control variant which was the least appreciated.

Positive feedback was received from tasters, who rated the value-added sauce as having a slightly perceptible beetroot taste and aroma.

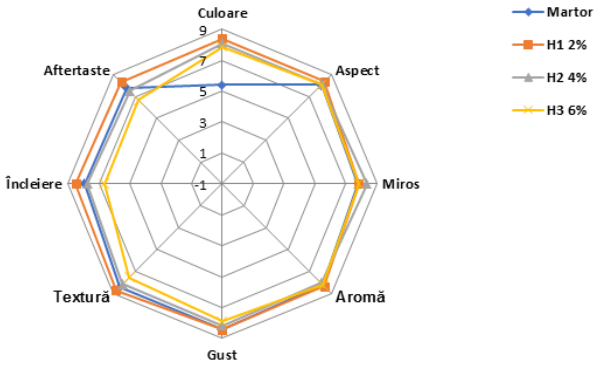

Figure 5.9. The comparative diagram of the specific sensory attributes of the beetroot samples: H- beetroot without the addition of beetroot powder, H1, H2, and H3 - beetroot with the addition of 2, 4 and 6% beetroot powder

The most appreciated marshmallow variant was H1, the one with a 2% powder content. It is observed that as the percentage of beetroot peel powder increased, the tasters appreciated their texture and aftertaste less.

The tendency of consumers to opt for the consumption of healthy, natural products has led to the development of a natural, innovative product with improved sensory properties (taste, aroma, color, texture), responding to the demand and needs of consumers. Thus, the obtaining of a new variety of sauce, with the addition of beetroot peel powder, is distinguished by a red-

violet color, conferred by the addition of powder rich in pigments (betalaine) from beetroot, attractive to consumers, especially for children.

The added value of the product is highlighted by the high intake of natural antioxidants present in beetroot peel, which have a remarkable antioxidant potential and are free of toxicity. In addition, the substitution of chemically synthesized additives with natural ones, present in beetroot peel, brings many benefits and directly contributes to increasing the quality of life.

5.6. Partial conclusions

The current study proposes a unique strategy for harnessing beetroot by-products as a source of bioactive substances to develop new value-added products. Characterization of the beetroot peel powder extract revealed a high concentration of polyphenolic compounds and showed significant antioxidant activity.

The functionality of beetroot peel powder has been evaluated by adding it to various value-added food products with enriched functional potential, such as mayonnaise, marshmallows, and pan.

The antioxidant activity of the mayonnaise samples increased with the content of beetroot peel powder and, due to the increased content of polyphenols, presented a higher nutritional quality compared to the control sample. Due to the presence of considerable levels of beetroot pigments, the addition of beetroot peel powder resulted in an increase in the a* parameter value (red color) from 13.82±0.22 for sample S1 by 1.5% powder at the value of 23.14±0.02 for sample S4 with 7% beetroot peel powder. There was also a reduction in the value of the parameter b* (yellow color) from the value of 6.72±0.28 of sample S1 to the value of -1.83±0.11 of sample S4. Furthermore, the addition of beetroot peel powder to mayonnaise samples resulted in greater firmness and improved stickiness and chewiness, giving the product a soft texture. The viscosity of the mayonnaise was also significantly improved, with the consistency index K showing increasing values with increasing beetroot powder concentration up to 3% (9.97 Pa□sn for 1.5% beetroot powder tomato and 14.35 Pa□sn for 3% beetroot peel powder). Sensory evaluation of value-added mayonnaise indicated that the addition of beetroot peel powder improved the color attributes of the product and caused no impact on the smell, taste, and overall acceptance score of the samples.

The results obtained in the present study support the multifunctionality of beetroot peel powder in marshmallows as a source of natural colorants with antioxidant activity that improves sensory characteristics. The addition of beetroot peel powder to the marshmallow samples did not change their texture. After storage, after 21 days, an increase in the values of the color parameters is observed for all three samples of marshmallows with added value, thus the value of the a* parameter increases between 25 and 29% in the case of marshmallows with addition of between 4 - 10% powder from beetroot peel. Sensory evaluation of value-added marshmallows indicated that the addition of beetroot peel powder also improved the color attributes of the product and caused no impact on the smell, taste, and overall acceptance score of the samples. The antioxidant activity of marshmallow samples increased with increasing beetroot peel powder content, respectively 39.06 ± 0.52 mM Trolox/100 g d.w. for sample B3 with 10% powder. Betalain content also increased (9.93 ± 0.38 mg/100g d.w.) for sample B3 with a 10% addition of beetroot peel powder and polyphenols (65.90 ± 0.68 mg EAG/100 g d.w.) compared to the control sample.

By characterizing the added-value pan, the multifunctionality of the powder obtained from beetroot peel in the composition of the pan is demonstrated, as an important source of natural compounds with antioxidant, coloring, and flavoring activity, which improves the sensory characteristics, such as the color, aroma, and texture of the product, contributing directly to increasing diversity and consumer appeal. This is evidenced by the increase in the total content

of betalains (3.77 ± 0.09 mg/g d.w.) and polyphenols (69.48 ± 2.88 mg EAG/100g d.w.), which lead to obtaining a pan with high antioxidant activity (73.89 ± 3.65 mM Trolox/100 g d.w.) for sample H3 with 6% addition of beetroot peel powder. Due to the increased presence of beetroot peel powder in the composition of the pan, has increased the product's appeal and consumer interest in this product due to its coloring power. The addition of beetroot powder increased the firmness and adhesion of the pan samples. Also, the addition of beetroot peel powder has a positive effect on the texture of the pan by improving firmness and facilitating chewing.

In addition, the use of by-products obtained from the industrial processing of red beet, considering them as a source of biologically active compounds, can become a viable alternative to the variants of synthetic dyes, flavorings, and antioxidants. They can have multiple uses in the food industry and can help reduce waste and implement a circular economic model for environmental protection.

Chapter 6. FINAL CONCLUSIONS

The goal of boosting global food production is not only challenging but requires tremendous commitment from national governments, agencies, organizations, industries, and individuals. However, while exploring strategies to achieve food sufficiency, it is important to adopt sustainable approaches that ensure adequate and rational use of available products.

It is a fact that by-products from food processing represent tons of waste that are often inappropriately wasted in the environment, leading to pollution, especially in developing countries. Numerous by-products from food production and processing contain valuable nutrients such as bioactive compounds, vitamins, lipids, proteins, and dietary fiber. By using biotechnological processes, factors such as the earthy taste of beetroot or the specific pungent smell of beetroot can be minimized for the widest possible use of this vegetable and the by-products resulting from its processing, as food additives or in the formulation of new value-added foods.

In this PhD thesis, the characterization of the main biologically active compounds from beetroot peel was aimed at using beetroot peel powder as a natural ingredient with added value that could have different purposes. Therefore, the studies carried out as part of the doctoral thesis, entitled "**VALUATION OF THE BY-PRODUCTS RESULTING FROM THE PROCESSING OF BEETS**" aimed at the valorization of the bioactive compounds from beetroot skin to develop some functional ingredients that would support the wishes of consumers regarding the consumption of food with benefits for health.

 Four different extraction methods were used. In all these methods, the concentration of ethanol, citric acid, the extraction time, and temperature were varied. Thus, the combination of 20% ethanol acidified with 0.1% citric acid allowed to obtain the highest amount of betalain compounds. The highest values of total polyphenols content were obtained by conventional extraction with 20% concentration ethanol and 1% citric acid as extraction parameters. The enzyme preparation with pectolytic activity (Zymorouge) led to a high extraction yield of betalain compounds, the obtained extract presented a high antioxidant activity.

 The Central Composite Design (CCD) projection matrix was used to investigate the effect of extraction parameters to optimize the extraction of betalains and total polyphenols from beetroot peel. The conditions optimized for the maximum extraction of betalains and polyphenolic compounds were: citric acid concentration of 1.5%, ethanol concentration of 50%, temperature of 52.52°C, and extraction time of 49.9 min.

 The chromatogram of the beetroot peel extract shows a distinct peak at a retention time of 1.87 ± 0.2 min properly correlated with the betanin standard.

 Following thermal stability studies, the total betalain content follows a downward trend in the temperature range of 20-170°C used. The content of total polyphenols in the beetroot peel extract showed a constant reduction throughout the studied temperature range from 20°C to 170ºC. Antioxidant activity has a similar decreasing trend as that of polyphenolic content. Following thermal stability studies, maintenance of the antioxidant activity is observed in the temperature range of 20-70ºC, followed by a decrease at a temperature of 170ºC, this phenomenon can be explained by a possible release of other types of biologically active compounds (flavonoids) from the matrix analyzed following heat treatment.

 Since phytochemical content and antioxidant capacity are temperature dependent, the choice of targeted use of biologically active beetroot compounds by-products incorporated in heat-treated foods must take into account their thermal stability.

 The results demonstrated that the beetroot peel extract acts as an inhibitor of α-glucosidase, α-amylase, lipase and lipoxygenase activity, suggesting that the presence of biologically active compounds has the potential to effectively contribute to the control of postprandial blood sugar, as well as for stress cellular oxidative stress related to diabetes, as well as on diseases related to hyperlipidemia.

 To evaluate the industrial application potential of the natural ingredients from the beetroot peel, different technological options were developed to obtain three value-added products, their functionality being evaluated through the content of phytochemical compounds and antioxidant activity.

 The addition of beetroot peel powder to mayonnaise samples led to an improvement in firmness, stickiness, and chewiness, giving the product a soft texture. The viscosity of the mayonnaise was also significantly improved. Sensory evaluation of the value-added mayonnaise indicated an improvement in the color attributes of the product and caused no impact on the overall acceptance score of the mayonnaise samples.

 The results obtained regarding the meringue product support the multifunctionality of beetroot peel powder as a source of natural dyes with antioxidant activity that improves sensory characteristics.

 The pan-type product demonstrates the multifunctionality of the powder obtained from beetroot peel in its composition, as an important source of natural compounds with antioxidant, coloring, and flavoring activity, which improve the sensory characteristics, such as the color, aroma, and texture of the product, directly contributing to the increase in diversity and consumer appeal.

Chapter 7. PERSONAL CONTRIBUTIONS AND PROSPECTS FOR FURTHER STUDIES

The doctoral thesis with the title "**VALUATION OF THE BY-PRODUCTS RESULTING FROM THE PROCESSING OF RED BEET**" represents an original research study, which allowed the passage of precise stages that had as a well-defined target the methods of recovery and reintegration into various food products of some bioactive compounds with benefits for human health, compounds that are of major interest.

The original contributions of the PhD thesis are based on aspects such as:

 Choosing an efficient extraction method in order to obtain extracts rich in biologically active compounds of major interest;

 Advanced characterization of beetroot peel extracts by determining the phytochemical profile and antioxidant activity, thermal stability, to determine the optimal conditions for obtaining, processing, and storing products rich in betalain compounds;

 In vitro testing of the biological activity of the beetroot peel extract;

 The development of different technological options for obtaining food products with added value by incorporating the bioactive compounds from the beetroot peel and their characterization from a physico-chemical, phytochemical, sensory point of view, etc., highlights the concept of circular economy in our country.

In the perspective of future research, the idea of using products derived from beetroot (extracts, powders) as functional ingredients for the formulation of other various food products (beverages such as juices; bakery products such as bread, biscuits, muffins, cakes, cake tops, etc.; fermented dairy products such as yogurt, cheese, cream cheese, kefir, sana, etc.).

It is also envisaged to determine the antibacterial and/or antifungal activity of beetroot peel extracts, as well as to test the cytotoxic, antiproliferative, and even probiotic potential of beetroot peel powders.

Beetroot peel extract is soluble in water facilitating its use in the food and even pharmaceutical industry, intending to produce nutraceuticals, and its high antioxidant capacity allows its use as a fortifier in food products.

Chapter 8. LIST OF PUBLICATIONS

The dissemination of the results of the research carried out throughout the doctoral studies took place in the following scientific papers published or communicated at national and international conferences as follows:

8.1. Articles published in ISI-indexed/rated journals

1. Lazăr (Mistrianu)*, S., Constantin, O.E., Stănciuc, N., Aprodu, I., Croitoru, C., Râpeanu, G. Optimization of Betalain Pigments Extraction Using Beetroot by-Products as a Valuable Source. *Inventions* 2021, 6(3), 50. https://doi.org/10.3390/inventions6030050

2. Lazăr (Mistrianu)*, S., Constantin, O.E., Horincar, G., Andronoiu, D.G., Stănciuc, N., Mureșan, C., Râpeanu, G. Beetroot By-Product as a Functional Ingredient for Obtaining Value-Added Mayonnaise. *Processes,* 2022, 10(2), 227. https://doi.org/10.3390/pr10020227 **Q3**, Impact Factor = 3,352

8.2. Patent applications

1. Lazăr (Mistrianu)*, S., Râpeanu, G., Horincar, G., Andronoiu, D.G., Stănciuc, N., Constantin, O.E., 2022. Mayonnaise with the addition of beetroot peel powder – value-added product and production technology, no. of registration at OSIM A/00384/03.06.2021, published in BOPI https://osim.ro/images/Publicatii/Inventii/2023/bopi_inv_022023.pdf.

2. Lazăr (Mistrianu)*, S., Râpeanu, G., Condurache (Lazăr) N.N., Stănciuc, N., Aprodu, I., Constantin, O.E., Andronoiu, D.G., Croitoru, C. 2022. Marshmallows with the addition of beetroot peel powder - value-added product and production technology, no. of registration at OSIM A/00132/16.03.2022

3. Lazăr (Mistrianu)*, S., Horincar, G., Andronoiu, D.G., Stănciuc, N., Râpeanu, G. 2022. Saucepan with the addition of beetroot peel powder - value-added product and production technology, no. of registration at OSIM A/00518/25.08.2022.

8.3. Participation in national and international conferences and symposiums

1. Lazăr (Mistrianu)*, S., Râpeanu, G., Stănciuc, N., Georgescu, L., Aprodu, I. 2020. Betalains from red beetroot: natural pigments with potential application in food industry, poster, *8th edition of Scientific Conference of Doctoral Schools SCDS-UDJG, "Dunarea de Jos" University of Galați*

2. Lazăr (Mistrianu)*, S., Râpeanu, G., Stănciuc, N., Constantin, O.E., Aprodu, I. 2020. Betalains recovery from beetroot skins using different extraction methods**,** poster, *Scientific Symposium "Young people and multidisciplinary research in applied life sciences", 7th edition, Section: Food Engineering, Banat`s University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania*" *Timisoara*

3. Lazăr (Mistrianu)*, S., Râpeanu, G., Stănciuc, N., Aprodu, I., Constantin, O.E. 2021. Thermal stability of betalains recovered from red beetroot peels, poster, *9th edition of Scientific Conference of Doctoral Schools SCDS-UDJG, "Dunarea de Jos" University of Galați*

4. Lazăr (Mistrianu)*, S., Râpeanu, G., Stănciuc, N., Aprodu, I., Constantin, O.E. 2021. Red beetroot by products as valuable source of natural colorants, poster, *9th edition of Scientific Conference of Doctoral Schools SCDS-UDJG, "Dunarea de Jos" University of Galați*

5. Lazăr (Mistrianu)*, S., Râpeanu, G., Stănciuc, N., Aprodu, I., Constantin, O.E. 2021 Thermostability of biological active compounds extracted red beetroot by products, poster, *Multidisciplinary Conference on Sustainable, Development, Section: Food Chemistry, Engineering & Technology, Faculty of Food Engineering Timişoara*

6. Lazăr (Mistrianu)*, S., Constantin, O.E., Râpeanu, G., Stănciuc, N., Aprodu, I., 2021 optimization of conventional extraction of betalain compounds from beetroot peels, poster, *EuroAliment, "Dunărea de Jos" University of Galați*

7. Lazăr (Mistrianu)*, S., Horincar, G., Andronoiu, D.G., Stănciuc, N., Constantin, O.E., Râpeanu, G. 2021. Value added mayonnaise enriched with red beetroot peels powder, poster, International scientific symposium "*Young researchers and scientific research in life sciences" Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" Timişoara*

8. Lazăr (Mistrianu)*, S., Horincar, G., Andronoiu, D.G., Stănciuc, N., Constantin, O.E., Râpeanu, G. 2021. Stability of mayonnaise enriched with red beetroot peels powder, poster, *PhD students' days Faculty of Food Engineering, Tourism, and Environmental Protection The First Edition, Arad*

9. Lazăr (Mistrianu)*, S., Râpeanu, G., Stănciuc, N., Aprodu, I., Constantin, O.E. 2022. Red beetroot peels valorisation as a source of natural dyes with health benefits, poster, 10th edition of *Scientific Conference of Doctoral Schools SCDS-UDJG, "Dunarea de Jos" University of Galați*

10. Lazăr (Mistrianu)*, S., Râpeanu, G., Constantin, O.E, Aprodu, I., Stănciuc, N. 2023. Valorisation of red beet by-products to develop functional food products peels as a source of natural dyes with health benefits, poster, 11th edition of *Scientific Conference of Doctoral Schools SCDS-UDJG, "Dunarea de Jos" University of Galați*

8.4. Awards

1. Third prize, Poster*, Betalains recovery from beetroot skins using different extraction methods*, Lazăr (Mistrianu)*, S., Râpeanu, G., Stănciuc, N., Constantin, O.E., Aprodu, I. Scientific Symposium "Young people and multidisciplinary research in applied life sciences", 7th edition, Section: Food Engineering, Banat`s University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" 27 november, 2020 Timisoara.

2. Honorable Mention, Presentation, *Thermal stability of betalains recovered from red beet peels*, Lazăr (Mistrianu)*, S., Râpeanu, G., Stănciuc, N., Aprodu, I., Constantin, O.E., 9th edition of Scientific Conference of Doctoral Schools SCDS-UDJG, "Dunarea de Jos" University of Galați, 10-11th of June 2021.

3. Gold medal, *Mayonnaise with the addition of beetroot peel powder – value-added product and production technology*, Lazăr (Mistrianu)*, S., Râpeanu, G., Horincar, G., Andronoiu, D.G., Stănciuc, N., Constantin, O.E., UGAL INVENT, 12-12 noiembrie 2021, Galați.

4. Third prize, poster, *Value added mayonnaise enriched with red beetroot peels powder*, Lazăr (Mistrianu)*, S., Horincar, G., Andronoiu, D.G., Stănciuc, N., Constantin, O.E., Râpeanu, G., simpozionul științific ”Young people and multidisciplinary research in applied life sciences, 25 noiembrie 2021, Timișoara.

5. Gold medal, *Mayonnaise with beetroot peels powder – value-added product and obtaining method*, Lazăr (Mistrianu)*, S., Râpeanu, G., Horincar, G., Andronoiu, D.G., Stănciuc, N., Constantin, O.E., INVENTICA, 22-24 iunie 2022, Iași.

6. Silver medal, *Alviță cu adaos de pudră din de sfeclă roșie – produs cu valoare adăugată și tehnologia de obținere,* Lazăr (Mistrianu)*, S., Horincar, G., Andronoiu, D.G., Stănciuc, N., Râpeanu, G., Salonul internațional al Cercetării Științifice, Inovării și Inventicii ”PRO INVENT”, 26-28 octombrie 2022, Cluj-Napoca.

7. Diploma of excellence and gold medal, *Pan with the addition of beetroot peel powder – value-added product and production technology,* Lazăr (Mistrianu)*, S., Horincar, G., Andronoiu, D.G., Stănciuc, N., Râpeanu, G., Salonul internațional al Cercetării Științifice, Inovării și Inventicii ”PRO INVENT”, 26-28 octombrie 2022, Cluj-Napoca.

8. Diploma of excellence and gold medal, *Meringue with added beetroot peel powder – value-added product and production technology,* Lazăr (Mistrianu)*, S., Râpeanu, G., Condurache (Lazăr) N.N., Stănciuc, N., Aprodu, I., Constantin, O.E., Andronoiu, D.G., Croitoru, C., Salonul internațional al Cercetării Științifice, Inovării și Inventicii ”PRO INVENT”, 26-28 octombrie 2022, Cluj-Napoca.

9. Special prize, *Pan with the addition of beetroot peel powder – value-added product and production technology,* Lazăr (Mistrianu)*, S., Horincar, G., Andronoiu, D.G., Stănciuc, N., Râpeanu, G., The International Exhibition of Research, Innovations and Inventions PRO INVENT, XX Edition, Cluj-Napoca, 26-28 octombrie 2022.

10. Diploma of excellence for the patent application *Pan with the addition of beetroot peel powder - value-added product and the technology for obtaining it,* Lazăr (Mistrianu)*, S., Horincar, G., Andronoiu, D.G., Stănciuc, N., Râpeanu, G., Susținerea cercetării de excelență în activitatea CDI din Universitatea ”Dunărea de Jos” din Galați – CEREX UDJG 2022.

11. Diploma of excellence for the patent application *Meringue with added beetroot peel powder – value-added product and production technology,* Lazăr (Mistrianu)*, S., Râpeanu, G., Condurache (Lazăr) N.N., Stănciuc, N., Aprodu, I., Constantin, O.E., Andronoiu, D.G., Croitoru, C., Susținerea cercetării de excelență în activitatea CDI din Universitatea ”Dunărea de Jos” din Galați – CEREX UDJG 2022.