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Doctoral School of Fundamental and Engineering Sciences

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DOCTORAL THESIS

Designing novel ingredients based on eggplants' bioactive compounds for different applications in the food industry

(Summary)

Ph.D. student,

Nina-Nicoleta CONDURACHE (LAZĂR)

Scientific coordinator,

Prof. Ph.D. Eng. Gabriela RÂPEANU

Work carried out within the project "Program for increasing performance and innovation in excellence doctoral and post-doctoral research – PROINVENT"
Contract no. 62487/06.03.2022, POCU/993/6/13 - SMIS Code: 153299

Series I.7: Food Engineering No. 18

GALAŢI

2023

Leader: Partners:

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Commission president	**Prof. Ph.D. Eng. Gabriela-Elena BAHRIM**
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Special appreciation to **my husband Alin** and **my family**, who supported me during these five challenging years!

With special gratitude and love, I dedicate this thesis to **my grandfather**, who is no longer among us but always in my heart **♥.**

I bow to you!

Ph.D. student **Nina-Nicoleta Condurache (Lazăr)**

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Keywords: eggplant peels; microencapsulation; anthocyanins; peptides; lactic acid bacteria; value-added food products;

INTRODUCTION

The doctoral thesis entitled "**Designing novel ingredients based on eggplants' bioactive compounds for different applications in the food industry**" aimed at studying and valorizing the biologically active compounds (mainly anthocyanins) through functional ingredients formulation for food industry application. The study focuses on eggplant peels, known for their biologically active compounds (especially anthocyanins) and antioxidant activity.

In the current context, the research carried out during the doctoral studies had the following objectives:

- extraction, identification, and quantification of biologically active compounds from eggplant peels;
- advanced characterization of eggplant peel extracts;
- enzymatic hydrolysis of whey proteins to supply peptides with antioxidant activity and prebiotic effect;
- study of the interaction between anthocyanins and whey proteins/peptides;
- formulation and characterization of functional ingredients via encapsulation of biologically active compounds from eggplant peels;
- formulation and characterization of value-added food products by adding functional powders.

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

I. Documentary study: consists of 7 chapters presenting recent data from the literature on eggplants, identification, and characterization of biologically active compounds methods, methods of generating bioactive peptides from whey proteins, benefits of bioactive compounds, peptides, and lactic bacteria on health, methods of encapsulation, and the concept of functional food.

II. Experimental study: consists of 6 chapters covering original investigations carried out during the doctoral research, as described above.

Chapter 8. "Extraction and characterization of biologically active compounds from eggplant peels"

Chapter 9. "Hydrolysis of whey proteins" presents data on the enzymatic hydrolysis of whey proteins by varying the parameters. The hydrolysates' characterization results in hydrolysis degree, antioxidant activity, and prebiotic effect are also presented.

Chapter 10. "Studies of binding and quenching of fluorescence of whey proteins and peptides by addition of biologically active compounds from eggplant peels" present the binding mechanism results between biologically active compounds from eggplant peel extracts and proteins/peptides.

Chapter 11. "Developing functional ingredients based on anthocyanins from eggplant peels and bioactive peptides from whey" presents data related to the microencapsulation of the compounds in the eggplant peel extract using different encapsulation materials and techniques. The microcapsules were characterized by encapsulation efficiencies, phytochemical content, antioxidant activity, storage stability, microstructure, *in vitro* digestibility, and cytotoxicity.

Chapter 12. "Technology development for added-value food" presents the technologies for developing functional foods enriched with microcapsules from eggplant peel extract. The value-added products were characterized by their phytochemical content and storage stability over time.

Each chapter of the experimental part is structured in a logical sequence, as follows:

- **Introduction**, which presents the research opportunity and the specific objectives of the study;
- **Materials and methods**, which describe the materials and reagents used, but also the methods of investigation, analysis, processing, and interpretation of experimental data;
- **Results and discussions**, in which the original data are highlighted compared to the existing data in the specialized literature;
- **Partial conclusions***;*
- **References**.

Chapter 13. "General conclusions" presents the conclusions resulting from the investigations carried out during the Ph.D. thesis.

The doctoral thesis comprises 140 pages, including 43 figures and 48 tables. The documentary study represents 25 % and the experimental part 75 % of the entire study.

At the end of the doctoral thesis are presented the original contributions, the perspectives for further research, and the dissemination of the results in the research field. Thus, the research results were capitalized by the elaboration of **4 scientific articles**, published in **ISI journals** (*Food and Bioprocess Technology, Food Chemistry, Plants, Inventions*), **3 national patent applications**, as well as **16 communications** at scientific events representative of the field of food science and biotechnology, from abroad and from the country.

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

During the doctoral studies, the student got involved in the research team of a project with topics converging to the doctoral thesis. The project was entitled "*Closing value chains in the bio-economy by obtaining innovative bioproducts required by the market, " acronym PRO-SPER,* PN-III-P1-1.2-PCCDI-2017-0569-PRO-SPER (10PCCI), project leader Prof. PhD. Eng. Gabriela Elena Bahrim.

The thesis was coordinated scientifically by **Prof. PhD. Eng. Gabriela RÂPEANU** as the doctoral supervisor and of the guidance commission composed of **Prof. PhD. Eng. Iuliana APRODU**, Prof. PhD. Eng. **Gabriela Elena BAHRIM**, and **Prof. PhD. Eng. Nicoleta STĂNCIUC**.

II. EXPERIMENTAL STUDY - PERSONAL CONTRIBUTIONS

CHAPTER 8. EXTRACTION AND CHARACTERIZATION OF BIOLOGICALLY ACTIVE COMPOUNDS FROM EGGPLANT PEELS

8.2. Objectives of the study

The main objective of this study was to achieve an extract from eggplant peels with a high concentration of biologically active compounds, especially anthocyanins, and to evaluate their phytochemical and functional properties.

Thus, the main objectives of this study were to:

- test four different methods such as conventional, ultrasound-assisted, microwave-assisted, and enzyme-assisted;
- vary several parameters and select the optimal extraction conditions;
- determine the total content of anthocyanins, flavonoids, polyphenols, and the antioxidant capacity;
- identify, and quantify the anthocyanins from the eggplant peel extracts by liquid chromatography techniques;
- monitor biologically active compounds' behavior by *in vitro* enzyme inhibition as well as during simulated *in vitro* digestion and heat treatment.

8.4. Results and discussion

8.4.1. Anthocyanins content of the extracts recovered using conventional and modern methods

Table 8.1 points out the effects that each combination of parameters has had on the process. The SLE generated the largest amounts of anthocyanins using 70% and 96 % ethanol, both acidified with HCl at 50 °C for 30 min, with no significant differences among the solid-liquid phase extraction efficiency ($p>0.05$). The lowest TAC was highlighted for the extraction with 96 % ethanol and CH_3COOH, regardless of the time or temperature used.

Table 8.1. *Total anthocyanin content (mg D3G/g dried eggplant peels) extracted with SLE*

Solvent	T°C	Acetic acid			Hydrochloric acid		
		30 min	60 min	120 min	30 min	60 min	120 min
EtOH	25	$0.93\pm0.07^{aA*1}$	$0.96\pm0.01^{aB*1}$	$0.93\pm0.04^{aC*1}$	$0.99\pm0.04^{cA*1}$	$0.98\pm0.03^{cB*1}$	$1.01\pm0.01^{cC*1}$
50 %	50	$1.07\pm0.13^{aA*4}$	$0.79\pm0.05^{bC\#4}$	$0.74\pm0.03^{bE\#4}$	$0.87\pm0.03^{cB\#4}$	$0.90\pm0.02^{cD\#4}$	$0.94\pm0.09^{cF*4}$
EtOH	25	$0.67\pm0.05^{aA\circ2}$	$0.72\pm0.04^{abC\circ2}$	$0.76\pm0.07^{aE\circ2}$	$0.84\pm0.06^{cB\circ2}$	$0.84\pm0.08^{cD\circ2}$	$0.87\pm0.04^{cF\circ2}$
70 %	50	$0.92\pm0.04^{aA\bullet5}$	$0.88\pm0.08^{aC\bullet4}$	$0.83\pm0.07^{aD\circ5}$	$1.01\pm0.07^{cB\bullet5}$	$0.96\pm0.08^{cC\bullet4}$	$1.02\pm0.08^{cE\bullet4}$
EtOH	25	$0.21\pm0.02^{aA\blacklozenge3}$	$0.19\pm0.01^{abC\blacklozenge3}$	$0.16\pm0.02^{aE\blacklozenge3}$	$0.93\pm0.07^{cB\blacklozenge1}$	$0.92\pm0.08^{cD\blacklozenge12}$	$1.01\pm0.11^{cF\blacklozenge1}$
96 %	50	$0.22\pm0.02^{aA\blacklozenge6}$	$0.26\pm0.02^{abC\heartsuit5}$	$0.28\pm0.02^{bE\heartsuit6}$	$1.11\pm0.09^{cB\heartsuit5}$	$1.13\pm0.11^{cD\heartsuit5}$	$1.06\pm0.07^{cF\blacklozenge4}$

The influence of time was highlighted in lowercase letters on the line; the influence of the acid was highlighted in capital letters on the line; the influence of temperature was highlighted by symbols (*, #; ○, ●; ♥, ♦) on the column, the influence of ethanol concentration was highlighted by numbers on the column. Values that share a lowercase/uppercase letter on the line /symbol per column/number per column are not significantly different ($p> 0.05$)

Table 8.2 points out the effects that certain combinations of parameters have had on the UAE process. UAE resulted in maximum anthocyanin content of 1.04 ± 0.10 mg D3G/g dw, using 96 % ethanol acidified with 1N HCl, after 30 min at 25 °C (**table 8.2**). However, 96% ethanol solution combined with CH_3COOH led to the lowest concentrations of anthocyanins, regardless of the time and temperature used.

Table 8.2. *Total anthocyanin content (mg D3G/g dried eggplant peels) generated by UAE*

Solvent	T°C	Acetic acid			Hydrochloric acid		
		15 min	30 min	45 min	15 min	30 min	45 min
EtOH 50 %	25	$0.81\pm0.05^{Aa*1}$	$0.78\pm0.07^{aB*1}$	$0.79\pm0.09^{aC*2}$	$0.77\pm0.07^{cA*1}$	$0.84\pm0.07^{cB*1}$	$0.82\pm0.08^{cC*1}$
EtOH 50 %	50	$0.70\pm0.06^{aA\#3}$	$0.75\pm0.07^{aC*3}$	$0.72\pm0.07^{aD*3}$	$0.80\pm0.04^{cB*3}$	$0.84\pm0.04^{cdC*34}$	$0.91\pm0.04^{dE\#3}$
EtOH 70 %	25	$0.79\pm0.03^{aA\circ1}$	$0.78\pm0.08^{aC\circ1}$	$0.85\pm0.07^{aD\circ2}$	$0.91\pm0.09^{cB\circ2}$	$0.90\pm0.09^{cC\circ1}$	$0.94\pm0.08^{cD\circ12}$
EtOH 70 %	50	$0.84\pm0.07^{aA\circ4}$	$0.73\pm0.05^{bB\circ3}$	$0.64\pm0.03^{bD\bullet4}$	$0.87\pm0.06^{cA\circ3}$	$0.93\pm0.08^{cC\circ3}$	$0.90\pm0.09^{cE\circ3}$
EtOH 96 %	25	$0.14\pm0.01^{aA\blacklozenge2}$	$0.20\pm0.01^{bC\blacklozenge2}$	$0.21\pm0.05^{bE\blacklozenge1}$	$0.98\pm0.08^{cB\blacklozenge2}$	$1.04\pm0.09^{cD\blacklozenge2}$	$0.97\pm0.07^{cF\blacklozenge1}$
EtOH 96 %	50	$0.20\pm0.03^{aA\blacktriangledown5}$	$0.22\pm0.02^{aC\blacklozenge4}$	$0.24\pm0.02^{aE\blacklozenge5}$	$0.79\pm0.06^{cB\blacktriangledown3}$	$0.75\pm0.09^{cD\blacktriangledown4}$	$0.95\pm0.08^{dF\blacklozenge3}$

The influence of time was highlighted in lowercase letters on the line; the influence of the acid was highlighted in capital letters on the line; the influence of temperature was highlighted by symbols (*, #; ◌, ●; ♥, ♦) on the column, the influence of ethanol concentration was highlighted by numbers on the column. Values that share a lowercase/uppercase letter on the line /symbol per column/number per column are not significantly different (*p> 0.05*)

MAE is another popular modern technology due to its high processing speed and low solvent consumption. The anthocyanin content as a function of the parameters' variation is shown in **table 8.3**. Regardless of the combination used, MAE produced lower levels of anthocyanins than the other two extraction methods. After 10 sec, the 70 % ethanol solution mixed with 1N HCl yielded the maximum TAC of 0.95 ± 0.09 mg D3G/g dw. On the other hand, 96 % ethanol solution with CH_3COOH addition, provided the lowest concentration of anthocyanins recovered by the MAE, as did the other two procedures (0.01 ± 0.01 mg D3G/g dw).

Table 8.3. *Total anthocyanin content (mg D3G/g dried eggplant peels) achieved with MAE*

Solvent	Acetic acid		Hydrochloric acid	
	10 sec	15 sec	10 sec	15 sec
EtOH 50 %	0.72 ± 0.05^{Aa1}	0.68 ± 0.05^{aB1}	0.77 ± 0.05^{bA1}	0.75 ± 0.05^{bC1}
EtOH 70 %	0.82 ± 0.06^{aA2}	0.76 ± 0.06^{aC2}	0.95 ± 0.09^{bB2}	0.83 ± 0.06^{cC1}
EtOH 96 %	0.01 ± 0.01^{aA3}	0.06 ± 0.01^{bC3}	0.76 ± 0.06^{cB1}	0.77 ± 0.03^{cD1}

The influence of time was highlighted in lowercase letters on the line; the influence of the acid was highlighted in capital letters on the line; the influence of ethanol concentration was highlighted in numbers on the column. The values that share a lowercase/uppercase letter on the line or number on the column are not significantly different (*p>0.05*)

EAE is notable for lowering solvent and energy usage while increasing the amount of biologically active compounds recovered. In this investigation, we used two enzymes, as shown in **table 8.4**, which displays the anthocyanin concentration following the EAE as a function of time.

Table 8.4. *Total anthocyanin content (mg D3G/g dried eggplant peels) released by EAE*

Enzyme \ Time	1 h	2 h	3h
Cellulase	0.29 ± 0.02^{a}	0.22 ± 0.02^{b}	0.15 ± 0.04^{c}
Pectolitic enzymes	nd	nd	nd

The influence of time was highlighted in lowercase letters on the line. Values that share a letter are not significantly different (*p>0.05*); nd – not detected;

However, after 1 h of hydrolysis, the maximum TAC with cellulase was 0.29 ± 0.02 mg D3G/g dried eggplant peels in our investigation. Pectolitic enzymes from *Aspergillus niger*, on the other hand, failed to liberate anthocyanins from cell walls after 3 h of hydrolysis or, probably, degraded them completely in only 1 h (**table 8.4**).

Since anthocyanins were the chemicals of interest in our investigation, it was essential to select the optimal method and combination of parameters for extracting them from the eggplant peels. Given that the study's ultimate purpose was to develop a value-added food

product based on eggplant peel extract, even though HCl produced the greatest anthocyanin results, it was omitted due to its potential toxicity. Furthermore, due to the poor yields of MAE and EAE in comparison to the other two, these were also not considered. As a result, only the SLE and UAE with acetic acid were considered. Only the combinations of factors from 30 minutes were used to statistically compare the data. The results are presented in **Table 8.5.**

Table 8.5. *Total anthocyanin content of eggplant peels (mg D3G/g dried eggplant peels) acquired by SLE and UAE after 30 min*

Solvent	T°C	Acetic acid	
		SLE	UAE
EtOH 50 %	25	$0.93\pm0.07^{a1*}$	$0.78\pm0.07^{b1*}$
	50	$1.07\pm0.13^{a1\bullet}$	$0.75\pm0.07^{b1\circ}$
EtOH 70 %	25	$0.67\pm0.05^{a2\#}$	$0.78\pm0.08^{b2*}$
	50	$0.92\pm0.04^{a3\blacktriangledown}$	$0.73\pm0.05^{b2\circ}$
EtOH 96 %	25	$0.21\pm0.02^{a4\circ}$	$0.20\pm0.01^{a3\#}$
	50	$0.22\pm0.02^{a4\blacklozenge}$	$0.22\pm0.02^{a3\bullet}$

The influence of the method was highlighted in lowercase letters on the line; the influence of ethanol concentration was highlighted by symbols (*, #; ◌, ●; ♥, ♦) on the column, the influence of temperature was highlighted by numbers on the column. Values that share a lowercase/uppercase letter on the line /symbol per column/number per column are not significantly different (*p>0.05*)

Table 8.5 suggests that, in contrast to the UAE, the conventional method allowed the recovery of extracts with the highest TAC. SLE provided the highest TAC after 30 min at 50°C with acetic acid addition in a 50% ethanol solution. However, no significant differences were seen between these concentrations and those measured at 25°C (*p>0.05*). Cos of the decreased energy consumption and risk of compound degradation, the latest combination was preferred. Although modern extraction techniques are generally preferable to traditional ones since they require significantly less time, SLE had a higher efficiency in our case than UAE.

8.4.2. Flavonoids content of the extracts acquired using conventional and modern methods

Table 8.6 depicts the TFC content obtained by varying the parameters during the SLE method. The highest TFC (2.42 ± 0.01 mg CE/g dw) was achieved with 50% aqueous ethanol acidified with CH_3COOH after 120 min at 25 °C. The lowest flavonoid concentration of 0.51 ± 0.05 mg CE/g dw was acquired with 96% ethanol solution acidified with 1N HCl after 30 min of extraction at 50 °C (**table 8.6**).

Table 8.6. *Total flavonoid content (mg CE/g dried eggplant peels) provided by SLE*

Solvent	T°C	Acetic acid			Hydrochloric acid		
		30 min	60 min	120 min	30 min	60 min	120 min
EtOH 50 %	25	$2.38\pm0.13^{Aa*1}$	$2.33\pm0.01^{aC*1}$	$2.42\pm0.01^{aD*1}$	$1.51\pm0.10^{bB*1}$	$2.05\pm0.20^{cC*1}$	$2.40\pm0.22^{cD*1}$
	50	$1.15\pm0.07^{aA\#4}$	$1.29\pm0.06^{bC\#4}$	$0.74\pm0.06^{cE\#4}$	$1.37\pm0.10^{dB*4}$	$1.11\pm0.07^{eD\#4}$	$1.14\pm0.15^{eF\#4}$
EtOH 70 %	25	$1.62\pm0.12^{aA\circ2}$	$1.67\pm0.06^{aC\circ2}$	$1.65\pm0.09^{aE\circ2}$	$0.70\pm0.03^{bB\circ2}$	$0.90\pm0.08^{bcD\circ2}$	$1.05\pm0.26^{cF\circ2}$
	50	$1.99\pm0.07^{aA\bullet5}$	$2.06\pm0.06^{aB\bullet5}$	$2.37\pm0.05^{bD\bullet5}$	$1.69\pm0.43^{dA\bullet4}$	$1.09\pm0.05^{eC\bullet4}$	$0.89\pm0.09^{eE\circ5}$
EtOH 96 %	25	$1.03\pm0.03^{aA\blacktriangledown3}$	$0.78\pm0.07^{bC\blacktriangledown3}$	$0.76\pm0.06^{bE\blacktriangledown3}$	$1.19\pm0.08^{cB\blacktriangledown3}$	$1.29\pm0.08^{cD\blacktriangledown3}$	$1.29\pm0.11^{cF\blacktriangledown2}$
	50	$0.83\pm0.05^{aA\blacklozenge6}$	$0.79\pm0.08^{aC\blacktriangledown6}$	$1.04\pm0.10^{bE\blacklozenge6}$	$0.51\pm0.05^{cB\blacklozenge5}$	$0.96\pm0.06^{dD\blacklozenge5}$	$1.13\pm0.06^{eF\blacklozenge4}$

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Table 8.7 provide the TFC values after using UAE by varying the parameters. Extraction with 50% aqueous ethanol solution and 1N HCl addition presented the highest TFC after only 15 min at 25 °C (3.18 ± 0.30 mg CE/g dw). By combining the 96 % ethanol solution with HCl, after 30 min at 25 °C, the lowest concentration of 0.58 ± 0.05 mg CE/g dw was measured.

Table 8.7. *Total flavonoid content (mg CE/g dried eggplant peels) generated by UAE*

Solvent	T°C	Acetic acid			Hydrochloric acid		
		15 min	30 min	45 min	15 min	30 min	45 min
EtOH	25	$2.78\pm0.06^{Aa*1}$	$2.47\pm0.25^{abC*1}$	$2.10\pm0.06^{bE*1}$	$3.18\pm0.30^{cA*1}$	$2.76\pm0.31^{cC*1}$	$1.66\pm0.08^{dF*1}$
50 %	50	$1.86\pm0.16^{aA\#4}$	$1.70\pm0.10^{abD\#4}$	$1.60\pm0.12^{bG\#4}$	$0.70\pm0.05^{cB\#4}$	$0.77\pm0.08^{cE\#4}$	$1.33\pm0.12^{dH\#4}$
EtOH	25	$1.18\pm0.10^{abA○2}$	$1.30\pm0.07^{aD○2}$	$1.13\pm0.07^{bG○2}$	$1.40\pm0.06^{cB○2}$	$1.07\pm0.05^{dE○2}$	$2.44\pm0.11^{eH○2}$
70 %	50	$2.19\pm0.03^{aA●5}$	$1.84\pm0.11^{bD●4}$	$1.84\pm0.08^{bG●5}$	$1.06\pm0.08^{cB●5}$	$1.07\pm0.08^{cE○5}$	$1.15\pm0.09^{cH●5}$
EtOH	25	$0.44\pm0.09^{aA♥3}$	$0.54\pm0.02^{bC♥3}$	$0.52\pm0.04^{abE♥3}$	$1.21\pm0.11^{cB♥2}$	$0.58\pm0.05^{dC♥3}$	$0.61\pm0.05^{dE♥3}$
96 %	50	$0.92\pm0.07^{aA♦6}$	$1.05\pm0.05^{bC♦5}$	$1.03\pm0.11^{bF♦6}$	$1.31\pm0.11^{cB♥6}$	$1.22\pm0.08^{cD♦6}$	$1.20\pm0.10^{cG♦45}$

The influence of time was highlighted in lowercase letters on the line; the influence of the acid was highlighted in capital letters on the line; the influence of temperature was highlighted by symbols (*, #; ○, ●; ♥, ♦) on the column, the influence of ethanol concentration was highlighted by numbers on the column. Values that share a lowercase/uppercase letter on the line /symbol per column/number per column are not significantly different (*p>0.05*)

Table 8.8 shows the TFC recovered with MAE by varying the parameters. After 10 sec in the microwave, 70 % aqueous ethanol solution acidified with acetic acid extracted the highest TFC of 2.76 ± 0.25 mg CE/g dw. The lowest flavonoid concentrations were attained with 96 % ethanol, the same as they were in the UAE. Thus, the combination with HCl yielded the lowest TFC (0.77 ± 0.06 mg CE/g dw), after 15 sec.

Table 8.8. *Total flavonoid content (mg CE/g dried eggplant peels) of MAE extract*

Solvent	Acetic acid		Hydrochloric acid	
	10 sec	15 sec	10 sec	15 sec
EtOH 50 %	2.59 ± 0.19^{Aa1}	2.14 ± 0.20^{bC1}	1.85 ± 0.16^{cB1}	1.89 ± 0.07^{cD1}
EtOH 70 %	2.76 ± 0.25^{aA1}	2.47 ± 0.17^{bC2}	2.56 ± 0.24^{cA2}	2.49 ± 0.23^{cC2}
EtOH 96 %	1.37 ± 0.13^{aA2}	1.44 ± 0.14^{aC3}	1.36 ± 0.13^{bA3}	0.77 ± 0.06^{cD3}

The influence of time was highlighted in lowercase letters; the influence of the acid was highlighted in capital letters on the line; the influence of ethanol concentration was highlighted in the column. The values that share a lowercase/uppercase letter on a line or number on a column are not significantly different (*p>0.05*)

Table 8.9 illustrates the TFC acquired by varying the parameters in the EAE approach. Cellulase also released the highest flavonoid concentration (1.54 ± 0.14 mg CE/g dw) after 2 h. However, as in the case of anthocyanins, this method had a lower yield than others

Table 8.9. *Total flavonoid content (mg CE/g dried eggplant peels) released by EAE*

Time / Enzyme	1 h	2 h	3 h
Cellulase	1.33 ± 0.12^{Aa}	1.54 ± 0.14^{aA}	1.42 ± 0.14^{abA}
Pectolitic enzymes	1.11 ± 0.05^{aB}	1.05 ± 0.06^{aB}	1.05 ± 0.10^{aB}

The influence of time was highlighted in lowercase letters; the influence of the enzyme was highlighted in capital letters on the column. The values that share a lowercase/uppercase letter per column are not significantly different (*p>0.05*)

8.4.3. Polyphenols content of the extracts provided by conventional and modern methods

Table 8.10 highlights the TPC resulting after the extractions performed. The highest TPC achieved with the SLE was 15.01 ± 1.85 mg GAE/g dw with 70 % aqueous ethanol solution and HCl after 60 min at 50 °C. The lowest TPC values were generated with 96 % ethanol solution and acetic acid.

Table 8.10. *Total polyphenol content (mg GAE/g dried eggplant peels) extracted by SLE*

Solvent	T°C	Acetic acid			Hydrochloric acid		
		30 min	60 min	120 min	30 min	60 min	120 min
EtOH	25	12.01±0.39^{Aa*1}	13.10±0.94^{aB*1}	13.86±0.55^{aC*1}	13.78±0.36^{bA*1}	13.66±0.55^{bB*1}	14.60±1.16^{bC*1}
50 %	50	12.68±0.28^{abA*4}	12.52±0.24^{aC*4}	12.94±0.27$^{bE\#4}$	13.16±0.19$^{cB\#4}$	13.15±0.38^{cD*4}	14.23±0.32^{dF*4}
EtOH	25	11.01±0.14$^{aA○2}$	11.29±0.40$^{abC○2}$	11.74±0.28$^{bE○2}$	13.54±0.37$^{cdB○1}$	13.14±0.53$^{cD○1}$	14.04±0.61$^{dF○12}$
70 %	50	12.64±1.01$^{aA●4}$	12.50±1.13$^{aC●4}$	12.56±1.05$^{aE○4}$	14.69±1.29$^{bB○5}$	15.01±1.85$^{bD●5}$	14.68±1.43$^{bF○4}$
EtOH	25	5.23±0.48$^{aA♥3}$	4.82±0.24$^{abC♥3}$	4.50±0.16$^{bE♥3}$	12.67±0.81$^{cB♥2}$	11.68±1.29$^{cD♥2}$	12.89±0.56$^{cF♥2}$
96 %	50	6.69±0.40$^{aA♦5}$	7.24±0.16$^{abC♦5}$	7.67±0.59$^{bE♦5}$	13.88±1.14$^{cB♥45}$	14.09±0.44$^{dD♦45}$	14.82±0.83$^{eF♦4}$

The influence of time was highlighted in lowercase letters on the line; the influence of the acid was highlighted in capital letters on the line; the influence of temperature was highlighted by symbols (*, #; ○, ●; ♥, ♦) on the column, the influence of ethanol concentration was highlighted by numbers on the column. Values that share a lowercase/uppercase letter on the line /symbol per column/number per column are not significantly different (*p>0.05*)

The highest TPC resulting after the UAE (**table 8.11**) was acquired with 50 % aqueous ethanol solution and CH_3COOH after 30 min of treatment at 50 °C (19.71 ± 0.82 mg GAE/g dw). As in the case of anthocyanins, the lowest TPC yield was produced with 96 % ethanol acidified with CH_3COOH, having values 76 % lower than those achieved with 50 % aqueous ethanol solution and glacial CH_3COOH.

Table 8.11. *Total polyphenol content (mg GAE/g dried eggplant peels) provided by UAE*

Solvent	T°C	Acetic acid			Hydrochloric acid		
		15 min	30 min	45 min	15 min	30 min	45 min
EtOH	25	13.38±0.58^{Aa*1}	13.18±0.62^{aB*1}	13.52±0.61^{aC*1}	13.29±0.65^{bA*1}	13.34±0.65^{bB*1}	13.76±0.72^{bC*12}
50 %	50	13.17±0.49^{aA*4}	19.71±0.82$^{bC\#4}$	12.52±0.28$^{aE\#4}$	14.29±0.53$^{cB\#4}$	13.62±0.85^{cD*4}	14.53±0.66^{cF*4}
EtOH	25	11.73±0.34$^{aA○2}$	11.42±0.53$^{aC○2}$	11.97±0.48$^{aE○2}$	13.94±0.31$^{bB○1}$	13.69±0.67$^{bD○1}$	14.29±0.86$^{bF○1}$
70 %	50	12.41±0.75$^{aA○4}$	12.47±1.19$^{aC○5}$	12.34±0.61$^{aE○4}$	13.79±0.86$^{bB○4}$	14.19±1.05$^{bD○4}$	14.64±0.97$^{bF○4}$
EtOH	25	4.79±0.39$^{aA♥3}$	5.00±0.42$^{aC♥3}$	4.77±0.41$^{aE♥3}$	13.93±1.18$^{bB♥1}$	14.01±1.87$^{bD♥1}$	13.05±0.72$^{bF♥2}$
96 %	50	5.97±0.46$^{abA♦5}$	6.33± 0.72$^{aC♦6}$	5.42±0.37$^{bE♦5}$	12.60±0.84$^{cB♦5}$	12.76±0.99$^{cD♥4}$	13.25±0.67$^{cF♥5}$

The influence of time was highlighted in lowercase letters on the line; the influence of the acid was highlighted in capital letters on the line; the influence of temperature was highlighted by symbols (*, #; ○, ●; ♥, ♦) on the column, the influence of ethanol concentration was highlighted by numbers on the column. Values that share a lowercase/uppercase letter on the line /symbol per column/number per column are not significantly different (*p>0.05*)

In the MAE's case (**table 8.12**), 96 % ethanol acidified with CH_3COOH had the lowest yield. In contrast, when CH_3COOH was replaced with HCl, the highest TPC of 14.44 ± 0.49 mg GAE/g dw was achieved after 15 sec of microwave treatment.

Table 8.12. *Total polyphenol content (mg GAE/g dried eggplant peels) acquired with MAE*

Solvent	Acetic acid		Hydrochloric acid	
	10 sec	15 sec	10 sec	15 sec
EtOH 50 %	12.8±0.93^{Aa1}	11.88±0.94^{aB1}	13.38±0.94^{bA1}	14.06±0.69^{bC1}
EtOH 70 %	12.06±1.45^{aA1}	11.54±0.84^{aB1}	13.35±0.82^{bA1}	14.08±0.76^{bC1}
EtOH 96 %	6.01±0.18^{aA2}	6.47±0.64^{aC2}	13.55±0.56^{bB1}	14.44±0.49^{cD1}

The influence of time was highlighted in lowercase letters on the line; the influence of the acid was highlighted in capital letters on the line; the influence of ethanol concentration was highlighted in

numbers on the column. The values that share a lowercase/uppercase letter on the line or number on the column are not significantly different (*p>0.05*)

Cellulase extraction yielded the highest amount of polyphenols of the two enzymes (**table 8.13**). Thus, cellulase released 10.68 ± 0.27 mg GAE/g dw after 1 h with no significant differences observed over time (*p>0.05*). However, the mixture of pectolytic enzymes reached half of the TPC recovered with cellulase, the highest concentration being 5.50 ± 0.28 mg GAE/g dw after 2 h of hydrolysis.

Table 8.13. *Total polyphenol content (mg GAE/g dried eggplant peels) released by EAE*

Enzyme \ Time	1 h	2 h	3 h
Cellulase	10.68 ± 0.27^{Aa}	10.85 ± 0.37^{aA}	10.44 ± 0.72^{aA}
Pectolitic enzymes	5.43 ± 0.40^{aB}	5.50 ± 0.28^{aB}	4.63 ± 0.48^{bB}

The influence of time was highlighted in lowercase letters; the influence of the enzyme was highlighted in capital letters on the column. The values that share a lowercase/uppercase letter per column are not significantly different (*p>0.05*)

8.4.4. Antioxidant activity of the extracts reached by conventional and modern methods

The results of the antioxidant activity of eggplant peel extracts provided with the SLE are shown in **table 8.14**. Results are expressed as µM TE/g dried eggplant peels. The SLE supernatants exhibited high antioxidant activity, regardless of the combination of parameters used (**table 8.14**). The antioxidant activity of 70% aqueous ethanol solution was the highest when combined with CH_3COOH at 50 °C after 120 min (42.84 ± 2.69 µM TE/g dw). This value is equivalent to a percentage of DPPH inhibition of 93.29 ± 0.15 %. However, the lowest antioxidant activity was highlighted by extraction with 70 % aqueous ethanol solution and HCl at 25 °C after 30 min (25.34 ± 1.88 µM TE/g dw). This value is equivalent to a percentage of DPPH inhibition of 74.97 ± 0.43 %.

Table 8.14. *Antioxidant activity (µM TE/g dried eggplant peels) of extracts provided by SLE*

Solvent	T°C	Acetic acid			Hydrochloric acid		
		30 min	60 min	120 min	30 min	60 min	120 min
EtOH	**25**	$35.46\pm2.42^{Aa*1}$	$35.34\pm1.26^{aC*1}$	$35.08\pm1.62^{aE*1}$	$32.31\pm0.98^{bB*1}$	$32.12\pm0.91^{bD*1}$	$32.50\pm0.43^{bF*1}$
50 %	**50**	$32.69\pm0.77^{aA\#3}$	$30.25\pm0.90^{bB\#2}$	$32.69\pm1.45^{aC\#2}$	$30.26\pm2.50^{cA*2}$	$31.12\pm2.12^{cB*3}$	$30.37\pm2.27^{cC\#3}$
EtOH	**25**	$30.51\pm1.02^{aA\circ2}$	$33.83\pm1.87^{bC\circ1}$	$34.73\pm1.44^{bE\circ1}$	$25.34\pm1.90^{cB\circ2}$	$28.80\pm2.45^{dD\circ2}$	$27.67\pm1.35^{cdF\circ2}$
70 %	**50**	$42.54\pm2.95^{aA\bullet4}$	$42.39\pm2.51^{aB\bullet3}$	$42.84\pm2.69^{aC\bullet3}$	$39.36\pm2.90^{bA\bullet3}$	$39.85\pm3.20^{bB\bullet4}$	$38.17\pm3.45^{bD\bullet4}$
EtOH	**25**	$36.60\pm3.36^{aA\blacktriangledown1}$	$34.34\pm0.78^{aC\blacktriangledown1}$	$33.70\pm0.57^{aE\blacktriangledown1}$	$31.89\pm3.01^{bB\blacktriangledown1}$	$30.96\pm2.80^{bD\blacktriangledown12}$	$29.75\pm2.05^{bF\blacktriangledown2}$
96 %	**50**	$38.76\pm2.51^{aA\blacktriangledown5}$	$38.44\pm2.53^{aB\blacktriangledown4}$	$38.01\pm2.50^{aC\blacktriangledown4}$	$34.92\pm3.45^{bA\blacktriangledown4}$	$38.97\pm1.00^{75Bc\blacktriangledown4}$	$32.61\pm2.55^{bD\blacktriangledown3}$

The influence of time was highlighted in lowercase letters on the line; the influence of the acid was highlighted in capital letters on the line; the influence of temperature was highlighted by symbols (*, #; ◌, ●; ♥, ♦) on the column, the influence of ethanol concentration was highlighted by numbers on the column. Values that share a lowercase/uppercase letter on the line /symbol per column/number per column are not significantly different (*p>0.05*)

The UAE allowed reaching antioxidant activities between 20.64 ± 1.93 and 37.90 ± 0.64 µM TE/g dw, the DPPH inhibition percentage being between 60.62 ± 1.45 % and 91.83 ± 0.57 % (**table 8.15**).

Table 8.15. *Antioxidant activity (µM TE/g dried eggplant peels) of extracts collected after the UAE*

Solvent	T°C	Acetic acid			Hydrochloric acid		
		15 min	30 min	45 min	15 min	30 min	45 min
EtOH	25	$37.90\pm0.64^{Aa*1}$	$37.81\pm0.59^{aB*1}$	$37.63\pm0.26^{aC*1}$	$37.06\pm0.66^{bA*1}$	$37.25\pm0.82^{bB*1}$	$35.30\pm0.25^{cD*1}$
50 %	50	$35.09\pm1.34^{aA\#4}$	$34.99\pm0.54^{aB\#4}$	$34.93\pm0.88^{aD\#4}$	$34.39\pm0.17^{bA\#4}$	$31.00\pm1.22^{bC\#4}$	$29.75\pm1.13^{cE\#4}$
EtOH	25	$37.17\pm0.43^{aA○2}$	$37.33\pm0.22^{aD○1}$	$36.62\pm0.22^{bG○2}$	$34.31\pm0.45^{cB○2}$	$32.74\pm0.40^{dE○2}$	$32.59\pm0.76^{dH○2}$
70 %	50	$36.25\pm0.61^{aA○5}$	$36.20\pm0.54^{aC●5}$	$36.25\pm0.61^{aE○4}$	$32.10\pm2.00^{bB○4}$	$33.54\pm0.65^{cD●5}$	$32.10\pm2.00^{cF○5}$
EtOH	25	$30.15\pm0.27^{aA♥3}$	$30.34\pm0.54^{aC♥2}$	$30.26\pm0.90^{aE♥3}$	$22.46\pm1.00^{bB♥3}$	$20.64\pm1.95^{bD♥3}$	$21.69\pm1.00^{bF♥3}$
96 %	50	$35.50\pm0.77^{aA♦4}$	$35.35\pm1.02^{aC♦45}$	$35.24\pm1.26^{aE♦4}$	$32.64\pm2.45^{bB♦4}$	$32.86\pm1.76^{bD♦45}$	$32.65\pm1.35^{bF♦5}$

The influence of time was highlighted in lowercase letters on the line; the influence of the acid was highlighted in capital letters on the line; the influence of temperature was highlighted by symbols (*, #; ○, ●; ♥, ♦) on the column, the influence of ethanol concentration was highlighted by numbers on the column. Values that share a lowercase/uppercase letter on the line /symbol per column/number per column are not significantly different (*p>0.05*)

The antioxidant activities of the extracts produced by the MAE technique are listed in **table 8.16.** Overall, the MAE with ethanol 50 % had the best yield, showing antioxidant activities with values ranging from 44.14 ± 0.47 µM TE/g dw to 45.18 ± 0.38 µM TE/g dw, not being significantly different (*p>0.05*) regardless of the time. These values are equivalent to values ranging from 89.81 ± 1.34 to 91.20 ± 0.56 % DPPH inhibition.

Table 8.16. *Antioxidant activity (µM TE/g dried eggplant peels) of extracts recovered by MAE*

Solvent	Acetic acid		Hydrochloric acid	
	10 sec	15 sec	10 sec	15 sec
EtOH 50 %	45.18 ± 0.32^{Aa1}	44.99 ± 0.39^{aB1}	45.08 ± 0.45^{bA1}	44.72 ± 0.69^{bBC1}
EtOH 70 %	36.65 ± 0.57^{aA2}	36.71 ± 0.38^{aB2}	36.12 ± 0.38^{bA2}	35.89 ± 0.42^{bC2}
EtOH 96 %	34.63 ± 0.41^{aA3}	32.67 ± 2.28^{aC3}	33.37 ± 0.52^{bB3}	33.83 ± 0.71^{bC3}

The influence of time was highlighted in lowercase letters on the line; the influence of the acid was highlighted in capital letters on the line; the influence of ethanol concentration was highlighted in numbers on the column. The values that share a lowercase/uppercase letter on the line or number on the column are not significantly different (*p>0.05*)

Table 8.17. *Antioxidant activity (µM TE/g dried eggplant peels) of extracts produced by EAE*

Enzyme \ Time	1 h	2 h	3 h
Cellulase	55.60 ± 3.36^{Aa}	55.12 ± 4.12^{aA}	53.77 ± 5.67^{aA}
Pectolitic enzymes	27.36 ± 0.77^{aB}	26.79 ± 2.09^{aB}	24.05 ± 1.32^{bB}

The influence of time was highlighted in lowercase letters; the influence of the enzyme was highlighted in capital letters on the column. The values that share a lowercase/uppercase letter per column are not significantly different (*p>0.05*)

Table 8.17 presents the antioxidant activities of the extracts acquired with the EAE. When compared to the pectolytic enzymes mixture, cellulase produced the highest yield in terms of antioxidant activity. One h of extraction resulted in 55.60 ± 3.36 µM TE/g dw, corresponding to 88.32 ± 0.15 % of DPPH inhibition. The antioxidant activity of the pectolytic enzymes extracts was considerably lower than that of cellulase extracts (*p<0.05*), also decreasing over time.

8.4.5. Chromatographic profile of eggplant peels bioactive compounds

Based on a comparison with the available standards, compounds' retention time, and already published data, in our study the anthocyanins from the eggplant peels were identified at a wavelength of 520 nm. The extracts from each method that delivered the best TAC values were chosen for HPLC after reviewing the results from the variation of all parameters, except

for the EAE extracts. Thus, the extract collected after stirring a 50 % aqueous ethanol solution with CH3COOH for 30 min at 25 °C was chosen from the SLE. The extract obtained from the UAE was chosen after mixing 50 % aqueous ethanol solution and acetic acid for 30 min at 25 °C. The extract employing 70 % aqueous ethanol and acetic acid, wave-treated for 10 seconds, was chosen from MAE.

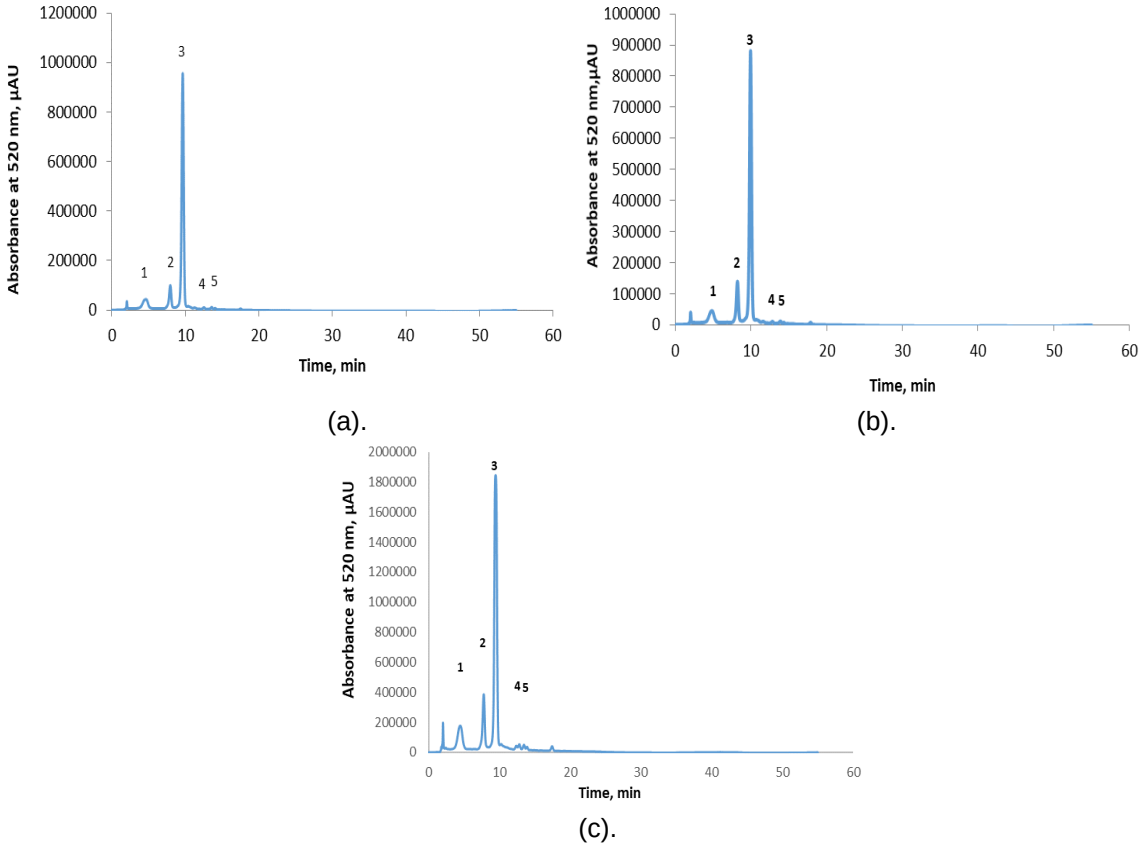


Figure 8.1. Chromatographic profile of eggplant peel extracts collected after SLE (A), UAE (B), and MAE (C): Peak 1 – delphinidin 3-*O*-rutinoside-5-glucoside; Peak 2 – delphinidin 3-*O*-glucoside; Peak 3 – delphinidin 3-*O*-rutinoside; Peak 4 – cyanidin 3-*O*-rutinoside, and Peak 5 - petunidin-3-*O*-rutinoside;

Figure 8.1 shows the chromatographic profile of the selected extracts. The HPLC analysis revealed the presence of delphinidin, cyanidin, and petunidin derivatives. Thus, for all three analyzed extracts the following compounds were identified: delphinidin 3-*O*-rutinoside-5-glucoside, delphinidin 3-*O*-glucoside, delphinidin 3-*O*-rutinoside, cyanidin 3-*O*-rutinoside, and petunidin 3-*O*-rutinoside (**figure 8.1**). The predominant component in all three extracts was delphinidin 3-*O*-rutinoside.

Following spectrophotometric and chromatographic examinations of extracts obtained using various procedures and parameters, the conventional method was chosen for the subsequent analyses.

8.4.6. In vitro enzymes inhibition capacity of eggplant peels extracts

In our study, the *in vitro* inhibition capacity of the eggplant peel compounds was assessed towards the enzymes associated with metabolic syndrome, namely LOX, lipase, and α-amylase. Thus, three extract concentrations were tested. The inhibitory percentages are presented in **figure 8.2**.

Average inhibition levels for all three enzymes were found in the eggplant peel extract. However, as expected, the inhibition of enzymes increased as the bioactive concentration rose (**figure 8.2**). In the case of LOX and lipase inhibition, nevertheless, there was no significant

difference between the 0.1 mg/mL and 0.5 mg/mL extracts (*p>0.05*). Furthermore, there was no significant difference in the inhibitory effects of the 0.5 mg/mL and 1 mg/mL eggplant peel extracts on amylase (*p>0.05*).

The eggplant peel compounds showed a significantly higher inhibition effect on α-amylase compared to the other enzymes (*p<0.05*). The lowest inhibitory effect was observed on lipase with values of approximately 20 % for all concentrations tested (**figure 8.2**).

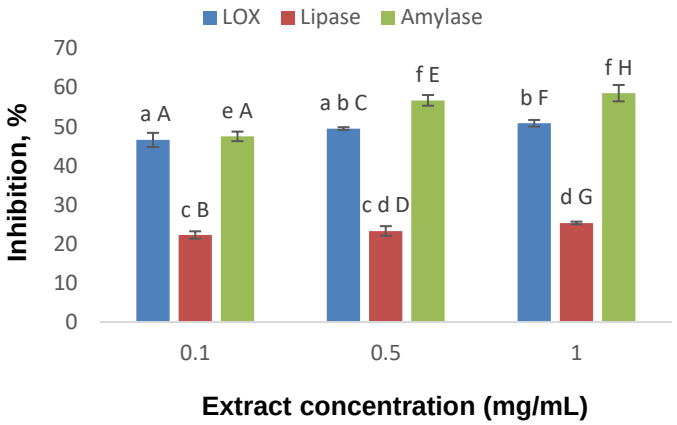
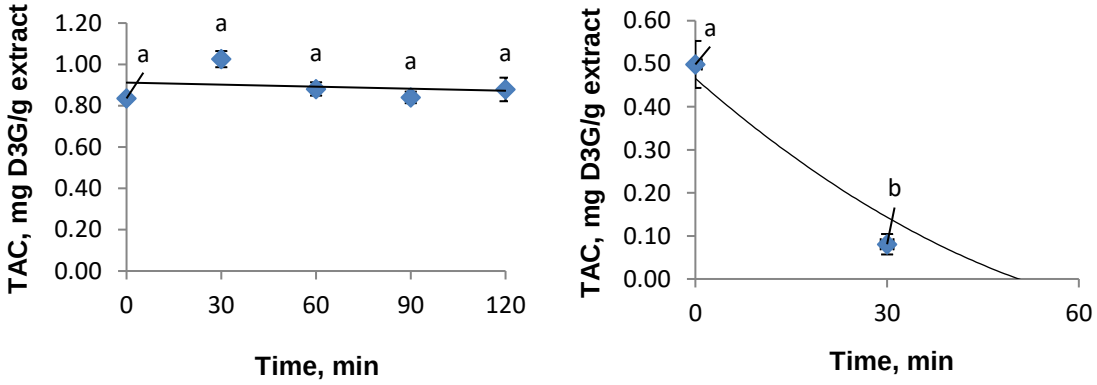


Figure 8.2. The enzyme inhibition percentage of 0.1 – 1 mg/mL eggplant peel extract. Lowercase letters highlight the inhibition differences between the concentrations. Uppercase letters highlight the inhibition differences between the enzymes. The values that share a letter are not significantly different (*p>0.05*) according to the ANOVA test

8.4.7. In vitro digestibility of anthocyanins from eggplant peels

In our study, the extract was subjected to *in vitro* simulated gastric and intestinal juices to understand how the anthocyanins from eggplant peel behave in gastrointestinal conditions. The results are illustrated in **figure 8.3**.



(a). (b).

Figure 8.3. Anthocyanins behavior under *in vitro* simulated gastric (a) and intestinal (b) digestion. The variation of TAC in time was highlighted in lowercase letters. The values that share a letter are not significantly different (*p>0.05*) according to the ANOVA test

Since the anthocyanins are pH-sensitive compounds, stable at low pH values, in the simulated gastric conditions, where the set pH was 3.0, the TACs from the eggplant peel extract were stable. **Figure 8.3a** reveals that after 120 min of digestion, no significant differences were found in the anthocyanin content (*p>0.05*). If in the gastric-simulated system the TACs had great stability, in the intestinal-simulated system, where the pH increased to 7.0,

a significant decrease happened ($p<0.05$). From **figure 8.3b** can be noted that no anthocyanin was recovered after 60 min of digestion.

8.4.8. Kinetics of thermal degradation of anthocyanins from eggplant peels

In our study, the eggplant peel extract was heat-treated at different temperature-time combinations to evaluate anthocyanins' behavior. Pasteurization, sterilization, and even baking-specific conditions were taken into consideration. A progressive decrease in TAC was observed over the whole temperature range investigated (**figure 8.4**).

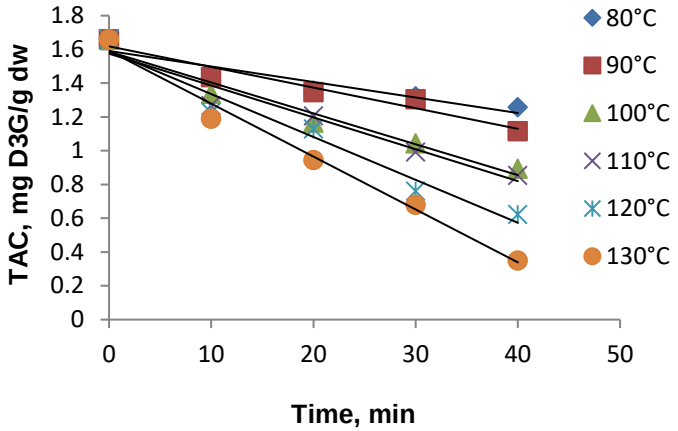


Figure 8.4. Thermal degradation of anthocyanins in eggplant peel extract-treated at different temperatures

Figure 8.4 points out an anthocyanin degradation increase with time and temperature. Thus, at 80 ºC, 14 % of TAC was degraded after the first 10 min of heat treatment, reaching a thermal degradation of 25 % after 40 min. At 130 °C, on the other hand, an acceleration of the anthocyanins' degradation was observed. In the first 10 min of heat treatment, 28 % of TAC was degraded, while a degradation of 77 % appeared after 40 min.

Kinetic parameters provide information on the impact of heat treatment on food quality and characteristics. This information is valuable for the food production process, helping to optimize the processes. Our study's target kinetic parameters were the degradation rate constant (k), the half-life (t1/2), and the activation energy (Ea). Thus, the first-order kinetic model was used to calculate the kinetic parameters for the degradation of eggplant peel anthocyanins. **Table 8.19** presents the values o these parameters.

Table 8.19. *Kinetic parameters of anthocyanins from the eggplant peel extract treated at different temperatures*

Temperature, °C	$k \cdot 10^{-2}$ (min^{-1})	$t_{1/2}$ (min)	Ea (kJ/mol)
80	0.74 ± 0.001	93.66 ± 1.05	34.63 ± 3.59
90	0.79 ± 0.004	87.74 ± 0.78	
100	1.53 ± 0.002	45.30 ± 0.44	
110	1.59 ± 0.001	43.59 ± 4.84	
120	2.42 ± 0.003	28.64 ± 0.33	
130	2.91 ± 0.01	23.81 ± 0.71	

Table 8.19 suggests that the k increased with the temperature. Heat processing caused anthocyanins to degrade due to oxidation, covalent bonding, or oxidation processes. Anthocyanins or their conjugated sugars probably decomposed into tiny molecules like aldehydes and benzoic acid derivatives when heated.

In our investigation, the half-life values were reduced with increasing temperature, and the activation energy was 39.87 kJ/mol (**table 8.19)**, indicating that anthocyanins were more temperature-dependent (f**igure 8.5**).

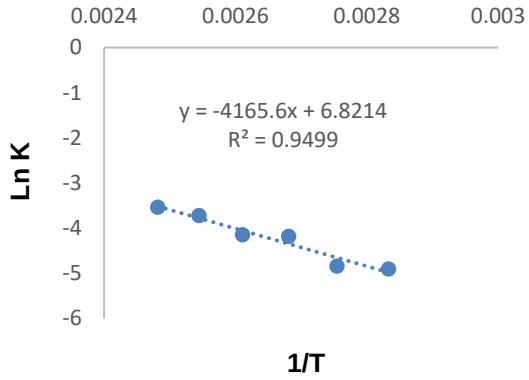


Figure 8.5. Arrhenius graphic for the temperature dependence of k vs. temperature

The thermodynamic parameters of interest in our study were the activation enthalpy (ΔH), the Gibbs free energy of inactivation (ΔG), and the activation entropy (ΔS). The calculated values of these parameters are detailed in **table 8.20**.

Table 8.20. *Thermodynamic parameters of anthocyanins from the eggplant peel extract-treated at different temperatures*

Temperature, °C	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (J·mol^{-1} × K^{-1})
80	31.69 ± 1.03	113.36 ± 9.56	-231.34 ± 11.05
90	31.61 ± 0.40	116.46 ± 11.61	-233.74 ± 18.80
100	31.53 ± 1.20	117.70 ± 10.98	-231.08 ± 15.21
110	31.44 ± 1.10	120.82 ± 9.03	-233.35 ± 11.55
120	31.36 ± 3.01	122.68 ± 9.43	-232.37 ± 15.13
130	31.28 ± 0.10	125.27 ± 11.26	-233.23 ± 17.01

The calculated ΔH values (**table 8.20**) were similar to increasing the temperature and positive. The ΔG values were also positive for all temperatures, while the ΔS values were negative. All these values of the thermodynamic parameters suggest that the molecules were more organized in the transition state than at the beginning of the reaction, the thermal degradation process being irreversible. In addition, the bioactive degradation was an endothermic and nonspontaneous reaction.

8.5. Partial conclusions

The goal of this study was to extract biologically active components from eggplant peels and determine the best approach and parameters for achieving the highest anthocyanin content achievable. Extractions were carried out using four different methods: conventional, ultrasound-assisted, microwave-assisted, and enzyme-assisted. The acid, time, temperature, and ethanol concentrations were also varied. The results allowed the elaboration of the following partial conclusions:

✓ The highest total anthocyanin content for the conventional method was reached with 96 % ethanol and HCl (1.13 ± 0.10 mg D3G/g dried eggplant peels after 60 min at a temperature of 50 °C).

✓ The ultrasound-assisted technique also led to the highest total anthocyanin content using 96 % ethanol and HCl (1.04 ± 0.10 mg D3G/g dried eggplant peels after 30 min at a temperature of 25 °C).

✓ After the microwave-assisted extraction, the highest total anthocyanin content was achieved using 70 % ethanol and HCl (0.95 ± 0.09 mg D3G/g dried eggplant peels after 10 sec).

✓ However, due to the HCl's toxicity, glacial acetic acid is preferable to it.

✓ The enzyme-assisted method reached 0.29 ± 0.02 mg D3G/g dried eggplant peels as the highest total anthocyanin content using cellulase for 1h of treatment.

✓ The lowest total anthocyanin contents were acquired with 96 % ethanol and CH_3COOH for conventional, ultrasound-assisted, and microwave-assisted methods, while for the enzyme-assisted method, no anthocyanins were detected after using the mixture of pectolytic enzymes.

✓ When comparing the ultrasound-assisted to the traditional method, it can be concluded that the latter allowed us to generate the maximum quantities of bioactive chemicals after 30 min at 25 ºC, using CH_3COOH mixed with 50 % ethanol. Thus, this extract was further used in our experiments.

✓ Each combination of parameters released biologically active compounds differently. However, independent of the time and temperature used, the lowest results were achieved when employing 96 % ethanol and CH_3COOH for all procedures.

✓ Following chromatographic analysis, five anthocyanins were identified in all extracts, the major one being delphinidin 3-*O*-rutinoside.

✓ The behavior of the eggplant peel anthocyanins during gastrointestinal digestion was pursued using a simulated *in vitro* method. Thus, the anthocyanins were stable during gastric digestion, while after 60 min of intestinal digestion, they completely degraded.

✓ Following the heat treatment of the eggplant peel extract in the temperature range of 25 - 130 °C, a gradual degradation of the anthocyanins was observed as the temperature and time increased. Thus, after 40 min of heat treatment at 130 °C, approximately 77 % of the anthocyanins were degraded. The degradation kinetics of anthocyanins were calculated using the first-order kinetic model. Thus, the degradation rate, half-life, and activation energy allow an accurate prediction of anthocyanins' thermal degradation. Among the thermodynamic parameters, they demonstrated that the temperature increase accelerated the degradation effect, allowing us to conclude the higher temperature dependence of anthocyanins.

✓ The addition of eggplant peel anthocyanins in food products should be followed by low temperatures and time to maintain their bioavailability if a thermal treatment is necessary.

Based on these results, the optimal parameters were established to formulate a food ingredient that will allow much more efficient use of anthocyanins in eggplant peel as natural pigments and, thus, increase their bioavailability.

CHAPTER 9. HYDROLYSIS OF WHEY PROTEINS

9.2. Objectives of the study

This study aimed to achieve biologically active peptides from whey proteins by enzymatic hydrolysis. The main objectives of this study were to:

- evaluate several hydrolysis enzymes in order to produce protein hydrolysates with higher degrees of hydrolysis;
- characterize the protein hydrolysates regarding the hydrolysis degree and antioxidant activity;
- test the prebiotic effect of the protein hydrolysates.

9.4. Results and discussion

9.4.1. Characterization of the protein hydrolysates

Table 9.1 present the DH and antioxidant activity values of the protein hydrolysates.

Table 9.1. *Protein hydrolysates characteristics*

Protein hydrolysate	Enzyme	Substrate	E/S ratio	Hydrolysis time (h)	DH (%)	DPPH scavenging activity (μM TE/g protein)
Hp(T)	Thermolysin	WPI	1:1000	6	18.73	4.44 ± 0.47^{b}
Hp(β-LgT)	Thermolysin	β-Lg	1:50	2	20.68	41.77 ± 4.08^{a}
Hp(CT)	Chymotrypsin	WPI	1:50	2	9.32	5.28 ± 0.34^{b}
Hp(LFCT)	Chymotrypsin	LF	1:50	2 ½	14.41	1.74 ± 0.12^{b}

The values that share a letter per column are not significantly different ($p>0.05$) according to the ANOVA test; E/S – enzyme/substrate;

The maximum DH was achieved for Hp that employed β-Lg as a substrate and thermolysin, as reported in **table 9.1**. The lowest degree of hydrolysis was observed for the Hp that used WPI as substrate but chymotrypsin for breaking polypeptide chains.

The highest antioxidant activities were exerted by the Hp provided by thermolysin through the cleavage of β-Lg proteins, as expected. The other three hydrolysates presented significantly lower antioxidant activities ($p<0.05$) compared to Hp(β-LgT) but without significant differences between them ($p>0.05$). However, despite the degrees of hydrolysis, the values of antioxidant activities for all hydrolysates were generally low.

For WPI hydrolysis using protease, several enzyme/substrate ratios were utilized to reach the greatest degree of hydrolysis. The DH acquired is illustrated in **figure 9.4**.

Figure 9.4a shows that for the three enzyme/substrate ratios, the DH increased with time but decreased with the increasing mass of the substrate. In the first 60 min of hydrolysis, DH increased in all three hydrolyses, and then the increase took place slowly (in terms of the last two ratios). After 2 h of hydrolysis, the maximum DH of 47.51 ± 1.00 % was recorded with the 1:10 ratio. Although the hydrolysis process continued for another hour, the DH remained the same, but the hydrolysates became very bitter due to the release of small peptides and amino acids. The 1:50 ratio displayed a much lower and slower increase in DH than previously reported. Thus, after 4 h of hydrolysis, the DH was 21.91 ± 2.51 %. The lowest degrees of hydrolysis was observed at a ratio of 1:100. In this case, DH increased more slowly, reaching 18.41 ± 1.03 % after 4 h. However, after 21 h of hydrolysis (**figure 9.4 b**), the DH increased to 27.14 ± 0.58 %.

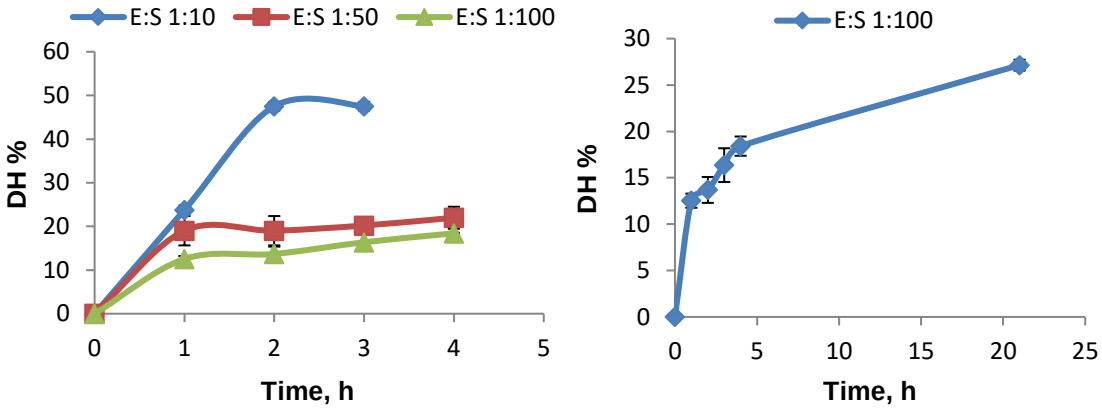


Figure 9.4. Degrees of hydrolysis obtained after enzymatic hydrolysis of IPZ with protease in different enzyme ratios: substrate

The antioxidant activity of the hydrolysate with protease after 21 h of hydrolysis was determined using both DPPH and ABTS as free radicals. When evaluating the antioxidant activity on the DPPH, an antioxidant effect of 3.97 ± 0.35 µM TE/g lyophilized hydrolysate was observed. The antioxidant activity against the ABTS radical exhibited a much higher value than in the case of DPPH. Thus, the hydrolysate showed antioxidant activity of 17.38 ± 0.02 µM TE/g lyophilized hydrolysate.

9.4.2. The prebiotic effect of the protein hydrolysates

In our study, the Hp(P) prebiotic effect, at a concentration of 2 % (w/v), on *L. paracasei* probiotic strain, during *in vitro* fermentation (48 h, at 37 °C) and storage (after 7, 14, and 21 days at 4 °C) was analyzed (**table 9.2**).

Table 9.2. *The prebiotic effect of hydrolysates (2 %) upon L. paracasei*

Sample	The prebiotic effect (log CFU/mL)			
	*	**	***	****
Control	1	1	1	1
Hp(P)	1.36 ± 0.01	1.31 ± 0.05	1.27 ± 0.07	1.27 ± 0.03

* Fermentation (48 h, 37 °C); ** 7th day of storage; ***14th day of storage; ***21th day of storage

The results proved that the commercial probiotic strain was able to grow in the MRS media supplemented with 2 % (w/v) hydrolysates. After 48 h of lactic fermentation, the prebiotic effect was shown. The prebiotic effect diminished slightly during the storage period, according to the data from **table 9.2**. Thus, after 21 days of storage, the prebiotic effect values of 1.270 ± 0.03 were recorded (**table 9.2**).

9.5. Partial conclusions

Enzymatic hydrolysis improves the functional properties and biological activity of proteins, which can still be used as ingredients for various foods. Various enzymes were examined in order to get protein hydrolysates with a high degree of hydrolysis from various substrates in this study. Thus, whey proteins, β-lactoglobulin, and lactoferrin were hydrolyzed with thermolysin, chymotrypsin, and protease. The results allowed the elaboration of the following partial conclusions:

- Various enzyme/substrate ratios were tested, and the resulting hydrolysates presented the degree of hydrolysis with values between 9.32 % and 47.51 %.

- Despite the high degree of hydrolysis, the evaluated antioxidant activities were low.
- With the increase in the substrate-enzyme ratio, protein hydrolysis with protease took place more slowly. At the same time, the degree of hydrolysis increased with the time of treatment.
- The antioxidant activity of the protein hydrolysate with protease increased with time and enzyme-to-substrate ratio.
- The protein hydrolysate achieved with protease after 21 h of hydrolysis presented a prebiotic effect on the *L. paracasei* strain.

CHAPTER 10. STUDIES OF BINDING AND QUENCHING OF FLUORESCENCE OF WHEY PROTEINS AND PEPTIDES BY THE ADDITION OF BIOLOGICALLY ACTIVE COMPOUNDS FROM EGGPLANT PEEL

10.2. Objectives of the study

The major objective of the study was to assess the binding mechanism between the whey proteins and peptides and the bioactive compounds from the eggplant peel. Thus, the objectives of this study were to:

- investigate the interaction between β-Lg, LF, WPI, and peptides with eggplant peels anthocyanins in the temperature range of 25–100 °C, considering the heating importance in the food industry;
- calculate the binding and thermodynamic parameters and approximate the main forces involved in the process.

10.4. Results and discussion

10.4.1. Quenching of β-Lg fluorescence

Figure 10.1 illustrates the changes in the emission spectra of the protein as the concentration of extract increased at different temperatures.

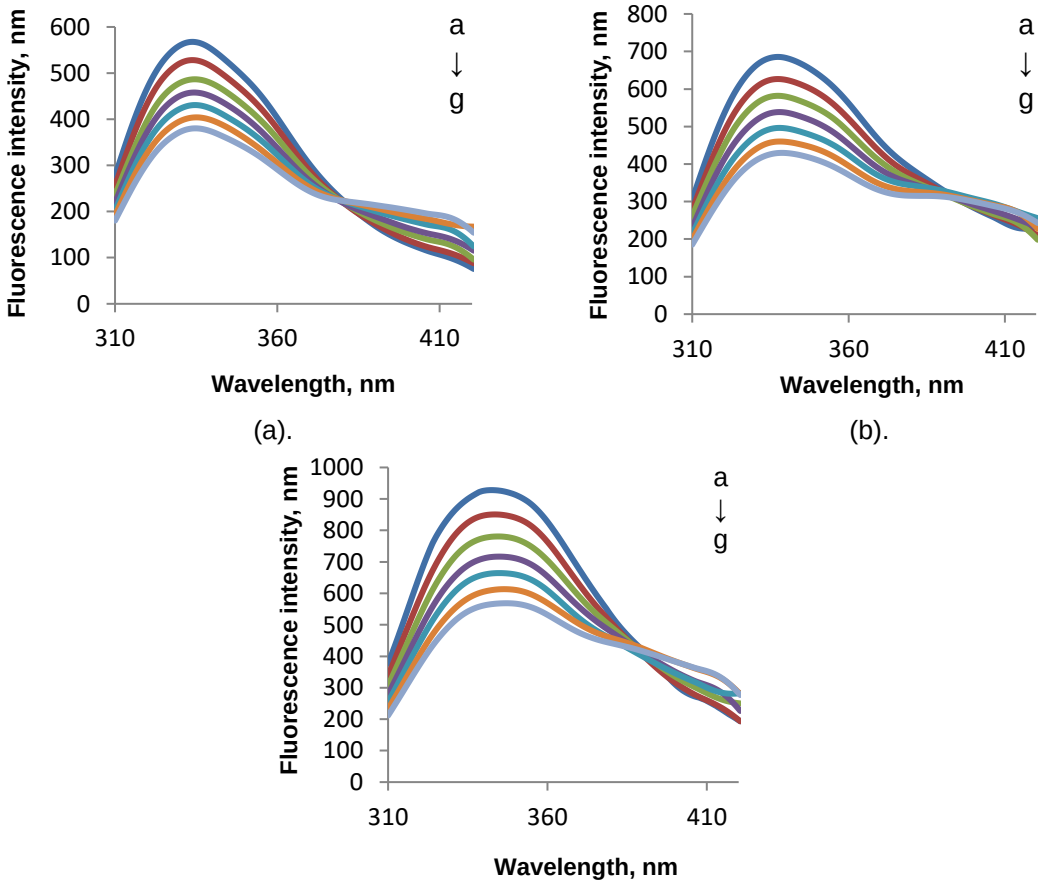


Figure 10.1. Fluorescence spectra of the interaction between heat-treated β-Lg at 25 °C (a), 70 °C (b), and 100 °C (c) and anthocyanins from eggplant peels in concentrations from 0 to 7.02 x 10^{-7} mol D3G (a→g)

Figure 10.1 shows the intensity of β-Lg fluorescence decreasing as the extract concentration increased. At a molecular level, energy transfer and binding interactions

between anthocyanins and proteins appeared and led to changes in the polarity of the environment around the Trp residues present in the polypeptide chains. At 25 °C, the anthocyanins quenched 33 % of the initial fluorescence of the protein (**figure 10.1 a**). As the temperature increased, an increase in the quenching effect of the fluorescence intensity appeared. Thus, at 70 °C, 37 % of the fluorescence intensity was quenched (**figure 10.1 b**), reaching 39 % in the case of heat treatment of the protein at 100 °C (**figure 10.1 c**).

In order to predict the fluorescence quenching mechanism (static or collisional), the binding parameters (**table 10.1**) were determined starting from the Stern-Volmer equation.

Table 10.1. *Binding parameters between heat-treated β-Lg in the temperature range of 25 °C to 100 °C and anthocyanins from eggplant peel*

Temperature (°C)	Ksv × 10^4 (L × mol^{-1})	Kb × 10^6 (L × mol^{-1})	n
25	73.96 ± 9.92^a	9.74 ± 0.46^b	0.91 ± 0.02^c
50	74.90 ± 7.75^a	9.58 ± 0.22^b	0.90 ± 0.04^c
60	79.66 ± 4.47^a	9.23 ± 0.54^b	0.89 ± 0.04^c
70	84.70 ± 2.36^a	8.83 ± 0.33^b	0.88 ± 0.02^c
80	86.30 ± 2.22^a	9.57 ± 0.57^b	0.96 ± 0.06^c
90	87.60 ± 7.25^a	10.56 ± 1.42^b	1.06 ± 0.16^c
100	87.65 ± 2.89^a	10.61 ± 1.84^b	0.90 ± 0.04^c

The influence of temperature was highlighted in lowercase letters per column. The values that share a letter are not significantly different (*p>0.05*) according to the ANOVA test.

The Ksv values did not increase significantly with the temperature (*p>0.05*), which indicates that at the molecular level, a low energy transfer happened, the process being static (**table 10.1**). The Kb values decreased slightly with increasing temperature up to 70 ºC, then increased with temperature up to 100 ºC. The same behaviour was followed by the number of binding sites. However, no significant differences were observed from a statistical point of view (*p>0.05*). This phenomenon is known as the „break-in Arrhenius plot" and is due to the overlapping forces involved in the binding process between protein and anthocyanins around a temperature of 70 °C.

Table 10.2 presents the thermodynamic parameters, and these allow the understanding of the type of forces involved in the binding mechanism of β-Lg with anthocyanins.

Table 10.2. *Thermodynamic binding parameters between heat-treated β-Lg in the 25 °C to 100 °C temperature range and anthocyanins from eggplant peel*

Temperature (°K)	ΔH (J/mol)	ΔS (J/mol × K)	ΔG (J/mol)
298	309.72	1.28	-71.72
323			-103.72
333			-116.52
343			-129.32
353	-1325.8	6.02	-3450.86
363			-3511.06
373			-3571.26

For the complex formed between β-Lg and anthocyanins from eggplant peel, the enthalpy and entropy were estimated in two temperature ranges, namely 25-70 ºC and 70-100 ºC. The values of the two thermodynamic parameters allow the appreciation of the fact that in the binding process, in the temperature range 25-70 ºC, hydrophobic forces (ΔH>0, ΔS>0) were involved (**table 10.2**), while in the range 70-100 ºC were electrostatic interactions (ΔH <0, ΔS> 0). The variation of free energy likewise exhibited negative values, indicating that the binding reaction occurs spontaneously and was dominated by enthalpy.

10.4.2. Quenching of Lf fluorescence

Figure 10.3 illustrates changes in Lf emission spectra as the extract concentration, temperature, and heat treatment time increased.

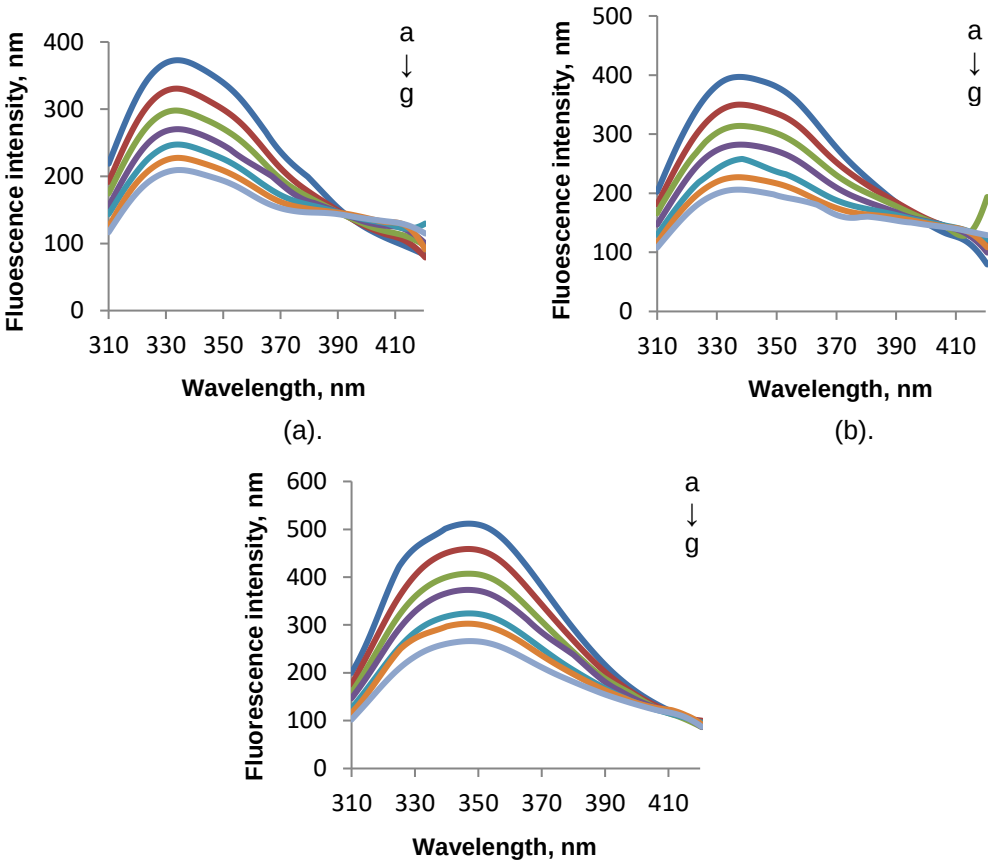


Figure 10.3. The fluorescence spectra of the interaction between Lf heat-treated at 25 °C (a), 60 °C (b), and 80 °C (c) and anthocyanins from eggplant peels in a concentration of 0 to 7.03 x 10^{-7} mol D3G (a→g)

A gradual decrease in fluorescence intensity was observed as the concentration of the extract increased. Thus, at 25 ºC, the anthocyanins quenched 44% of the intensity of LF's fluorescence and at 60 ºC and 80 ºC in a proportion of 48 %. The quenching effect increased as the temperature rose. This suggests that there were binding interactions as well as energy transfer between the anthocyanins from the eggplant peel and Lf.

The binding parameters of anthocyanins to Lf are detailed in **table 10.3**.

Table 10.3. *Binding parameters between heat-treated Lf in the temperature range of 25 °C to 80 °C and anthocyanins from eggplant peel*

Temperature (°C)	Ksv × 10^4 (L × mol^{-1})	Kb × 10^5 (L × mol^{-1})	n
25	106.43 ± 9.12^{a}	80.29 ± 4.47^{b}	0.88 ± 0.03^{c}
50	110.13 ± 3.32^{a}	80.74 ± 1.42^{b}	0.91 ± 0.05^{bc}
60	117.13 ± 7.42^{a}	83.08 ± 6.19^{b}	0.97 ± 0.03^{abc}
70	130.19 ± 6.29^{a}	83.77 ± 7.47^{b}	1.01 ± 0.01^{ab}
80	134.95 ± 7.84^{a}	84.57 ± 3.37^{b}	1.02 ± 0.03^{a}

The influence of temperature was highlighted in lowercase letters per column. The values that share a letter are not significantly different ($p>0.05$) according to the ANOVA test.

The increase in temperature led to a slight increase in the values of the Ksv (**table 10.3**), indicating a low energy transfer between Trp and anthocyanin molecules, specific to a

static process. The kb values increased slightly with temperature, but there was no significant influence of temperature on this constant (*p>0.05*). The n values increased significantly (*p<0.05*) with increasing temperature, with Lf having at least one site with a high binding affinity for anthocyanins.

Table 10.4. *Thermodynamic parameters between heat-treated LF from 25 °C to 80 °C and the anthocyanins from the eggplant peel*

Temperature (°K)	ΔH (J/mol)	ΔS (J/mol × K)	ΔG (J/mol)
298			-1791.65
323			-1920.65
333	-253.97	5.16	-1972.25
343			-2023.85
353			-2075.45

After calculating the thermodynamic parameters (**table 10.4**), a ΔH of -253.91 J/mol and ΔS of 5.16 J/mol × K resulted. These values indicate that electrostatic interactions were involved in the binding mechanism. At the same time, it indicates that the reaction between molecules was exothermic. The ΔG had negative values, signifying that the binding process occurred spontaneously from right to left, with enthalpy dominating.

10.4.3. Quenching of whey proteins fluorescence

Figure 10.5 display the changes in the emission spectra of the WPI solution as the extract concentration increased.

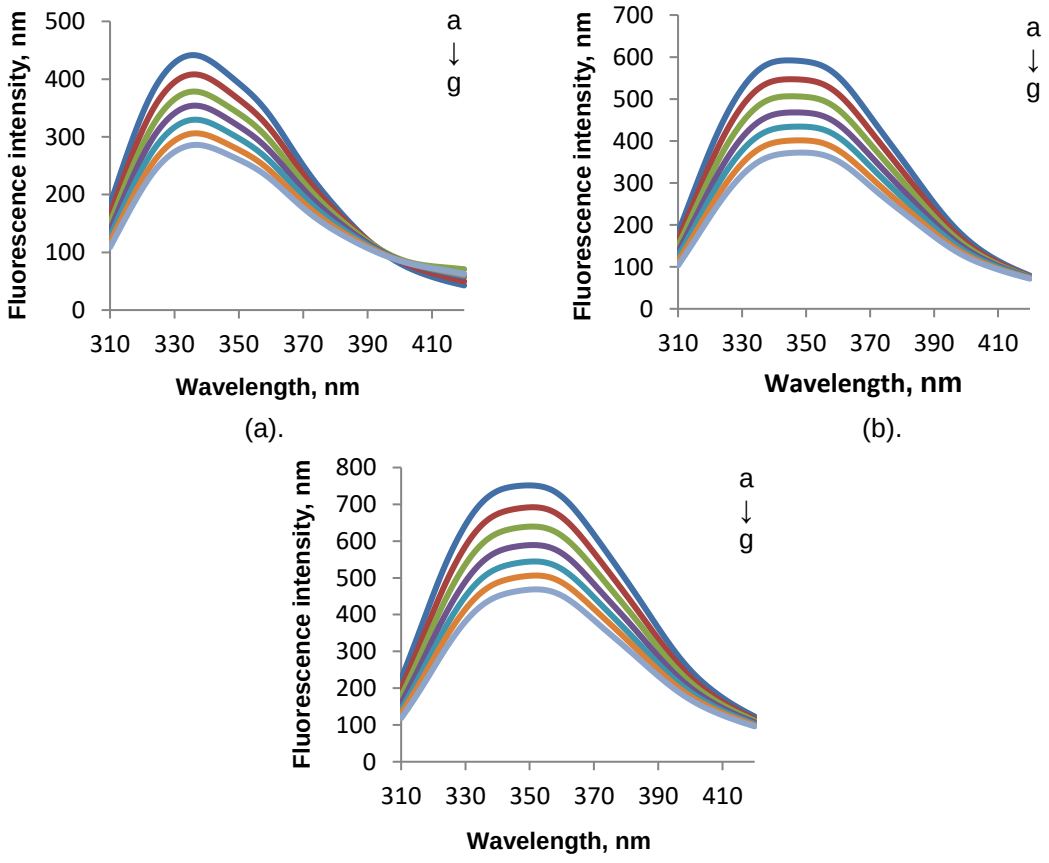


Figure 10.5. Fluorescence spectra of the interaction between heat-treated WPI at 25 °C (a), 70 °C (b), and 90 °C (c) and anthocyanins from eggplant peels in a concentration of 0 to 8.47 × 10^{-7} mol D3G (a→g)

At a temperature of 25 °C, the anthocyanins in the eggplant peel extract quenched 35 % of the initial fluorescence of the proteins. As the temperature increased, an increase in the fluorescence quenching effect was observed by the anthocyanins in the eggplant peel, reaching 38 % in the case of heat treatment of the protein at 90 °C. It indicates that binding and energy transfer interactions occur between anthocyanins in eggplant peel and WPI proteins.

The binding parameters given in **table 10.5** have been determined to predict the fluorescence quenching mechanism.

***Table 10.5**. Binding parameters between heat-treated WPI in the temperature range of 25 °C to 90 °C and anthocyanins from eggplant peel*

Temperature (°C)	Ksv × 10^4 (L × mol^{-1})	Kb × 10^6 (L × mol^{-1})	n
25	66.40 ± 3.67^a	9.83 ± 0.59^b	0.87 ± 0.07^c
50	68.45 ± 1.30^a	9.87 ± 0.47^b	0.89 ± 0.04^c
60	68.80 ± 5.52^a	10.01 ± 0.09^b	0.91 ± 0.04^c
70	71.00 ± 3.04^a	9.75 ± 0.27^b	0.89 ± 0.01^c
80	73.10 ± 3.14^a	9.55 ± 0.19^b	0.89 ± 0.01^c
90	79.90 ± 0.56^a	9.02 ± 0.11^b	0.88 ± 0.02^c

The influence of temperature was highlighted in lowercase letters per column. The values that share a letter are not significantly different (*p>0.05*) according to the ANOVA test.

The temperature increase led to a slight Ksv value increase (**table 10.5**), specific to a static process, indicating a low energy transfer between Trp and anthocyanin molecules. The values of kb increased slightly with the temperature up to 60 ºC and then decreased slightly with increasing temperature. This phenomenon is called the "break-in Arrhenius plot" due to the β-Lg reaction (found in the highest percentage in WPI) to the heat treatment.

Table 10.6 presents the enthalpy and entropy values estimated for the interaction between the heat-treated WPI and anthocyanins in two temperature ranges, namely 25-60 ºC and 70-90 ºC.

***Table 10.6.** Thermodynamic binding parameters between heat-treated WPI from 25 °C to 90 °C and anthocyanins in eggplant peel*

Temperature (°K)	ΔH (J/mol)	ΔS (J/mol × K)	ΔG (J/mol)
298			-908.53
323	-118.83	2.65	-974.78
333			-1001.28
343			-209.31
353	284.61	1.44	-223.71
363			-238.11

The values for the two thermodynamic parameters demonstrate that both electrostatic interactions (ΔH <0, ΔS> 0) and hydrophobic forces (ΔH> 0, ΔS> 0) were involved in the binding process (**table 10.6**). At the same time, ΔG values decreased in two temperature ranges, namely 25-60 ºC and 70-90 ºC, suggesting that the binding reaction took place spontaneously and almost completely, being dominated by enthalpy more than entropy.

10.4.4. Quenching of whey peptides fluorescence

Figure 10.7 illustrates the fluorescence spectra of heat-treated peptides at different temperatures, titrated with an extract from eggplant peels.

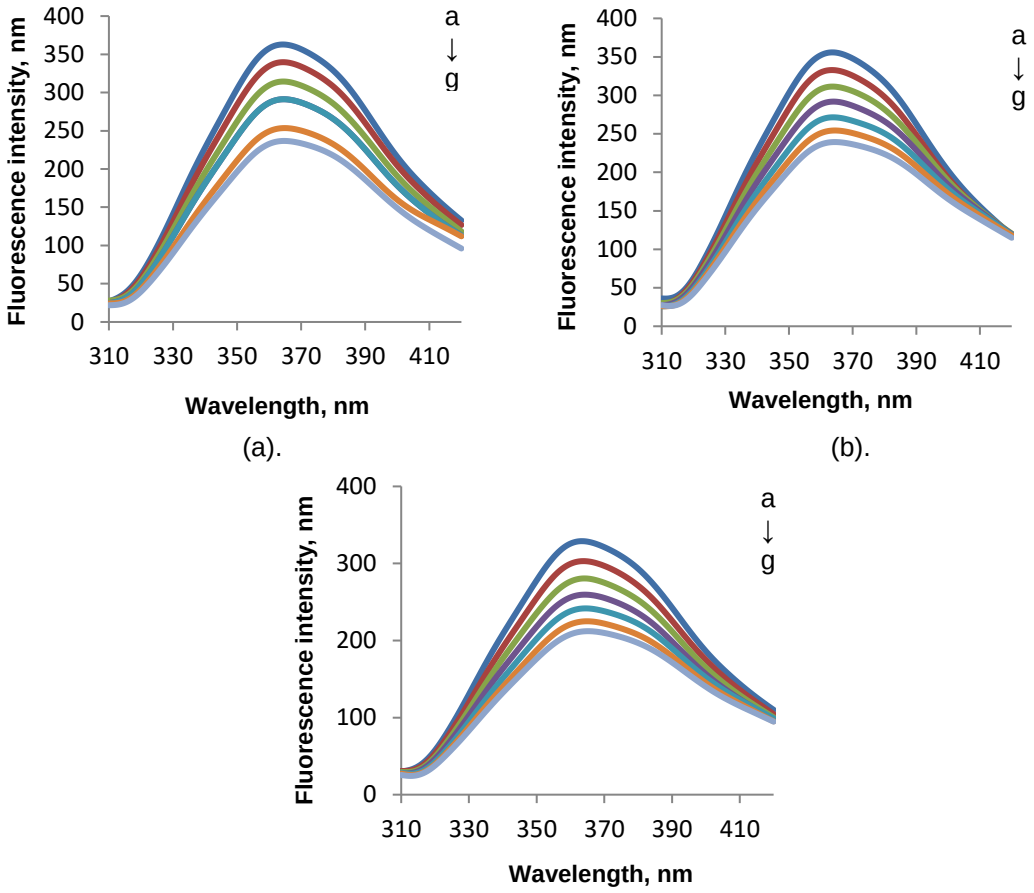


Figure 10.7. The fluorescence spectra of the interaction between Hp(P) 3 Da heat-treated at 25 °C (a), 70 °C (b), and 100 °C (c) and the anthocyanins from eggplant peels in a concentration of 0 to 8.48 x 10^{-7} mol D3G (a→g)

At a temperature of 25 °C, the anthocyanins from the eggplant peel extract quenched 29 % of the peptides' initial fluorescence. As the temperature increased, an increase in the quenching effect of fluorescence was observed by the anthocyanins from eggplant peel. At 70 °C, the fluorescence quenching effect was 33 %, reaching 36 % in the case of heat treatment of peptides at 100 °C. It indicates that binding and energy transfer happened between the anthocyanins from the eggplant peel and the peptides.

Table 10.7 details the values of the binding parameters between heat-treated Hp(P) in the temperature range of 25 °C to 100 °C and anthocyanins from eggplant peel.

Table 10.7. *Binding parameters between heat-treated Hp(P) in the temperature range of 25 °C to 100 °C and anthocyanins from eggplant peel*

Temperature (°C)	Ksv × 10^4 (L × mol^{-1})	Kb × 10^6 (L × mol^{-1})	n
25	60.63 ± 2.07^{a}	10.73 ± 0.^{27a}	0.93 ± 0.02^{b}
50	61.73 ± 2.25^{a}	10.21 ± 0.33^{ab}	0.88 ± 0.02^{b}
60	64.10 ± 4.02^{a}	9.80 ± 0.10^{ab}	0.86 ± 0.02b
70	72.10 ± 2.96^{a}	9.56 ± 0.82^{ab}	0.85 ± 0.04^{b}
80	67.70 ± 0.56^{a}	9.81 ± 0.57^{b}	0.87 ± 0.01^{b}
90	67.33 ± 4.42^{a}	9.84 ± 0.18^{ab}	0.88 ± 0.05^{b}
100	64.83 ± 3.31^{a}	9.93 ± 0.16^{ab}	0.89 ± 0.04^{b}

The influence of temperature was highlighted in lowercase letters per column. The values that share a letter are not significantly different (*p>0.05*) according to the ANOVA test.

From **Table 10.7** it stands out a slight increase of the Ksv values with increasing the temperature up to 70 ºC, indicating a low energy transfer between the Trp residues and anthocyanin molecules and a static process. Following heat treatment at higher temperatures, Ksv values began to decrease. The kb values increased with the temperature up to 70 ºC and then decreased with increasing the temperature. It was noted that the number of binding sites followed the same pattern, indicating the existence of an affinity for the binding site of the anthocyanins from eggplant peel extract.

Table 10.8 presents the thermodynamic parameters estimated for the interaction between the heat-treated Hp(P) and the anthocyanins from eggplant peel.

In the case of the complex formed between 3 kDa peptides and anthocyanin, enthalpy and entropy were estimated in two temperature ranges, namely 25-70 °C and 80-100 °C, as can be seen in **table 10.8**. The reported values prove that hydrophobic forces were involved in the binding process for the first temperature range, the reaction between the molecules being endothermic. Electrostatic forces were involved in the second temperature range, the reaction being exothermic. The variation of ΔG decreased with the temperature, suggesting that the binding reaction occurs spontaneously and was dominated by enthalpy.

Table 10.8. *Thermodynamic binding parameters between the heat-treated Hp(P) 3 kDa in the 25 ºC – 100 ºC range and the anthocyanin from eggplant peel*

Temperature (°K)	ΔH (J/mol)	ΔS (J/mol × K)	ΔG (J/mol)
298	259.17	1.5	-187.73
323			-225.23
333			-240.23
343			-255.23
353	-156.02	2.71	-1112.65
363			-1139.75
373			-1166.85

10.5. Partial conclusions

The results allowed the elaboration of the following partial conclusions:

✓ Fluorescence spectroscopy showed that anthocyanins from eggplant peel influenced the secondary structure of proteins and peptides by binding to them.

✓ A strong binding process has also been observed between the proteins/peptides and anthocyanins at certain temperatures.

✓ Hydrophobic forces at low temperatures and electrostatic interactions at higher temperatures participated in the binding process between the anthocyanins from eggplant peel extract and β-lactoglobulin.

✓ Electrostatic interactions have occurred in the interactions between anthocyanins and lactoferrin.

✓ The binding of anthocyanins to proteins in whey protein isolate was based on electrostatic interactions at low temperatures and hydrophobic forces at high temperatures.

✓ Between the anthocyanins and the peptides resulted by enzymatic hydrolysis of the whey protein isolate with protease, at low temperatures, hydrophobic forces were involved, while at high temperatures above 80 ºC, electrostatic interactions participated.

CHAPTER 11. DEVELOPING FUNCTIONAL INGREDIENTS BASED ON ANTHOCYANINS FROM EGGPLANT PEELS AND BIOACTIVE PEPTIDES FROM WHEY

11.2. Objectives of the study

This study aimed to valorize anthocyanins from eggplant peels as potential functional ingredients for food industry applications. It involved increasing the stability and functionality of anthocyanins by microencapsulating them along with bioactive peptides. The ultimate goal of microencapsulation was to release components to be absorbed efficiently into the human digestive system.

Thus, the objectives of this study were to:

- Encapsulate and co-microencapsulate the biological actives compounds from eggplant peel using different encapsulation materials (proteins, protein hydrolysates, lactic acid bacteria strains) and by various techniques;
- achieve phytochemical characterization of the particles in terms of anthocyanin, flavonoid, and polyphenol content, antioxidant activity, encapsulation efficiency, and storage stability over time;
- analyze the morphological structure of the new ingredients by confocal laser microscopy;
- test the functionality of the microparticles in terms of *in vitro* digestibility.

11.4. Results and discussion

11.4.1. Phytochemical characterization and encapsulation efficiency of powders

Phytochemical characterization and encapsulation efficiency of the powders designed by the freeze-drying technique with the variation of the encapsulation materials

Anthocyanin-rich extracts were microencapsulated in various combinations of CMC, P, Hp, and WPI. Variations in polysaccharides and protein concentrations have led to different anthocyanin encapsulation efficiencies of 98.67 ± 2.31 % for V2 and 96.64 ± 0.42 % for V3.

***Table 11.1**. Content of phytochemicals in microcapsules processed by varying the encapsulation materials*

Sample	TAC (µg D3G/g dw)	TFC (mg CE/g dw)	TPC (mg GAE/g dw)	Activitate antioxidantă (µM TE/g dw)
Extract	561.56 ± 8.64	2.87 ± 0.02	13.13 ± 0.81	25.70 ± 0.75
V2	392.26 ± 22.92^{a}	4.15 ± 0.13^{b}	10.74 ± 0.70^{a}	36.34 ± 0.58^{a}
V3	290.95 ± 3.53^{b}	3.66 ± 0.54^{ab}	12.30 ± 0.39^{b}	35.42 ± 0.73^{a}

Values that share a lowercase letter per column are not significantly different (*p>0.05*) according to the ANOVA test; V2 – CMC + P; V3 – WPI + Hp(P);

Out of both experimental variants, the one with only CMC and P as encapsulating agents showed the highest TAC (**table 11.1**). V2 also has the highest flavonoid content but the lowest polyphenol content. In terms of antioxidant activity, there were no significant differences between the three powders (*p>0.05*). The results were included in patent request A00532/08.10.2021.

Phytochemical characterization and encapsulation efficiency of the powders designed by the freeze-drying technique with the variation of the encapsulation materials' ratio

The efficiency of anthocyanin microencapsulation was 69.90 ± 1.90 % for the control, 70.03 ± 2.17% for I1, 77.60 ± 1.92 % for I2, and 73.78 ± 1.71 % in the case of I3. The content of phytochemicals and antioxidant activity of the microparticles are listed in **table 11.2**.

Table 11.2. *Content of phytochemicals in microcapsules designed by varying the ratio of encapsulation materials*

Sample	TAC (µg D3G/g dw)	TFC (mg CE/g dw)	TPC (mg GAE/g dw)	Antioxidant activity (µM TE/g dw)
Extract	580.0 ± 0.03	4.48 ± 0.80	13.64 ± 0.19	157.82 ± 9.46
Control	1250.0 ± 0.25^{a}	25.50 ± 0.60^{ab}	67.78 ± 9.29^{a}	94.71 ± 2.80^{a}
I1	520.0 ± 0.02^{b}	20.81 ± 0.96^{a}	62.49 ± 9.32^{a}	62.45 ± 2.74^{b}
I2	610.0 ± 0.01^{b}	23.77 ± 1.22^{ab}	62.07 ± 0.39^{a}	55.43 ± 7.17^{b}
I3	890.0 ± 0.02^{ab}	28.67 ± 1.95^{b}	69.15 ± 0.60^{a}	64.58 ± 0.49^{b}

Values that share a lowercase letter per column are not significantly different (*p>0.05*) according to the ANOVA test; Control – CMC/P/WPI in 1:1:0.4 ratio, I1 - CMC/P/Hp(T) in 1:1:0.4 ratio; I2 - CMC/P//Hp(T) in 2:1:0.4 ratio; I3 - CMC/P/Hp(T) in 1:2:0.4 ratio;

The highest TAC was found in the control variant, followed by I3. The latter also displayed the highest TPC and TFC (**table 11.2**). Samples I1 and I2 had a lower content of phytochemicals than the other two. Because of the high concentration of biologically active compounds, the control and I3 showed an antioxidant activity greater than 94.71 ± 2.57 µM TE/g dw and 91.31 ± 0.98 µM TE/g dw compared to I1 and I2.

Phytochemical characterization and encapsulation efficiency of the powders designed by the freeze-drying technique with the variation of the protein hydrolysate's type

The microencapsulation efficiency of anthocyanins was 88.57 ± 6.69% in the control, 86.54 ± 5.20% for VCT, and 64.12 ± 5.41% for VT. It can be observed that, contrary to our expectations, a hydrolyzate with a higher degree of hydrolysis led to a lower encapsulation efficiency.

Table 11.3*. Content of phytochemicals in microcapsules prepared by varying the type of protein hydrolysate*

Sample	TAC (µg D3G/g dw)	TFC (mg CE/g dw)	TPC (mg GAE/g dw)	Antioxidant activity (µM TE/g dw)
Extract	340.0 ± 0.51	13.44 ± 0.97	19.52 ± 0.51	157.82 ± 9.46
Control	110.0 ± 0.01^{ab}	67.74 ± 1.43^{a}	13.69 ± 1.10^{a}	38.62 ± 3.87^{ab}
VCT	90.0 ± 0.01^{a}	74.48 ± 4.15^{b}	19.82 ± 3.07^{b}	34.39 ± 5.69^{a}
VT	130.0 ± 0.02^{b}	77.46 ± 0.55^{b}	17.26 ± 1.29^{ab}	46.38 ± 1.28^{b}

Values that share a lowercase letter per column are not significantly different (*p>0.05*) according to the ANOVA test; Control – CMC + P + WPI; VCT – CMC + P + Hp(CT); VT – CMC + P + Hp(T);

The content of phytochemicals and the antioxidant activity of the fine particles are presented in **table 11.3**. The highest TAC was found for VT, followed by the control variant. The highest TFC was also found in the case of VT, followed by VCT. VCT exhibited the highest TPC in polyphenol concentration, followed by VT. Because of the high concentration of anthocyanins, the control and VT displayed an antioxidant activity greater than 38.62 ± 3.87 µM TE/g dw and 46.38 ± 1.28 µM TE/g dw compared to VCT.

Phytochemical characterization and encapsulation efficiency of the powders developed by the freeze-drying technique with the variation of the lactic acid bacteria strains

The efficiency of anthocyanin microencapsulation was 95.76 ± 0.67% for the control, 94.85 ± 0.38% for VB, and 95.89 ± 0.38% for VBP. The EE of LAB was 91.30 % for VB and 97.14 % for VBP. The content of phytochemicals and the antioxidant activity of the powders are indicated in **table 11.4**.

Table 11.4. *Content of phytochemicals in microcapsules designed by varying the strain of LAB*

Sample	TAC (µg D3G/g dw)	TFC (mg EC/g dw)	TPC (mg EAG/g dw)	Antioxidant activity (µM TE/g dw)	LAB (CFU/g dw)
Extract	662.40 ± 13.14	15.11 ± 0.14	24.15 ± 0.34	258.04 ± 0.51	-
Control	360.0 ± 0.01^{a}	4.33 ± 0.74^{a}	4.77 ± 0.26^{a}	86.55 ± 0.66^{a}	-
VB	270.0 ± 0.01^{b}	5.02 ± 0.88^{a}	5.88 ± 0.72^{ab}	73.77 ± 0.50^{b}	1.96 x 10^{8b}
VBP	320.0 ± 0.01^{c}	4.94 ± 0.86^{a}	6.34 ± 0.70^{b}	85.25 ± 0.89^{a}	1.12 x 10^{9a}

Values that share a lowercase letter per column are not significantly different (*p>0.05*) according to the ANOVA test; Control – WPI + CMC + P + Hp(β-LgT); VB – WPI + CMC + P + Hp(β-LgT) + *L. bifermentans*; VBP - VB – WPI + CMC + P + Hp(β-LgT) + *L. bifermentans* + *L. plantarum* (1:1);

The highest TAC was found for control, followed by co-culture. In terms of polyphenol concentration, VBP exhibited the highest TPC, followed by VB. Due to the high concentration of anthocyanins, the control and VBP displayed an antioxidant activity greater than 86.55 ± 0.66 µM TE/g dw and 85.25 ± 0.89 µM TE/g dw compared to VB. However, as reported in **table 11.4**, there are no significant differences in TFC from the three experimental variants. Therefore, it can be appreciated that the co-microencapsulation technique and the compounds used allowed the designing of ingredients with a high content of biologically active compounds and LAB, with a high encapsulation efficiency. Therefore, all variants showed high values of antioxidant activity. Results were included in patent request A00481/01.08.2019.

Phytochemical characterization and encapsulation efficiency of the powders prepared by the gelation technique

Following the encapsulation of the bioactives from eggplant peel by the gelation method, encapsulation efficiencies of 64.68 ± 0.68 % for GV1 and 96.44 ± 3.43 % for GV2, respectively, were calculated.

Table 11.5 details the content of phytochemicals in the variants generated by the gelling approach. In this case, the highest TAC was found in the technological versions with higher polysaccharide concentrations than proteins. In the case of encapsulation of flavonoids in eggplant peel extract, no significant differences were observed between the two ingredients (*p>0.05*). The highest TPC was encapsulated in GV1. This powder version also showed the highest antioxidant activity.

Table 11.5. *Content of phytochemicals in microcapsules produced by gelation [19]*

Sample	TAC (µg D3G/g dw)	TFC (mg CE/g dw)	TPC (mg GAE/g dw)	Antioxidant activity (µM TE/g dw)
Extract	406.47 ± 37.41	2.99 ± 0.12	12.79 ± 0.66	193.14 ± 1.25
GV1	50.41 ± 2.13^{a}	1.53 ± 0.06^{a}	8.03 ± 0.18^{a}	41.96 ± 0.28^{a}
GV2	94.94 ± 7.94^{b}	1.64 ± 0.14^{a}	7.22 ± 0.18^{b}	36.60 ± 0.83^{b}

Values that share a lowercase letter per column are not significantly different (*p>0.05*) according to the ANOVA test; GV1 – CMC/P/WPI IN A 1:1:2 ratio; GV2 – CMC/P/WPI in a 1:1:0.6;

11.4.2. Storage stability of powders

Storage stability of phytochemicals from the powders designed by the freeze-drying technique with the variation of the encapsulation materials

A significant release of TAC from the encapsulation matrices happened during the 28 days of storage at 4 ºC (*p<0.05*). Anthocyanins in the polysaccharides matrix were discharged at 15 %, while the matrix consisting only of proteins led to a 33 % release of anthocyanins (**figure 11.8 a**). However, a 23 % increase in the TFC was noticed in V2, while V3 presented stability (**figure 11.8 b**). Good stability in time was also observed in the case of TPC for V2. In V3 approximately a 12 % increase in the total polyphenols appeared (f**igure 11.8 c**). The increase of biologically active compounds from the encapsulation matrices caused an increase in antioxidant activity. Thus, there was a significant increase (*p<0.05*) in the values of antioxidant activity against the DPPH radical for both powders after 28 days (**figure 11.8 d**).

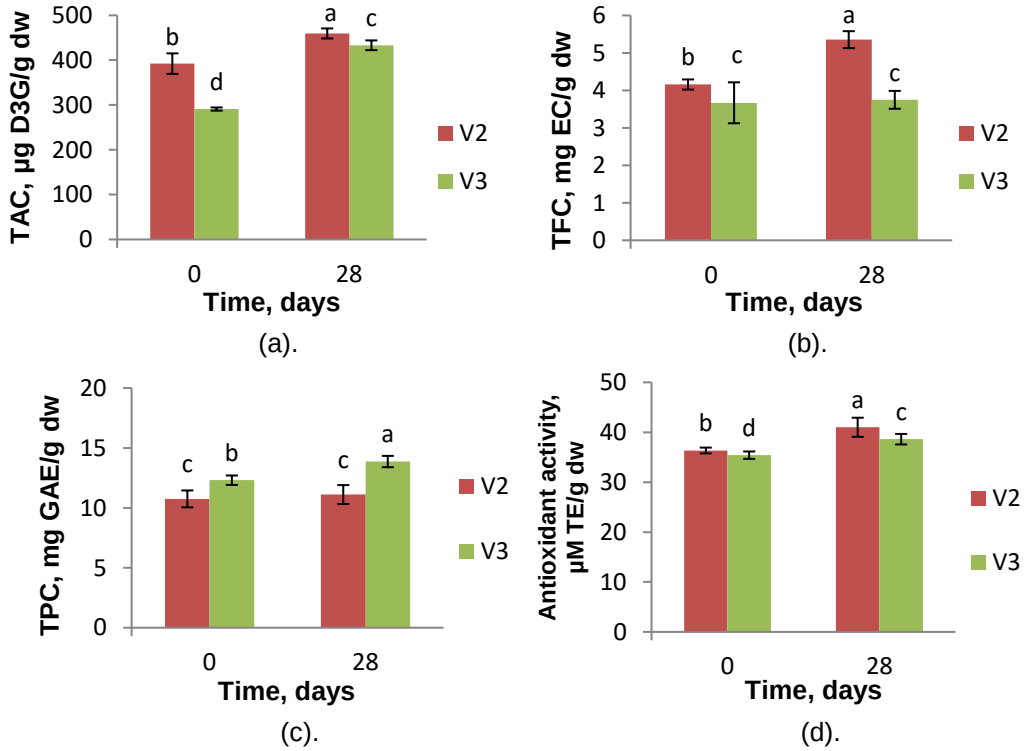


Figure 11.8. Storage stability of TAC (a), TFC (b), TPC (c), and *antioxidant* activity (d) from the powders produced by the freeze-drying technique with the variation of the encapsulation materials; V2 – CMC + P; V3 – WPI + Hp(P); Values that share a letter are not significantly different (*p>0.05*) according to the ANOVA test

Storage stability of phytochemicals from the powders developed by the freeze-drying technique with the variation of the encapsulation materials' ratio

Figure 11.9 illustrates a significant decrease of approximately 31% in TAC in the control sample, while for I1 there was a slight increase of 8 %. In I2, there was a slight decrease of 11%, while I3 showed the highest stability, with a slight decrease of 1% in the TAC content. TPC also decreased between 30.88 ± 0.68 % in I1 and 36.89 ± 1.88% in I3, while I2 showed good stability with a slight decrease to 0.12 ± 0, 01% (**figure 11.9**). TFC increased in all samples, mainly in variant I1, leading to an increase in antioxidant activity.

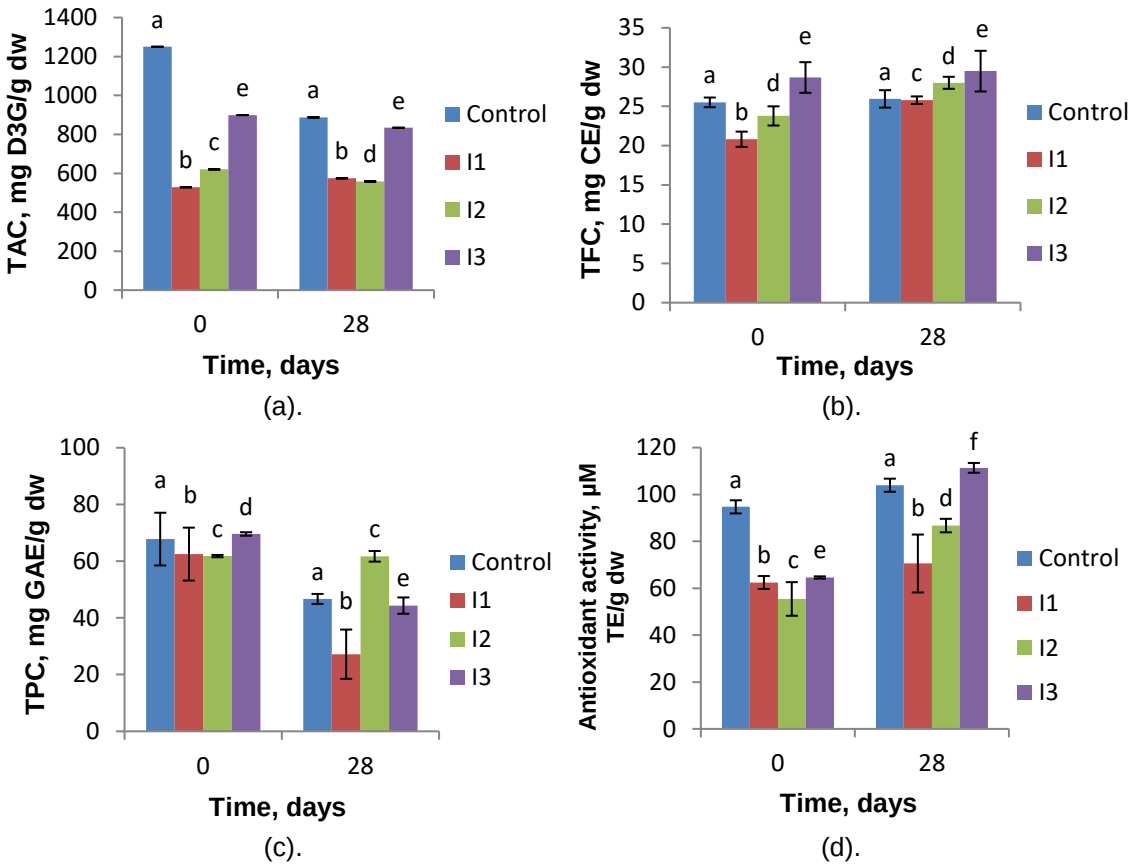
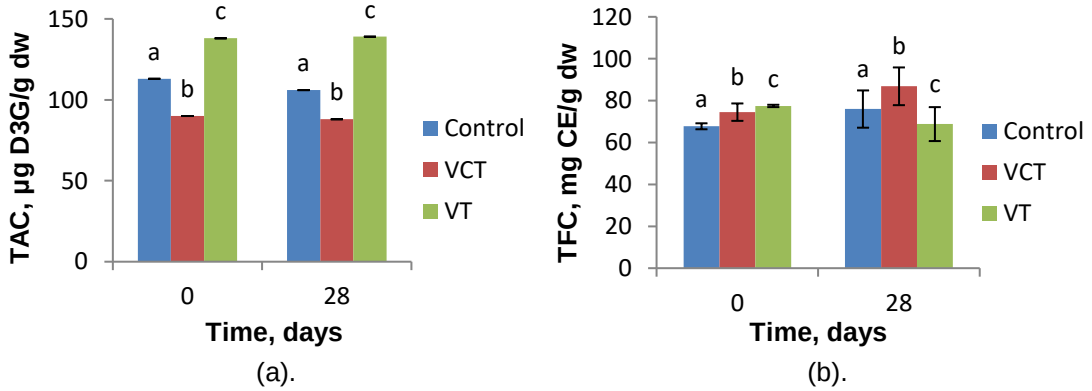
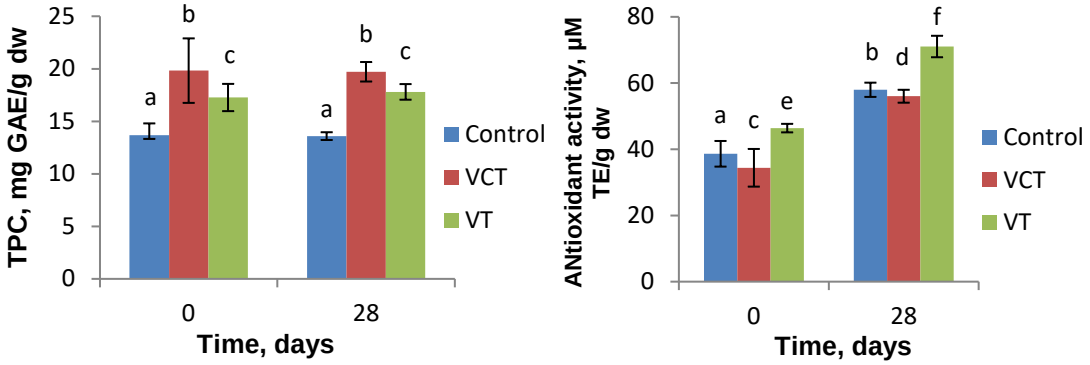


Figure 11.9. Storage stability of TAC (a), TFC (b), TPC (c), and antioxidant activity (d) from the powders designed by the freeze-drying technique with the variation of the encapsulation material's ratio; Control – CMC/P/WPI in 1:1:0.4 ratio, I1 - CMC/P/Hp(T) in 1:1:0.4 ratio; I2 - CMC/P/Hp(T) in 2:1:0.4 ratio; I3 - CMC/P/Hp(T) in 1:2:0.4 ratio; Values that share a letter are not significantly different (*p>0.05*) according to the ANOVA test

Storage stability of phytochemicals from the powders produced by the freeze-drying technique with the variation of the protein hydrolysate's type

The storage stability of the ingredients as a function of the hydrolysate type variation is represented in **figure 11.10**. Regarding TAC, TFC, and TPC, the remarkable stability of biologically active compounds was noticed in all variants (**figure 11.10**). Though, a significant increase in antioxidant activity appeared in all experimental variants of our study, especially in the case of the VCT, where a 38.60 % increase was observed after 28 days of storage (*p<0.05*). The antioxidant activity of the control sample increased by 33.35 %, while VT by 34.70 %.



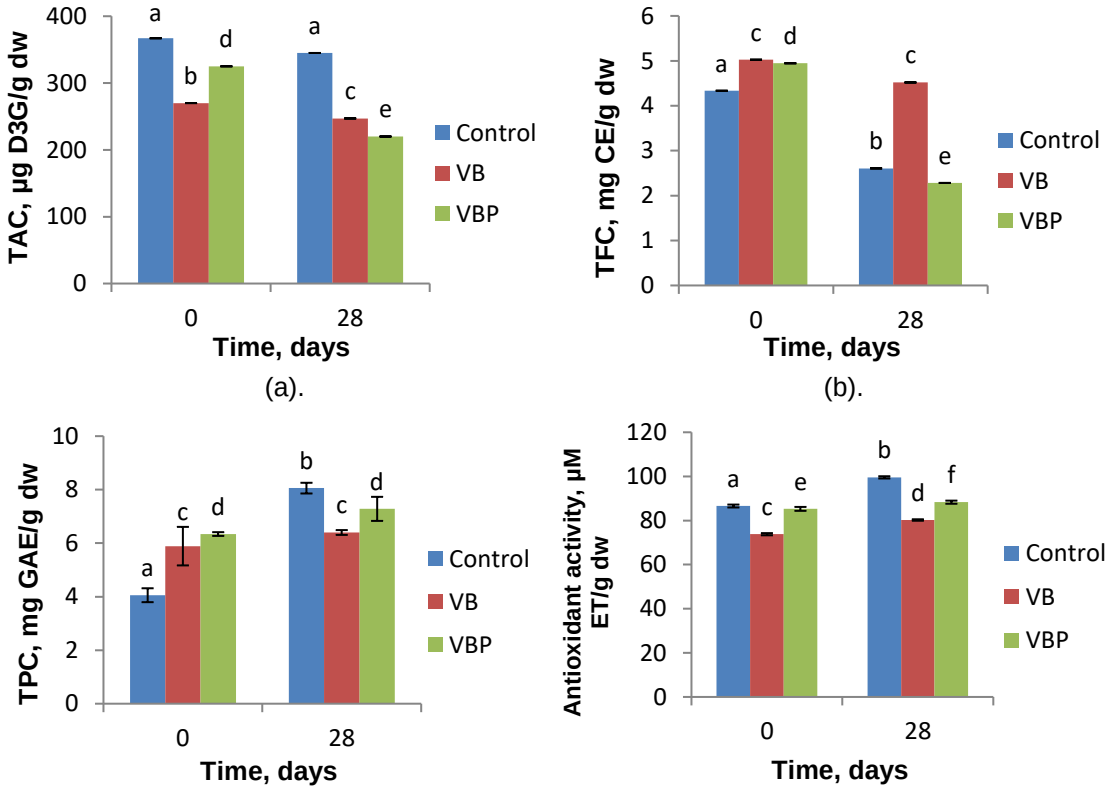


(c). (d).

Figure 11.10. Storage stability of TAC (a), TFC (b), TPC (c), and antioxidant activity (d) from the powders realized by the freeze-drying technique with the variation of the protein hydrolysate's type; Control – CMC + P + WPI; VCT – CMC + P + Hp(CT); VT – CMC + P + Hp(T); Values that share a letter are not significantly different (*p>0.05*) according to the ANOVA test

Storage stability of phytochemicals from powders realized by the freeze-drying technique with the variation of the lactic acid bacteria strains

The storage stability of the ingredients realized by varying the LAB strains is represented in **figure 11.11**. Storage for 28 days decreased the content of biologically active compounds, especially in VBP, where the TAC decreased by 32%, and the TFC in control and VB by 40% and 54%, respectively. All three samples released polyphenolic compounds, which led to an increase in antioxidant activity. All samples retained antioxidant activity, over 80 µM TE/g dw (**figure 11.11**). Our results comply with other studies.



(a). (b).

(c). (d).

Figure 11.11. Storage stability of TAC (a), TFC (b), TPC (c), and antioxidant activity (d) from the powders prepared by the freeze-drying technique with the variation of the lactic acid bacteria strains; Control – WPI + CMC + P + Hp(β-LgT); VB – WPI + CMC + P + Hp(β-LgT) + *L. bifermentans*; VBP -

VB – WPI + CMC + P + Hp(β-LgT) + *L. bifermentans* + *L. plantarum* (1:1); Values that share a letter are not significantly different (*p>0.05*) according to the ANOVA test

In both our ingredients, the LABs' viability decreased with 1 log, according to **table 11.6**. Our findings are in agreement with other authors.

Table 11.6. *Storage stability of LAB from the powders*

Powder variant	Cell viability (CFU/g dw)	
	0 days	28 days
Control	-	-
VB	$1.96 \times 10^{8\,a}$	$7.88 \times 10^{7\,b}$
VBP	$1.12 \times 10^{9\,a}$	$1.78 \times 10^{8\,b}$

Values that share a lowercase per line are not significantly different (*p>0.05*) according to the ANOVA test; Control – WPI + CMC + P + Hp(β-LgT); VB – WPI + CMC + P + Hp(β-LgT) + *L. bifermentans*; VBP - VB – WPI + CMC + P + Hp(β-LgT) + *L. bifermentans* + *L. plantarum* (1:1);

Storage stability of phytochemicals from powders provided by the gelation technique

The microparticles stored at 4 ºC for 28 days were characterized by phytochemical content and antioxidant activity. Changes in TAC, TFC, TPC, and antioxidant activity of both ingredients during storage are illustrated in **figure 11.12**.

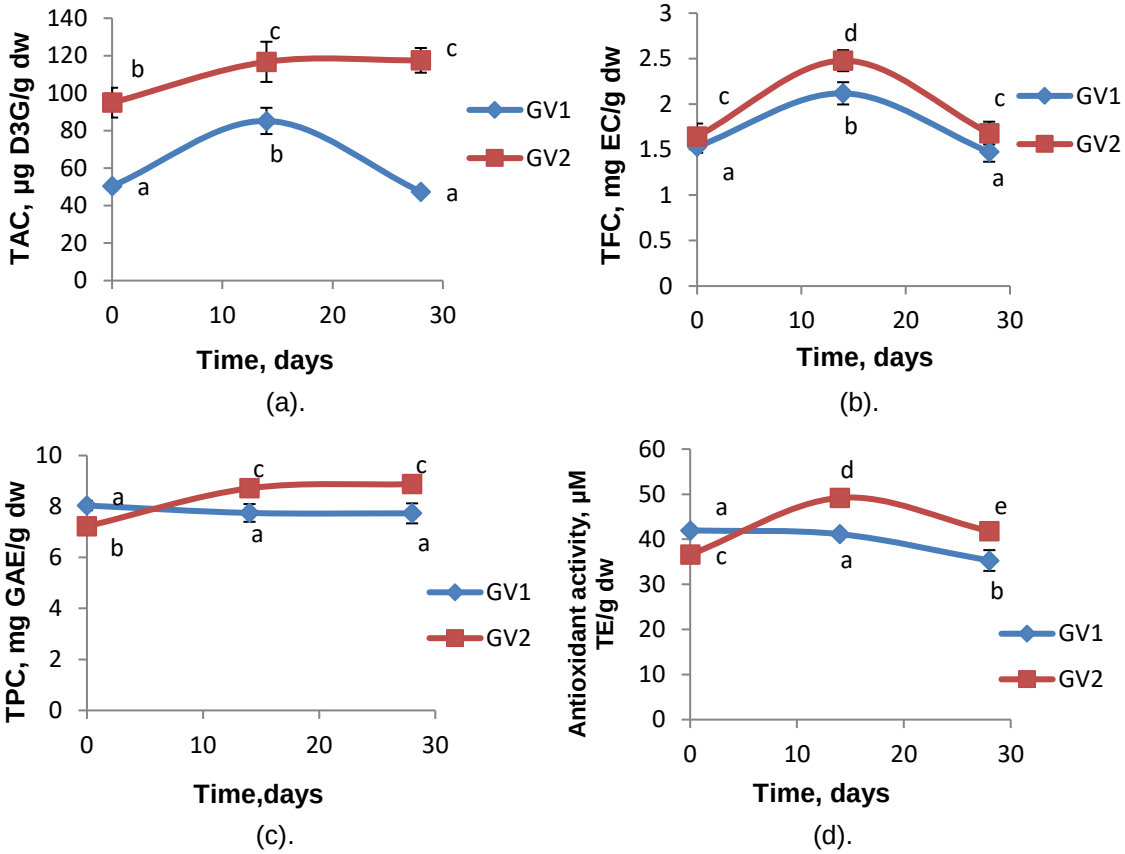


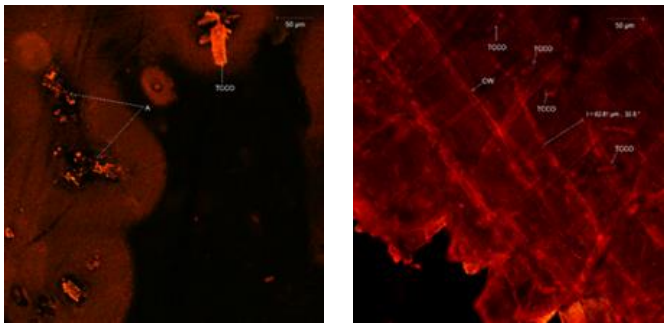
Figure 11.12. Storage stability of TAC (a), TFC (b), TPC (c), and antioxidant activity (d) from the powders produced by the gelation technique; GV1 – CMC/P/WPI IN A 1:1:2 ratio; GV2 – CMC/P/WPI in a 1:1:0.6; Values that share a lowercase letter per column are not significantly different (*p>0.05*) according to the ANOVA test; Values that share a letter are not significantly different (*p>0.05*) according to the ANOVA test;

Both the TAC (**figure 11.12 a**) and TFC (**figure 11.12 b**) of the first variant did not change significantly during the 28 days of storage ($p>0.05$). In contrast, for GV2, there was a release of total matrix anthocyanins of approximately 20 %. GV2's TPC also increased by approximately 18 %, while GV1's TPC remained constant (**figure 11.12 c**). Regarding antioxidant activity (**figure 11.12 d**), GV1 decreased by 16 % after 28 days of storage, while GV2 showed an increase of approximately 13 %. Although GV1 encapsulated a lower concentration of biologically active compounds than GV2, it has the best storage stability over time.

11.4.3. Morphological structure of powders

Morphological structure of the powders realized by freeze-drying technique with the variation of the encapsulation materials

Figure 11.13 displays the biologically active compounds, especially anthocyanins, evenly distributed in the thick polymeric matrix and calcium oxalate macules, specific to the pericarp of eggplant peels. **Figure 11.13** shows a uniform distribution of the biologically active compounds from eggplant peel in the complex microencapsulation matrix. The anthocyanin particles presented average sizes between 7 and 15 microns. Thus, the encapsulation process was considered uniform.



(a). (b).

Figure 11.13. Morphological structure of powders produced by freeze-drying technique with the variation of the encapsulation materials: V2 - CMC + P (a - coloured); V3 - WPI + Hp(P) (b - coloured);

It can be seen that the microencapsulation process in the protein matrix protected the plant tissue. At the same time, fragments of plant tissue with an intact cell wall and in sizes between 60-65 µm could be frequently viewed.

Morphological structure of the powders provided by the freeze-drying technique with the variation of the encapsulation materials' ratio

The morphological structure of the four variants is presented in the native CLSM images from **figure 11.14**. In all technological versions, the presence of asymmetric and thin scales with emission in all spectral ranges could be observed due to the complexity of the samples (small peptides, anthocyanins, other polyphenols, or carbohydrates). In the control, large formations with irregular contours and variable dimensions (between 26.54 and 40.52 µm), with porous structures (such as micro-cavities or cracks), with some small vesicles at the periphery, with diameters of 2 - 3 µm were noticed (**figure 11.14 a**). In I1 and I2, the morphological pattern of the microparticles was predominantly filamentous, probably due to the presence of peptides. Finger or needle-shaped extensions varied in size from 8.54 to 20.20 µm in I2 (**figure 11.14 c**) or even larger than 28.53 - 46.16 µm in I1 (Figure 11.14 b). In I3

(**figure 11.14 d**), probably due to a higher proportion of pectin, the patterns were different, with large clusters (46.32 - 81.16 μm) formed by the fusion of spherosomes.

Images of coloured microcapsules with both DAPI and Congo red showed aggregate anthocyanins inside the matrix. The biologically active compounds formed small red particles with a diameter of 1-2 μm (**figure 11.14 e**), being distributed in the matrix (coloured in blue/green). No significant differences could be observed between the control and I1 (**figure 11.14 f**). However, in I2 (**figure 11.14 g**), individual microvesicles with diameters between 6.7 and 14.82 μm were noticed. Microspherosomes, with a tendency to agglutinate, were visible inside them, suggesting a possible double encapsulation. In I3 (**figure 11.14 h**), dense, large, irregular clusters with dimensions between 64.43 and 102.10 μm were observed, possibly resulting from the confluence of microspherosomes with variable diameters (4-5 μm on average).

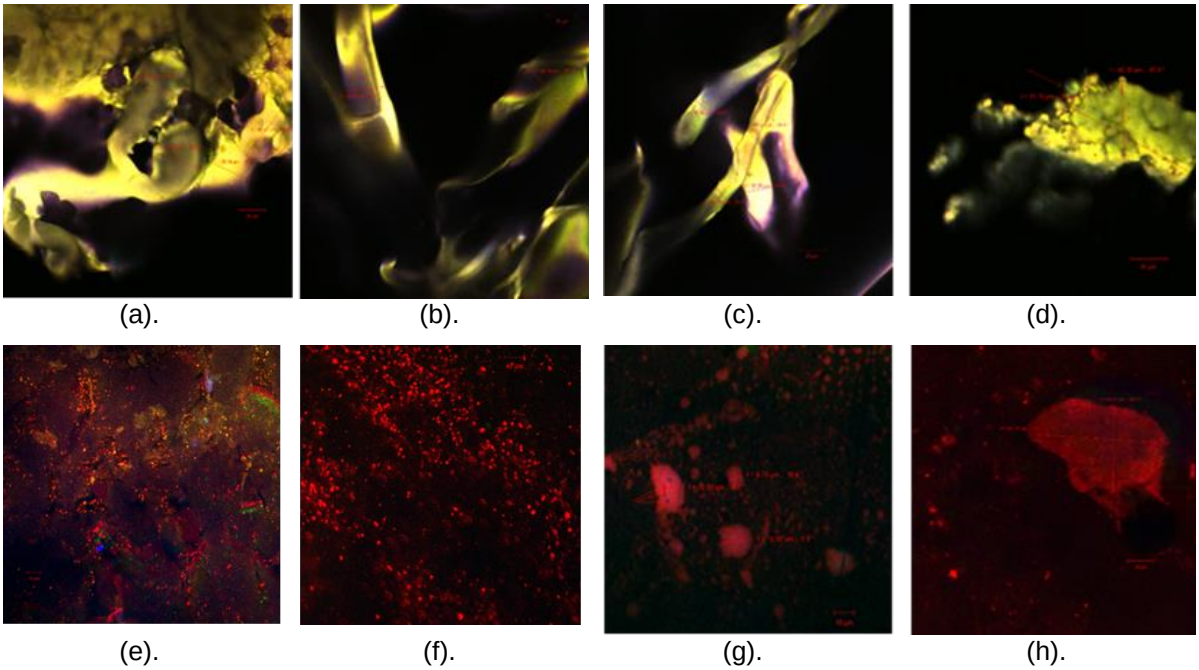
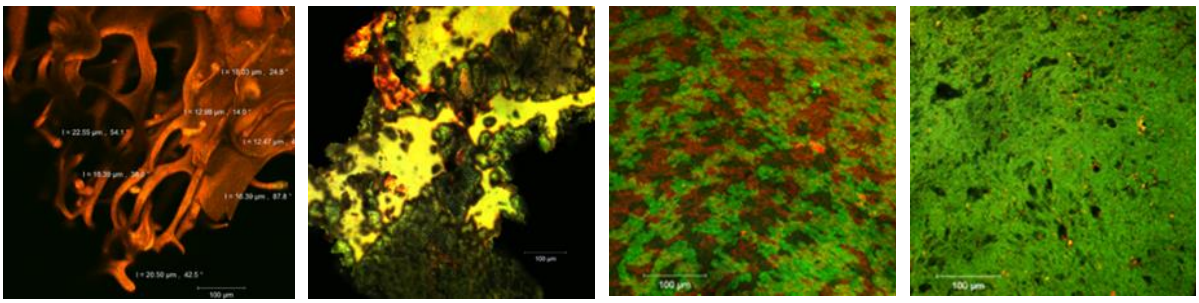


Figure 11.14. Morphological structure of powders designed by freeze-drying technique with the variation of the encapsulation materials' ratio: Control - CMC/P/WPI in 1:1:0.4 ratio (a - native, e - coloured); I1 - CMC/P/Hp(T) in 1:1:0.4 ratio (b - native, f - coloured); I2 - CMC/P/Hp(T) in 2:1:0.4 ratio (c - native, g - coloured), and I3 - CMC/P/Hp(T) in 1:2:0.4 ratio (d - native, h - coloured)

Morphological structure of the powders realized by the gelation technique

Figure 11.15 shows that the bioactive in the eggplant exocarp, by encapsulation, generated a digitiform appearance, laced in GV1 (**figure 11.15 a**) or compact scales, irregular in GV2 (**figure 11.15 b**). Interestingly, the same plant extract acquired different autofluorescent properties, depending on the proportion of biopolymers in the microencapsulating matrix, probably due to the transient bonds created. The higher percentage of proteins in GV1 determined a frameshift of the emission to the range 640-680nm, while in the presence of a richer carbohydrates matrix (GV2), the emission spectrum of phytopigments in the extract was in the green-yellow range (520-540nm).



(a). (b). (c). (d).

Figure 11.15. Morphological structure of powders produced by gelation technique: GV1 – CMC/P/WPI IN A 1:1:2 ratio (a - native, c - coloured) and GV2 – CMC/P/WPI in a 1:1:0.6 (b - native, d - coloured)

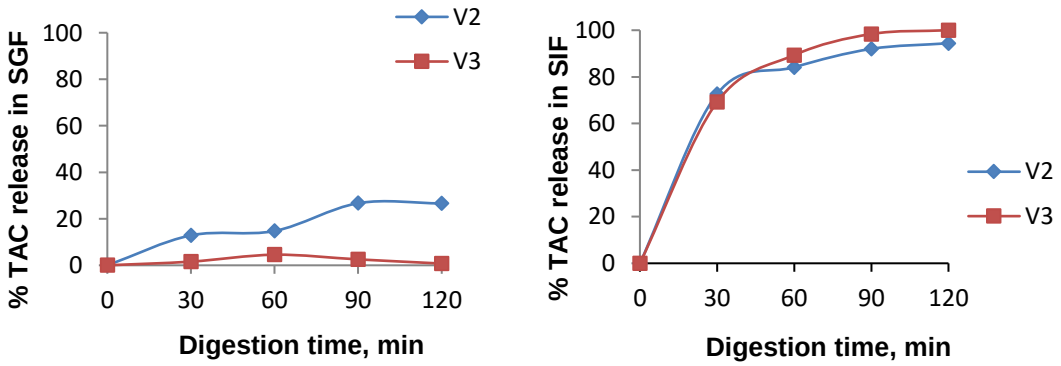
The microcapsules formed more or less homogeneous biofilms by marking them with Congo red (**figure 11.15 c** and **d**). Bioactive compounds (in green) were observed in the predominant protein network (in red) in the GV1 sample matrix (**figure 11.15 c**). As the protein content decreased and the carbohydrate polymer content in the matrix increased, the powder became more hydrophilic, finer, and more homogeneous, and the Congo red fluorescent label was weaker (**figure 11.15 d**).

11.4.4. *In vitro* digestibility of the anthocyanins from powders

In vitro digestibility of anthocyanins from the powders realized by freeze-drying technique with the variation of the encapsulation materials

The release of TAC from microencapsulated ingredients by freeze-drying was studied by *in vitro* simulation of gastric and intestinal digestion (**figure 11.16**).

Following the simulation of the gastric digestion of the ingredients from the eggplant peel (**figure 11.16 a**), an anthocyanin increase of 26.61% of V2 was observed after 2 h of digestion. In the case of V3, the matrix consisting only of proteins and peptides allowed a maximum increase of total anthocyanins of 4.55% after 60 min of digestion. In addition, in the case of simulated gastric digestion, the matrix of proteins and peptides (V3) had a greater protective effect on anthocyanins in eggplant peels than the matrix of carbohydrates only (V2). However, this changed during simulated intestinal digestion (**figure 11.16 b**). In this case, V3 led to the complete release of the anthocyanins from the matrix after 2 h of digestion, while for the anthocyanins from V2, the release took place more slowly, being only 94.41% after the 2 h of simulated digestion. This may be due to the type of bonds between anthocyanins and the materials used for encapsulation.

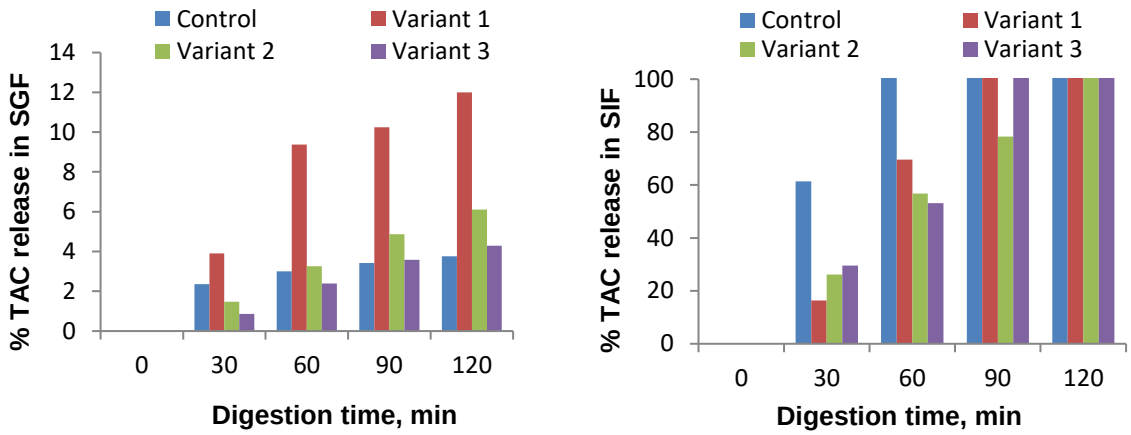


(a). (b).

Figure 11.16. *In vitro* gastric (a) and intestinal (b) digestibility of powders produced by freeze-drying technique with the variation of the encapsulation materials; V2 – CMC + P; V3 – WPI + Hp(P);

In vitro digestibility of anthocyanins from the powders prepared by freeze-drying technique with the variation of the encapsulation materials' ratio

The release of anthocyanins from the capsules was achieved *in vitro* by simulating gastric and intestinal digestion. *In vitro* digestibility in SGF proved that the selected encapsulation matrices showed a protective effect on anthocyanins in all experimental variants (**figure 11.17**). A maximum increase of up to 12.43 ± 0.15% after 120 min of digestion was observed in the case of I1 for the TAC due to matrix release. Among the samples investigated, in the case of control and I3, the highest protective effect was noticed, with a slight decrease of a maximum of 3.76 ± 0.09% and 4.28 ± 0.01%, respectively, after 120 min of digestion (**figure 11.17 a**).



(a). (b).

Figure 11.17. *In vitro* gastric (a) and intestinal (b) digestibility of powders produced by freeze-drying technique with the variation of the encapsulation materials' ratio: control (CMC/P/WPI in 1:1:0.4 ratio); variant 1 (I1 - CMC/P//Hp(T) in 1:1:0.4 ratio); variant 2 (I2 - CMC/P/Hp(T) in 2:1:0.4 ratio); variant 3 (I3 - CMC/P/Hp(T) in 1:2:0.4 ratio);

In SIF, the results showed that a maximum amount of anthocyanins was released after 120 min of digestion in all ingredients, with a maximum value recorded for I3 of 41.47 ± 1.40%, followed by I2 with 25, 86 ± 1.85%, and I1 by 22.40 ± 1.13%, respectively (**figure 11.17 b**).

11.4.5. *In vitro* biocompatibility of powders

Testing the in vitro biocompatibility of powders designed by freeze-drying technique with the variation of the encapsulation materials' ratio

The results found after 24 h and 48 h of cultivation are exhibited in **table 11.7** and **11.8**.

Table 11.7. *Viability of the cultured fibroblast cells in the presence of the microparticles for 24 h, determined by the Neutral Red method (percentage of control culture, %)*

Concentration (µg/mL)	10	50	100	250	500	750	1000
Control	118.34	108.92	113.89	117.56	116.41	85.28	82.73
I1	110.47	102.83	102.83	104.05	101.65	100.47	98.66
I2	116.80	115.35	105.39	113.64	106.20	100.47	94.95
I3	112.89	111.49	117.08	109.58	79.25	49.96	21.71

Control – CMC/P/WPI in 1:1:0.4 ratio, I1 - CMC/P/Hp(T) in 1:1:0.4 ratio; I2 - CMC/P/Hp(T) in 2:1:0.4 ratio; I3 - CMC/P/Hp(T) in 1:2:0.4 ratio;

After 24 h of L929 fibroblast cell culture in the presence of the samples, the cell viability was high, with values between 79.25 % and 118.34 %, except for I3. Thus, all samples were

non-cytotoxic in L929 fibroblast culture (cell viability values >80 %), in the range of tested concentrations, between 10-1000 µg/mL, except for I3.

Table 11.8. *Viability of the cultured fibroblast cells in the presence of the microparticles for 48 h, using the Neutral Red method (percentage of control culture, %)*

Concentration (µg/mL)	10	50	100	250	500	750	1000
Control	103.65	100.18	103.36	100.13	99.95	98.47	99.32
I1	117.36	114.50	111.63	111.79	108.38	116.07	114.93
I2	106.89	114.66	118.71	115.29	108.21	107.51	91.44
I3	100.37	101.19	101.53	102.75	88.24	28.42	8.09

Control – CMC/P/WPI in 1:1:0.4 ratio, I1 - CMC/P/Hp(T) in 1:1:0.4 ratio; I2 - CMC/P/Hp(T) in 2:1:0.4 ratio; I3 - CMC/P/Hp(T) in 1:2:0.4 ratio;

After 48 h of culture, all samples were non-cytotoxic in L929 fibroblast culture (cell viability values > 80 %), in the range of tested concentrations, between 10-1000 µg/mL, except for I3 for which it was observed, at concentrations higher than 500 µg/mL, a sudden decrease in cell viability up to 8.09 %. The results demonstrate that I3 was biocompatible only in the range of 10-500 µg/mL and cytotoxicity at higher concentrations.

Cell morphology was analyzed after 48 h of culture in the presence of different particle concentrations and the images are shown in **figure 11.18**.

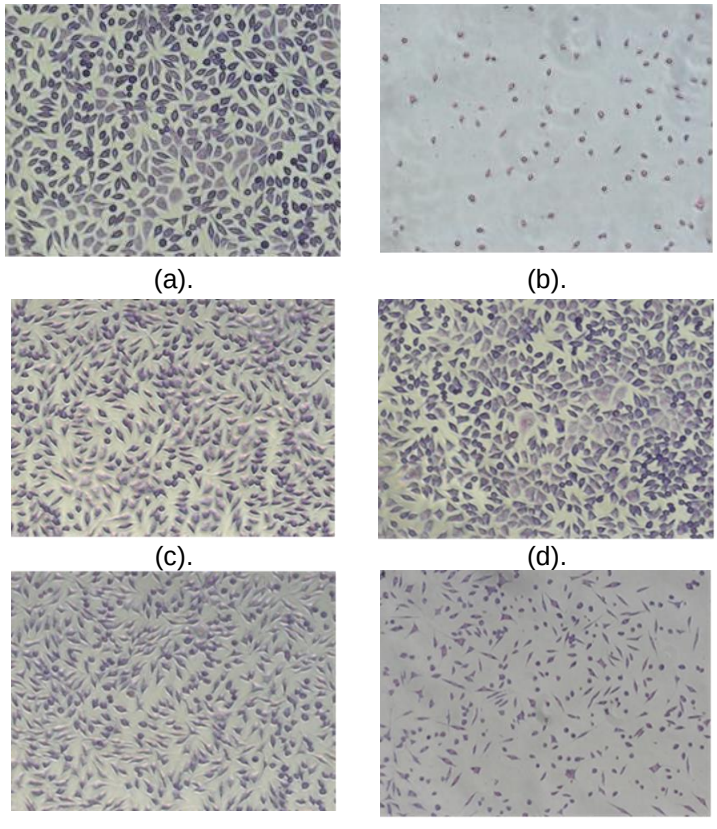


Figure 11.18. Optical microscopy images of fibroblast cells cultured in the presence of powders for 48 h, under standard conditions (37 ºC, atmosphere with 5 % CO_2) by Giemsa staining: Blank (a); H_2O_2 (b); Control - 1000 µg/mL (c); I1 – 1000 µg/mL (d), I2 - 1000 µg/mL (e), I3 - 1000 µg/mL (f)

Optical microscopy images illustrated that the anthocyanin-treated cells maintained the normal fusiform phenotype, characteristic of fibroblast cells, similar to the untreated culture control. The cells were evenly distributed on the culture plate, and the cell density was similar

to or even higher compared to the untreated culture control. In contrast, cultures of fibroblasts treated with 0.003 % H_2O_2 and I3 with a concentration of 1000 µg/mL showed an altered morphology, with numerous degenerated and lysed cells, with a low cell density, which indicated a cytotoxic effect of the sample (**figure 11.18**). These observations confirmed the quantitative data achieved by the Neutral Red method.

Testing the in vitro biocompatibility of powders produced by the freeze-drying technique with the variation of the lactic acid bacteria strains

The *in vitro* biocompatibility results of the powders produced by the freeze-drying technique with the variation of the lactic acid bacteria strains are presented in **tables 11.9** and **11.10.** The results of the *in vitro* biocompatibility assessment demonstrated that all variants were biocompatible because, compared to the control sample (cells in culture medium), cells cultured in the presence of functionalized ingredients showed high viability values and proliferation, and their morphology in culture was normal, characteristic of the fibroblast cell phenotype.

Table 11.9. *Viability of the cultured fibroblast cells in the presence of functionalized ingredients for 24 h, determined by the Neutral Red method (percentage of control culture, %)*

Concentration (µg/mL)	10	50	100	250
Control	92.37	94.87	96.30	90.58
VB	96.43	101.50	103.18	102.43
VBP	107.16	108.78	109.16	111.69

Control – WPI + CMC + P + Hp(β-LgT); VB – WPI + CMC + P + Hp(β-LgT) + *L. bifermentans*; VBP - VB – WPI + CMC + P + Hp(β-LgT) + *L. bifermentans* + *L. plantarum* (1:1);

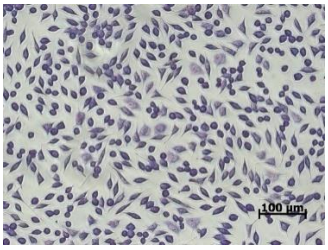
Table 11.10. *Viability of the cultured fibroblast cells in the presence of microparticles for 48 h, determined by the Neutral Red method (percentage of control culture, %)*

Concentration (µg/mL)	10	50	100	250
Control	110.25	108.85	107.06	103.66
VB	104.79	103.08	101.02	94.03
VBP	92.17	94.06	92.78	93.41

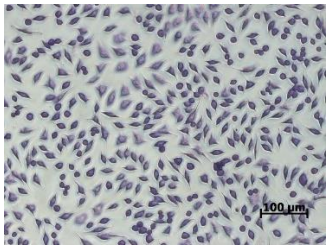
Control – WPI + CMC + P + Hp(β-LgT); VB – WPI + CMC + P + Hp(β-LgT) + *L. bifermentans*; VBP - VB – WPI + CMC + P + Hp(β-LgT) + *L. bifermentans* + *L. plantarum* (1:1);

From **tables 11.9** and **11.10**, it can be pointed out that the control sample was compatible in the range of 10-250 µg/mL, both after 24 h and 48 h of cultivation.

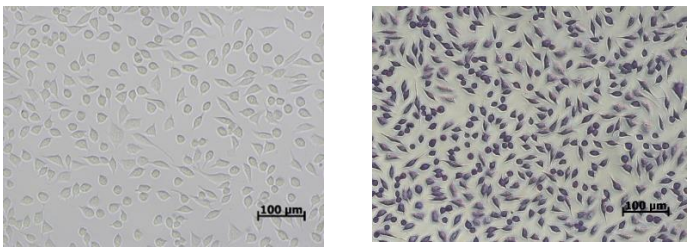
Cell morphology was analyzed after 48 h of cultivation in the presence of microencapsulated extracts (**figure 11.19**). The images displayed that the cells treated with 10-250 µg/mL microencapsulated eggplant peel extracts maintained their normal fusiform phenotype, characteristic of fibroblast cells, similar to the untreated culture.



(a).



(b).



(c). (d).

Figure 11.19. Optical microscopy images of fibroblast cells cultured in the presence of powders for 48 h, under standard conditions (37 ºC, atmosphere with 5 % CO_2) by Giemsa staining: Blank (a); Control - 250 µg/mL (b); VB – 250 µg/mL (c), VBP - 250 µg/mL (d)

11.5. Partial conclusions

The results of this study support the multifunctionality of the ingredients designed by encapsulating the ethanolic extract from eggplant peels. Two encapsulation methods were tested: the freeze-drying and gelation combined with the freeze-drying techniques. A combination of encapsulation matrices that exploit the biological, functional, and technological potential of whey proteins, peptides, CMC, and P was used. Thus, in the case of freeze-drying encapsulation, several experimental microcapsules were made in which the encapsulation materials and their concentrations were varied. Variants have also been designed in which different types of protein hydrolysates and strains of lactic acid bacteria have been added.

The results allowed the elaboration of the following partial conclusions:

✓ The encapsulation methods and materials successfully encapsulated the biologically active compounds from the eggplant peel and LAB, respectively.

✓ The microparticles had high encapsulation efficiencies and content of biologically active compounds, having high antioxidant activity, and also high LAB viability where applicable.

✓ Higher concentrations of polysaccharides provided higher EE than proteins, for both freeze-drying and gelation methods.

✓ The best results in terms of EE, phytochemical contents, and antioxidant activity were observed for the ingredients developed by freeze-drying, then gelation combined with freeze-drying.

✓ The higher DH of Hp did not positively influence the encapsulation efficiency of anthocyanins, with higher EE being noticed when a Hp with a lower DH was used.

✓ Relatively good stability was observed in all powder variants.

✓ The high encapsulation efficiency of anthocyanins from the powder variants was also confirmed by CLSM analysis.

✓ Microcapsules were produced with dimensions ranging between 5 – 100 µm.

✓ A release of anthocyanins from powders was noticed in the simulated gastrointestinal tract conditions, leading to the conclusion that the selected encapsulation methods together with the chosen parameters lead to an increase in the bioavailability of anthocyanins from eggplant peels.

✓ The tested microparticles were cytocompatible, stimulating the proliferation of the fibroblast cell culture.

CHAPTER 12. TECHNOLOGY DEVELOPMENT FOR ADDED-VALUE FOOD

12.2. Objectives of the study

This study aimed to design value-added food products by adding functional food ingredients prepaired by the microencapsulation of the eggplant peel extract.

The main objectives of this study were to realize:

- value-added meringues;
- value-added ice cream;
- value-added dessert sauce;
- characterize the value-added food products in terms of phytochemical content, antioxidant activity, phytochemicals stability, and texture;

12.4. Results and discussion

12.4.1. Phytochemical characterization and storage stability of phytochemicals from value-added meringues

To verify the functionality of the microencapsulated ingredients by varying the protein hydrolysate, they were used to formulate a food product, namely meringues, in a proportion of 2 %. Three experimental meringues, corresponding to the three variants, coded B1, B2, and B3, were prepaired. The samples were then analyzed for initial TAC and antioxidant activity and after storage for 7 days at room temperature (**table 12.1**). Results were included in patent request A25042/05.10.2018.

Table 12.1. *Variation of TAC (µg D3G/g dw) and antioxidant activity (µM TE/g dw) in meringue variants, during 7 days of storage at 25ºC*

Characteristics	Storage time, days	Meringues		
		B1	B2	B3
TAC (µg D3G/g dw)	0	28.33 ± 2.26^{a}	36.49 ± 1.46^{a}	92.11 ± 2.28^{a}
	7	13.45 ± 5.17^{b}	19.76 ± 1.67^{b}	11.18 ± 0.12^{b}
Antioxidant activity (µM TE/g dw)	0	7.02 ± 0.19^{a}	8.41 ± 0.27^{a}	8.84 ± 0.14^{a}
	7	1.35 ± 0.24^{b}	2.04 ± 0.67^{b}	1.03 ± 0.24^{b}

Variations in phytochemicals over time are highlighted using a lowercase letter per column. Values that share a letter are not significantly different (*p>0.05*) according to the ANOVA test; B1 – control without encapsulated eggplant peel extract: B2 - meringues with 2 % VCT microcapsules; B3 – meringues with 2 % VT powder;

From **table 12.1** can be seen that the products containing hydrolysates (B2 and B3) had a higher concentration of anthocyanins than B1 (without hydrolysate). The same behavior can be observed for the antioxidant activities. Regardless of the experimental variant, meringues with the addition of microcapsules showed a high TAC and antioxidant activity. In addition, these experimental meringues presented low stability of phytochemicals, with TAC decreasing by 46 % in B2 and 88 % in B3, while the antioxidant activity decreased by 76 % for B2 and 88 % for B3 after 7 days of storage at room temperature (**table 12.1**).

12.4.2. Phytochemical characterization and storage stability of phytochemicals from value-added ice cream

The experimental variants of functionalized co-microencapsulated ingredients were added in a proportion of 2 % in ice cream. The initial phytochemical profile and viability of LAB during storage, taking into account a shelf life average of 21 days, were tested. The products

were stored in containers with UV protection at -18 °C. The ice cream kept the coding system for the ingredients, adding the letter I to avoid confusion. Thus, IVB contains powder with *L. bifermentans*, while IVBP contains ingredients with a co-culture of *L. bifermentans* and *L. plantarum*. The initial results and after 21 days of storage are detailed in **table 12.2**. Results were included in patent request A00481/01.08.2019.

Table 12.2. *Phytochemical and functional characteristics of value-added ice cream*

Phytochemical characteristics	Storage time, days	IM	IVB	IVBP
TAC (mg D3G/100 g dw)	0	-	2.26 ± 0.14^{bB}	3.92 ± 0.33^{aB}
	28	-	6.97 ± 0.39^{bA}	8.97 ± 0.16^{aA}
TPC (mg GAE/100 g dw)	0	-	85.16 ± 4.02^{bD}	99.65 ± 7.06^{aD}
	28	-	139.76 ± 5.42^{bC}	207.12 ± 8.18^{aC}
Antioxidant activity (µM TE/100 g dw)	0	142.97 ± 6.85^{cF}	389.49 ± 29.51^{bF}	496.90 ± 44.52^{aF}
	28	449.81 ± 4.72^{bE}	831.82 ± 79.01^{aE}	924.67 ± 42.94^{aE}
LAB viability (CFU/g dw)	0	-	$5.55 \times 10^{7\ aG}$	$5.05 \times 10^{7\ aG}$
	28	-	4.80×10^{7H}	1.60×10^{7H}

The values that share one letter per column/line are not significantly different (*p>0.05*) according to the ANOVA test. The lowercase letters on the line highlight the differences between the samples in terms of phytochemical content before storage; Uppercase letters on the column highlight storage stability of phytochemicals; IM - ice cream without eggplant peel extract; IVB - ice cream with 2 % of VB with *L. bifermentans*; IVBP - ice cream with 2 % VBP with *L. bifermentans* and *L. plantarum* (1:1).

According to the results from **table 12.2**, significant differences between the three ice creams in phytochemical content can be noted. Thus, significantly higher concentrations of anthocyanins and polyphenols were observed in the variant IVBP compared to IVB (*p<0.05*). In addition, as expected, the phytochemical compounds analyzed were not detected in the powder-free sauce. In contrast, in terms of antioxidant activity, the control ice cream had the lowest value, while IVBP showed the highest scavenging activity, with 22 % higher than IVB (**table 12.2**). Both technological ice creams functionalized with LAB displayed a concentration of bacteria of order 7 log/g product. In the selected matrix, freezing storage led to a significant release of biologically active compounds from co-microcapsules, leading to double antioxidant activity values for the enriched variants. Lactic bacteria have maintained their viability in storage for the variant containing both lactobacilli species (**table 12.2**).

12.4.3. Phytochemical characterization and storage stability of phytochemicals from value-added dessert sauce

The functionality of the topping was tested by adding it to a food matrix. The technological variant of the chosen ingredient was the powder designed by freeze-drying which had only carbohydrates (CMC and P) as encapsulation materials due to the high anthocyanin content compared to the other ones. The food matrix chosen was a topping for various desserts, and the ingredient was added in a ratio of 0.5 and 1 %. Three sauces were prepaired, one of which was the control without added ingredients. The phytochemical profile of the topping was analyzed both immediately and after an average storage period of 28 days at 4 ºC. The sauces were also analyzed in terms of texture.

The value-added topping was analyzed from a physicochemical point of view, the results being presented in **table 12.3**. Results were included in patent request A00532/08.10.2021.

Table 12.3. *Physico-chemical characteristics of value-added topping*

Sample	Proteins (g/100 g)	Lipids (g/100 g)	Glucides (g/100 g)	Humidity (g/100 g)	Fibers (g/100 g)	Energetic value (kcal)
M	4.24 ± 0.15	7.90 ± 0.61	66.05 ± 2.21	21.80 ± 0.20	8.10 ± 0.05	361.65 ± 8.57
T1	4.24 ± 0.14	7.91 ± 0.99	66.74 ± 3.41	21.11 ± 0.25	9.38 ± 0.17	364.57 ± 7.99
T2	4.24 ± 0.21	7.91 ± 0.79	67.22 ± 2.75	20.63 ± 0.20	9.86 ± 0.36	366.54 ± 8.12

M- topping without added powder; T1 – topping with 0.5 % added V2 powder; T2 – topping with 1 % added V2 powder; V2 - CMC + P (1:1);

Table 12.3 shows that the addition of the ingredient designed by microencapsulating the bioactive compounds from the eggplant peel extract into the dessert topping had an important contribution to the fiber content. It increased by 14 % and 18 % compared to the control, respectively. There was also a slight increase in the carbohydrate content in both toppings with added ingredients.

The sauces were stored in glass jars with airtight lids at 4 °C for 28 days. The results of the phytochemical characterization of the three topping variants, before and after storage over time, are shown in **table 12.4**. Results were included in patent request A00532/08.10.2021.

Table 12.4*. Phytochemical content of dessert sauces enriched with microcapsules before and after storage at 4 °C for 28 days*

Sample	Time days	TAC (µg D3G/100 g dw)	TFC (mg CE/100 g dw)	TPC (mg GAE/100 g dw)	Antioxidant activity (µM TE/100 g dw)
M	0	nd	nd	nd	32.63 ± 1.02^{aA}
	28	nd	nd	nd	26.54 ± 7.60^{B}
T1	0	147.17 ± 19.83^{a}	21.84 ± 1.12^{aA}	90.12 ± 3.60^{aA}	94.78 ± 2.74^{bC}
	28	nd	8.59 ± 0.40^{B}	51.61 ± 2.46^{B}	99.83 ± 3.42^{C}
T2	0	78.56 ± 6.18^{bA}	11.70 ± 1.39^{bC}	62.42 ± 3.97^{bC}	161.84 ± 4.19^{cE}
	28	128.74 ± 14.01^{B}	17.40 ± 2.64^{C}	37.15 ± 1.58^{D}	181.29 ± 6.15^{F}

The values that share one letter per column/line are not significantly different ($p>0.05$) according to the ANOVA test. The lowercase letters highlight the differences between the samples in terms of phytochemical content before storage. Uppercase letters show changes in phytochemical content after storage at 4 ° C for 28 days; M- topping without added powder; T1 – topping with 0.5 % added V2 powder; T2 – topping with 1 % added V2 powder; V2 - CMC + P (1:1);

According to the results from **table 12.4**, significant differences between the three toppings in phytochemical content can be noted. Thus, significantly higher concentrations of anthocyanins, flavonoids, and polyphenols were observed in the variant with 0.5 % microcapsules (T1) compared to the topping with 1 % ($p<0.05$). In addition, as expected, the phytochemical compounds analyzed were not detected in the powder-free sauce. In contrast, in terms of antioxidant activity, the control sauce had the lowest value, and there were no statistically significant differences between the two variants of powdered sauce ($p>0.05$). Although T2 had the highest particle concentration, it showed the lowest values of phytochemicals. This can be explained by the fact that probably in T1, being less ingredient, it solubilized faster than in T2.

After storing the sauces at 4 °C, significant changes in the content of phytochemicals were observed after 28 days ($p<0.05$). Regarding T1, after 28 days of storage, the concentration of anthocyanins decreased until no longer detected spectrophotometrically. **Table 12.4** also details a significant decrease in TFC and TPC in T1 ($p<0.05$), but the antioxidant activity remained constant. On the other hand, the concentration of anthocyanins in T2 increased significantly in the 28 days ($p<0.05$), probably due to the complete solubilization of the powder. Although in the case of this sauce, TFC remained constant during

the 28 days of storage, TPC decreased significantly ($p<0.05$). The increase in TAC can be correlated with antioxidant activity, the latter showing a significant increase after 28 days of storage ($p<0.05$).

12.4.4. Texture characterization of value-added dessert sauce

Table 12.5 lists the values of the analysis of the textural parameters of the dessert sauce enriched with functional ingredients from eggplant peel. Results were included in patent request A00532/08.10.2021.

Table 12.5. *Textural parameters of sauce samples with the addition of eggplant peel powder*

Textural parameters	M	T1 (0.5 %)	T2 (1 %)
Firmness (N)	1.59	1.97	2.58
Adhesion (mJ)	10.41	16.38	16.66
Cohesiveness	0.65	0.57	0.53
Elasticity (mm)	14.49	13.16	13.27

M- topping without added powder; T1 – topping with 0.5 % added V2 powder; T2 – topping with 1 % added V2 powder; V2 - CMC + P (1:1);

Firmness is the maximum resistance force opposed by the sample during penetration. From **table 12.5**, it can be noticed that the addition of powder from eggplant peel extract improved the firmness by approximately 24 % in T1, and by 62 % in T2. The increase in firmness can be associated with the increase in resistance to deformation, which indicates good stability. Adhesion is defined as the energy required to remove the sample from the test device. The lowest adhesion value of 10.41 mJ was recorded for the control (M), which indicates that the addition of powder increased the adhesion of the sauce. Cohesion is a measure of the internal bonds between the constituent elements of the sauce. This parameter recorded values inversely proportional to the concentration of addition, which indicated an improved capacity for disintegration during chewing. Elasticity is the ability of the samples to recover the deformation. This parameter recorded the highest value for the M.

12.5. Partial conclusions

The previous ingredients were tested in terms of functionality and food matrices, and the ones chosen were meringues, ice cream, and dessert sauce.

The results allowed the elaboration of the following partial conclusions:

✓ Compared with the control variants, the value-added products presented functionalities through a high content of biologically active compounds and antioxidant activity.

✓ The multifunctional ingredients showed good stability in the tested food systems, their use is not limited by the complexity of the matrix, except for the meringues where a decrease in the total anthocyanin content and antioxidant activity was observed.

✓ The viability of lactic acid bacteria was maintained above 10^7 for both ice cream variants.

✓ The addition of the ingredient realized by freeze-drying using the bioactive compounds from the eggplant peel and carboxymethyl cellulose and pectin as encapsulation materials into the dessert topping increased 14-18 % of the topping fiber content compared to the control sample.

✓ The texture analysis of the toppings revealed that the addition of powder designed by microencapsulating the eggplant peel extract improves the textural characteristics of the sauce.

CHAPTER 13. GENERAL CONCLUSIONS

Nowadays, a balanced diet represents the main objective to prevent deficiencies and diet-related diseases. The major challenge of science is to improve life quality. In this context, the approaches presented in the doctoral thesis entitled „**Designing novel ingredients based on eggplants' bioactive compounds for different applications in the food industry**" aimed to identify, quantify, and valorize the biologically active compounds from eggplant peels along with whey peptides, and even LAB. The development of potential functional ingredients for use in foods with health benefits, using the biologically active compounds from eggplant peels and whey peptides, represents the ultimate goal of this study.

Therefore, the main objectives of the doctoral thesis were the:

- Quantification and identification of biologically active compounds from eggplant peels produced by various extraction methods in which the parameters were varied to establish the optimal extraction conditions.
- Microencapsulation of biologically active compounds from eggplant peels for integration into functional foods to produce ingredients with high functionality by increasing their stability and controlled release.
- Development of value-added food technologies by adding functional microencapsulated ingredients.

All three objectives were achieved, which allowed the formulation of the following general conclusions:

- Four extraction methods were compared in terms of the content of biologically active compounds, with an emphasis on anthocyanins, in which the solvent concentration, acid, extraction time, and temperature were varied.
- The chromatographic profile of the eggplant peel extracts indicated the presence of five anthocyanins, derivatives of delphinidin, cyanidin, and petunidin. However, the major compound in the extracts selected for conventional, ultrasound-assisted, and microwave-assisted was delphinidin 3-*O*-glucoside.
- Thermal processing stability testing and simulated *in vitro* digestibility have shown increased instability of anthocyanins in extracts.
- The process of enzymatic hydrolysis of whey proteins has led to peptides with varying degrees of hydrolysis, antioxidant activity, and prebiotic effect.
- Fluorescence spectroscopy techniques were used for the characterization of the binding mechanisms between whey proteins and anthocyanins from eggplant peel extract, as the preliminary stage of microencapsulation, to predict the optimal conditions and the peculiarities of binding from the perspective of the preparation of composites with functionality.
- This instability has led to techniques to increase the stability of the compounds in the eggplant peel extract that are based on the microencapsulation process. Thus, freeze-drying and gelation have led to fine particles with high biological activity, especially antioxidant activity.
- The ingredients were enriched with peptides and, in some cases, lactic acid bacteria.
- The microparticles had different morphological structures and sizes due to different encapsulation methods and materials. Increased stability of anthocyanins in the simulated gastrointestinal system was also observed, the powders stimulating cell proliferation in the tested concentration range.
- The functionality was tested by adding the ingredients to meringues, ice cream, and dessert sauce. Powders have been shown to add value to products in which they have been added by increasing the content of biologically active compounds and antioxidant activity, in some cases positively influencing the texture.

- Therefore, the study demonstrated the potential development of multifunctional ingredients based on biologically active compounds from eggplant peels, whey peptides, and lactic acid bacteria for applications in the food industry.
- However, further study of these ingredients is needed to demonstrate their effectiveness on human health, such as antidiabetic and anti-inflammatory effects, etc.

CHAPTER 14. ORIGINAL CONTRIBUTIONS AND PROSPECTS FOR FURTHER STUDIES

14.1. Original contributions

In this study, the original contributions are:

- Extraction and analysis of the composition in biologically active compounds and antioxidant activity of eggplant peel.
- The study of the degradation kinetic parameters is needed to optimize industrial-technological processes conditions and minimize the loss or degradation of bioactive components with a functional role of thermally treated food products.
- Study of the behaviour of bioactive compounds from eggplant peel extracts during digestion to increase bioactivity and bioavailability in the finished product to be absorbed and thus exert possible beneficial effects on the human body.
- The biochemical investigation by fluorescence spectroscopy to study the stability of anthocyanin-protein complexes in order to formulate functional ingredients.
- Producing peptides from whey proteins with biological and prebiotic properties.
- Formulation of ingredients based on biologically active compounds from eggplant peels, enriched with peptides and lactic acid bacteria, with high phytochemicals and antioxidant activity that ensure their good stability in the gastrointestinal system and controlled release.
- Testing powders in real food matrices to highlight their quality as a functional ingredient.

14.2. Prospects for further studies

The future perspectives are:

- Rheological analysis of the food products for which this investigation is suitable.
- Achieving the colourimetric profile of microparticles and value-added products.
- Testing the antidiabetic, anti-inflammatory, anti-hyperlipidemia, and anti-tumoral effects of the powders since they are a complex matrix of compounds.
- Testing of the value-added products in terms of sensory properties.
- Study of the biologically active compounds from heat-treated eggplant peels as they would result from the canning industry and comparison with the results obtained on non-heat-treated peels.
- Study of the food safety assessment of the obtained ingredients in terms of emerging contaminants (pesticides, toxins, heavy metals, nanoplastics). The contaminants' possible transfer from the raw material to the valorized product needs to be assessed.

CHAPTER 15. DISSEMINATION OF RESULTS

A. Articles

A.1. ISI – articles with impact factor

1. **Condurache Nina Nicoleta**, Croitoru Constantin, Enachi Elena, Bahrim Gabriela Elena, Stănciuc Nicoleta, Râpeanu Gabriela. *Eggplant peels as a valuable source of anthocyanins: Extraction, thermal stability, and biological activities*. **Plants 2021**, 10(3), pp. 1 – 17, DOI 10.3390/plants10030577, **Q1** – **4.654 impact factor**.
2. **Condurache Nina Nicoleta**, Aprodu Iuliana, Grigore-Gurgu Leontina, Petre Brindusa Alina, Enache Elena, Râpeanu Gabriela, Bahrim Gabriela Elena, Stănciuc Nicoleta. *Fluorescence spectroscopy and molecular modeling of anthocyanins binding to bovine lactoferrin peptides.* **Food Chemistry 2020**, 318, 126508, DOI https://doi.org/10.1016/j.foodchem.2020.126508, **Q1** – **9.231 impact factor**.
3. **Condurache Nina Nicoleta**, Aprodu Iuliana, Crăciunescu Oana, Tatia Rodica, Horincar Georgiana, Barbu Vasilica, Enachi Elena, Râpeanu Gabriela, Bahrim Gabriela Elena, Oancea Anca, Stănciuc Nicoleta. *Probing the Functionality of Bioactives from Eggplant Peel Extracts Through Extraction and Microencapsulation in Different Polymers and Whey Protein Hydrolysates*. **Food and Bioprocess Technology 2019**, 12, pp. 1316–1329, DOI https://doi.org/10.1007/s11947-019-02302-1, **Q1** – **5.581 impact factor**.

A.2. ISI-indexed articles

1. **Condurache Nina Nicoleta**, Turturică Mihaela, Enachi Elena, Barbu Vasilica, Bahrim Gabriela Elena, Stănciuc Nicoleta, Croitoru Constantin, Râpeanu Gabriela. *Impact of wall materials on physico-chemical properties and stability of eggplant peels anthocyanin hydrogels*. **Inventions 2021**, 6(3), 47, DOI 10.3390/inventions6030047, **ISI indexed**.

B. Patent requests

1. **Condurache (Lazăr) Nina Nicoleta**, Râpeanu Gabriela, Stănciuc Nicoleta, Andronoiu Georgeta Doina, Bahrim Gabriela Elena. *Dessert sauce with the addition of microencapsulated powder from anthocyanin extract from eggplant peel (Solanum melongena L.) - value-added product and production technology*. **Request registration no. OSIM A/00532/2021**.
2. **Condurache Nina Nicoleta**, Bahrim Gabriela Elena, Râpeanu Gabriela, Stănciuc Nicoleta. *Multifunctional natural ingredients based on anthocyanin extracts from eggplant peels, co-microencapsulated lactic acid bacteria, and their applications*. **Request registration no. OSIM A/00481/2019.**
3. **Condurache Nina Nicoleta**, Bahrim Gabriela Elena, Râpeanu Gabriela, Stănciuc Nicoleta. *Microencapsulated ingredients based on anthocyanin extracts from eggplant husks and high-function bioactive whey peptides for use in the food industry*. **Request registration no. OSIM 25042/2018.**

C. Conferences

C.1. International conferences in Romania

1. **Condurache (Lazăr) Nina Nicoleta**, Râpeanu Gabriela. *Bioeconomical valorization of eggplant peel anthocyanins and whey proteins*. PhD Students' Days Faculty of Food Engineering, Tourism And Environmental Protection The Second Edition, November 25-26, 2022, Arad, Romania, online.
2. **Condurache (Lazăr) Nina-Nicoleta**, Aprodu Iuliana, Bahrim Gabriela Elena, Stănciuc Nicoleta, Râpeanu Gabriela. *Eggplant peel anthocyanins as food enhancers.* „Dunarea de Jos" University of Galati Scientific Conference of Doctoral Schools SCDS – UDJG, 9-10 June 2022, Galati, Romania.

3. **Condurache (Lazăr) Nina-Nicoleta**, Aprodu Iuliana, Bahrim Gabriela Elena, Stănciuc Nicoleta, Râpeanu Gabriela. *Functionalization of food through the incorporation of bioactive compounds from eggplant peels.* „Dunarea de Jos" University of Galati Scientific Conference of Doctoral Schools, 9-10 June 2022, Galati, Romania.
4. **Condurache (Lazăr) Nina Nicoleta**; Aprodu Iuliana; Bahrim Gabriela Elena; Stănciuc Nicoleta; Râpeanu Gabriela. *Anthocyanins and bioactive peptides based multifunctional ingredients: an integrative approach*. „Dunarea de Jos" University of Galati, The 10th International Symposium EuroAliment, Faculty of Food Science and Engineering, Galati 7-8 October 2021, online.
5. **Condurache Nina Nicoleta**, Bahrim Gabriela Elena, Râpeanu Gabriela, Stănciuc Nicoleta. *Ice cream enriched with microencapsulated bioactive compounds from eggplant peels and LAB.* „Dunarea de Jos" University of Galati, Scientific Conference of Doctoral Schools SCDS – UDJG 10-11 June 2021, online.
6. **Condurache Nina Nicoleta**, Bahrim Gabriela Elena, Râpeanu Gabriela, Stănciuc Nicoleta. *Impact of wall materials on phytochemical properties and stability of peels anthocyanin hydrogels*. „Dunarea de Jos" University of Galati, Scientific Conference of Doctoral Schools SCDS – UDJG 10-11 June 2021, online.
7. **Condurache (Lazăr) Nina Nicoleta**, Milea Stefania Adelina, Râpeanu Gabriela, Bahrim Gabriela Elena, Stănciuc Nicoleta. *Innovative natural ingredients with multiple functionalities and applications in the food and pharmaceutical fields*. Ingredients Show by Ro.aliment 17-21 May 2021, online.
8. **Condurache Nina Nicoleta**, Bahrim Gabriela Elena, Râpeanu Gabriela, Stănciuc Nicoleta. *"Natural ingredients with multiple functionality based on anthocyanin extracts from eggplant shells and comic encapsulated lactic acid bacteria and their applications"*. Ingredients Show Bucharest 5-9 October 2020, online.
9. **Condurache Nina Nicoleta**, Aprodu Iuliana, Bahrim Gabriela Elena, Stănciuc Nicoleta, Râpeanu Gabriela. *Studies on binding mechanism between phenolic compounds from eggplant peels and lactoferrin*. "Dunarea De Jos" University Of Galati, Scientific Conference of Doctoral Schools SCDS-UDJG 8th Edition 18-19 June 2020, online.
10. **Condurache Nina Nicoleta**, Enachi Elena, Bahrim Gabriela Elena, Stănciuc Nicoleta, Râpeanu Gabriela. *Influence of parameters on different extraction methods of anthocyanins from eggplant peels*. "Dunarea De Jos" University Of Galati, Scientific Conference of Doctoral Schools SCDS-UDJG 8th Edition 18-19 June 2020, online.
11. Milea Ștefania Adelina, **Condurache Nina Nicoleta**, Râpeanu Gabriela, Bahrim Gabriela Elena, Stănciuc Nicoleta. *Ingredients co-encapsulated with lactic acid bacteria and bioactive compounds*. International Expo-Conference Ingredients Show, 3rd Edition, 17-18 October 2019, Sinaia, Romania.
12. **Condurache Nina Nicoleta**, Râpeanu Gabriela, Stănciuc Nicoleta, Bahrim Gabriela Elena. *Functionality of ingredients obtained by microencapsulation of anthocyanins from eggplant peels with polisaccharides and bioactive peptides*. Dunarea de Jos University of Galati, Faculty of Food Science and Engineering, The 9th International Symposium EuroAliment 5-6 September 2019, Galati, Romania.

C.2. National conferences

1. **Condurache Nina Nicoleta**, Bahrim Gabriela Elena, Râpeanu Gabriela, Stănciuc Nicoleta. *Fluorescence quenching of whey proteins and peptides by eggplant peels' anthocyanins*. Faculty of Food Engineering, Tourism and Environmental Protection Arad, Students Scientific Communication Session 18th Edition, 26-27 November 2020, online.
2. **Condurache Nina Nicoleta**, Râpeanu Gabriela, Bahrim Gabriela Elena, Stănciuc Nicoleta. *Anthocyanins from food by-products as alternative for synthetic additives? A big challenge for food and related industries*. "Spiru Haret" University of Bucharest, Second European One Health Conference 21-22 June 2019, Bucharest, Romania.
3. **Condurache Nina Nicoleta**, Bahrim Gabriela Elena, Stănciuc Nicoleta, Râpeanu Gabriela. *Bioactive compounds and antioxidant activity of extracts from eggplant peel using different extraction methods*. "Dunarea de Jos" University of Galati, Scientific Conference of Doctoral Schools the 7th edition 13-14 June 2019, Galati, Romania.

D. Invention fairs

D.1. International invention fairs

1. **Condurache (Lazăr) Nina-Nicoleta**, Râpeanu Gabriela, Stănciuc Nicoleta, Andronoiu Doina Georgeta, Bahrim Gabriela-Elena. *Dessert topping with antioxidant activity and high fiber content. International Fair of Inventions and Innovations in the field of food "Inovaliment"* 2nd edition, 22-26 November, 2021, online.
2. **Condurache Nina-Nicoleta**, Bahrim Gabriela-Elena, Stănciuc Nicoleta, Andronoiu Doina, Râpeanu Gabriela. *Sweet sauce enriched with fiber and biologically active compounds from eggplant peel - A/00532.* Innovation and Research Salon "UGAL INVENT" 5th edition, 10-12 November, 2021, online.
3. **Condurache Nina Nicoleta**, Bahrim Gabriela-Elena, Stănciuc Nicoleta, Râpeanu Gabriela. *Natural ingredients with multiple functionality based on anthocyanin extracts from eggplant peels co-microencapsulated with lactic acid bacteria and their applications*. *International Fair of Inventions and Innovations in the field of food "Inovaliment" 1st edition*, 23-27 November 2020, online.
4. **Condurache Nina Nicoleta**, Bahrim Gabriela-Elena, Rapeanu Gabriela, Stanciuc Nicoleta. *Natural ingredients with multiple functionality based on anthocyanin extracts from eggplant peels co-microencapsulated with lactic acid bacteria and their applications.* "INFOINVENT„ Specialized International Exhibition 16th Edition, 20 - 23 November 2019, Chisinău, Rep. Moldavia.
5. **Condurache Nina Nicoleta**, Bahrim Gabriela-Elena, Rapeanu Gabriela, Stanciuc Nicoleta. *Natural ingredients with multiple functionality based on anthocyanin extracts from eggplant peels co-microencapsulated with lactic acid bacteria and their applications. „*Dunarea de Jos" University of Galati, „UGAL INVENT" innovation and research show, 16-18 October 2019, Galati, Romania.
6. **Condurache Nina Nicoleta**, Râpeanu Gabriela, Bahrim Gabriela Elena, Stănciuc Nicoleta. *Natural ingredients with multiple functionality based on anthocyanin extracts from eggplant peels co-microencapsulated with lactic acid bacteria and their applications".* Technical University "Gheorghe Asachi" of Iași and National Institute of Inventions Iasi (INI), The 23rd International Exhibition of inventics "Inventica" 26-28 June 2019, Iasi, Romania.

E. Awards and distinctions

1. **Second prize** at PhD Students' Days Faculty of Food Engineering, Tourism And Environmental Protection The Second Edition, November 25-26, 2022, Arad, Romania, for the work *Bioeconomical valorization of eggplant peel anthocyanins and whey proteins*. **Condurache (Lazăr) Nina Nicoleta**, Râpeanu Gabriela.
2. **Honorable mention** at „Dunarea de Jos" University of Galati Scientific Conference of Doctoral Schools, 9-10 June 2022, Galati, Romania, for the work *Functionalization of food through the incorporation of bioactive compounds from eggplant peels.* **Condurache (Lazăr) Nina-Nicoleta**, Aprodu Iuliana, Bahrim Gabriela Elena, Stănciuc Nicoleta, Râpeanu Gabriela.
3. **Mention** at the competition "Awarding the research results of PhD students from IOSUD-UDJG" for 2021 for the article *Eggplant peels as a valuable source of anthocyanins: extraction, thermal stability and biological activities*, published in the journal Plants. **Condurache (Lazăr) Nina-Nicoleta**, Croitoru Constantin, Enachi Elena, Bahrim Gabriela-Elena, Stănciuc Nicoleta, Râpeanu Gabriela.
4. **Silver medal** at „Dunarea de Jos" University of Galati, „UGAL INVENT" Innovation and Research show, 10-12 November 2021, Galati, Romania, for the work *Sweet sauce enriched with fibers and biologically active compounds from eggplant peels*. **Condurache (Lazăr) Nina Nicoleta**, Bahrim Gabriela Elena, Stanciuc Nicoleta, Andronoiu Georgeta Doina.
5. **First prize** at "Dunarea de Jos" University of Galati, Scientific Conference of Doctoral Schools SCDS – UDJG, 10-11 June 2021 for the research *Ice cream enriched with microencapsulated bioactive compounds from eggplant peels and LAB*. **Condurache Nina Nicoleta**, Bahrim Gabriela Elena, Râpeanu Gabriela, Stănciuc Nicoleta.
6. **Diploma of excellence** at the CEREX Gala for excellent results in the research activity within the "Dunărea de Jos" University of Galati.

7. **First prize** at the competition "Awarding the research results of PhD students from IOSUD-UDJG" for 2020 for the article *Fluorescence spectroscopy and molecular modeling of anthocyanins binding to bovine lactoferrin peptides*, published in the journal Food Chemistry.
8. **Special Prize** at the International Fair of Inventions and Innovations in the field of food "Inovaliment" 1st edition, November 23-27, 2020 for the paper *Natural ingredients with multiple functionality based on anthocyanin extracts from eggplant peels co-microencapsulatedwith lactic acid bacteria and their applications*. **Condurache Nina Nicoleta**, Bahrim Gabriela Elena, Stănciuc Nicoleta, Râpeanu Gabriela.
9. **Second prize** in the posters section at Students Scientific Communication Session 18th Edition, Faculty of Food Engineering, Tourism and Environmental Protection Arad, November 26-27, 2020 for the work *Fluorescence quenching of whey proteins and peptides by eggplant peels' anthocyanins*. **Condurache Nina Nicoleta**, Bahrim Gabriela Elena, Râpeanu Gabriela, Stănciuc Nicoleta.
10. **Gold medal** at the International Specialized Exhibition "Infoinvent" 16th edition, Chisinau, Rep. Moldavia, November 20-23, 2019 for the work *Natural ingredients with multiple functionality based on anthocyanin extracts from eggplant peels co-microencapsulated with lactic acid bacteria and their applications*. **Condurache Nina Nicoleta**, Bahrim Gabriela, Rapeanu Gabriela, Stănciuc Nicoleta.
11. **Second prize** at International Expo-Conference Ingredients Show, 3rd Edition, 17-18 October 2019, Sinaia for the paper *Ingredients co-encapsulated with lactic acid bacteria and bioactive compounds.* Milea Ștefania Adelina, **Condurache Nina Nicoleta**, Râpeanu Gabriela, Bahrim Gabriela Elena, Stănciuc Nicoleta.
12. **Mention** at Scientific Conference of Doctoral Schools SCDS-UDJG 8th Edition, "Dunarea De Jos" University Of Galati, 18-19 June 2019 for the paper *Studies on binding mechanism between phenolic compounds from eggplant peels and lactoferrin*. **Condurache Nina Nicoleta**, Aprodu Iuliana, Bahrim Gabriela Elena, Stănciuc Nicoleta, Râpeanu Gabriela.
13. **Second prize** at Scientific Conference of Doctoral Schools the 7th edition, "Dunarea de Jos" University of Galati, 13th-14th June 2019 for the paper *Bioactive compounds and antioxidant activity of extracts from eggplant peel using different extraction methods*. **Condurache Nina Nicoleta**, Bahrim Gabriela Elena, Stănciuc Nicoleta, Râpeanu Gabriela.
14. **Prof. Constantin Moraru prize** for the paper *Bioactive compounds and antioxidant activity of extracts from eggplant peel using different extraction methods*. **Condurache Nina Nicoleta**, Bahrim Gabriela Elena, Stănciuc Nicoleta, Râpeanu Gabriela.

F. Projects

1. 31.05.2022 - 15.12.2022. **Research assistant** in project „*Innovative strategies for capitalizing agri-food by-products into products with economic value promoting the principles of the circular economy*", financing contract no. 14888/11.05.2022.
2. 01.03.2021 – 31.12.2023. **Research assistant** in project "*New emerging concepts for food functionalization, through the transition from probiotics to metabiotics, as a health promotion strategy (BIOTICS +)*", financing contract no. PCE 159/2021.
3. 08.10.2021 – 30.09.2022. **Research assistant** in project „*Innovative and emerging solutions for the design of natural co-microcomposites to improve food functionality*", financing contract no. RF 3637/30.09.2021.
4. 01.07.2018 – 15.09.2020. **Research assistant** in project PN-III-P1-1.2-PCCDI-2017-056, 10PCCDI/2018 PRO-SPER „*Closing value chains in the bioeconomy by obtaining innovative bioproducts required by the market*" Project 3 - Tribotic products - probiotics, prebiotics, postbiotics - with multiple uses, obtained from by-products from the industrialization of vegetables – 3-4Life.

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