IOSUD – „DUNĂREA DE JOS" UNIVERSITY OF GALAȚI

Doctoral School of Fundamental and Engineering Sciences

Project co-financed from the European Social Fund Operational Human Capital 2014-2020

DOCTORAL THESIS

Food-grade functional composites based on yellow onion skins, peptides and probiotics

(Doctoral Thesis Summary)

Ph.D. student,

Ștefania-Adelina MILEA

Scientific coordinator,

Prof. Ph.D. Eng. Nicoleta STĂNCIUC

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. 19

GALAŢI

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INTRODUCTION

Nowadays, the plant-based food market is expanding, generating a significant amount of by-products and waste. Improved waste management is an essential step in the transition to a bioeconomy since it helps to reduce the negative environmental consequences of the fruit and vegetable processing sectors. As a result, there is a widespread aim to develop new ways to valorize these resources, as well as green valorization strategies (Sabater et al., 2020). Agricultural waste, co-products, and by-products are utilized in animal feed production all over the world, while biomass wastes are widely employed in bioenergy production. A responsible bioeconomy, on the other hand, must emphasize the production of high-quality foods (Valenti et al., 2020). According to the Food Wastage Footprint and Climate Change Report (FAO, 2019), in the early part of the food supply chain, about 15% of all fruits and 25% of all vegetables are discarded (Basri et al., 2021). Food waste is predicted to be 89 million tonnes every year in the European Union. In the next four years, an increase in food waste by 40% was suggested (Stenmarck et al., 2016). The zero-waste concept is an effective approach to improve the valorization of generated agro-industrial wastes into value-added goods, which may be used in the food industry as colorants, antioxidants, preservatives, and many other ones (Saini et al., 2019). Wastes that aren't being used (skins, seeds, rinds, and pomace) often include beneficial bioactive components such as amylopectin, phytochemicals, enzymes, enzymes, dietary fibers, and oils. Fruit and vegetable wastes (FVW) contain significant amounts of nutrients and other nutritional ingredients that help in animal feed development, bioactive ingredient formulation, and ethanol production (Basri et al., 2021).

More advanced valorization strategies attempt to recover high-value components from fruit and vegetable by-products, which are commonly used as natural sources of bioactive molecules in pharmaceuticals, medicine, and functional food formulations (Lu et al., 2019). Bioactive compounds provide basic nutritional requirements and have a positive health effect on the human body. They are known to have antioxidant, antimicrobial, or anti-inflammatory characteristics; however, these qualities are highly dependent on their bioactivity, chemical structure, dose, etc. Because bioactive components are abundant in wastes, they may be extracted and used in the production of food, ingredients, or nutraceuticals, with enhanced functionality. However, when considering the added value of these bioactives, several extraction processes may be considered, taking into account the extraction yields and the use of different solvents. Additionally, the bioactive compounds' stability and bioavailability are often compromised, involving the need to design a viable and efficient delivery system to boost their specific functionality in the body (Saini et al., 2019). These by-products offer a variety of natural antioxidants such as flavonoids (hesperetin, quercetin, genistein, and kaempferol), carotenoids (lutein and zeaxanthin), and phenolic acids.

Yellow onions have high levels of bioactive constituents in their skins or outer layer, mostly in the form of glycosides, such as phenolic and flavonoid compounds. Flavonoids are natural pigments that contribute to the color of fruits and vegetables. Their biological properties, such as their anti-oxidant, anti-carcinogenic, anti-inflammatory, and anti-mutagenic activities, are widely recognized. Due to its capacity to inhibit and/or delay the production of free radicals or reactive species brought on by oxidative stress, quercetin is perhaps one of the most researched natural antioxidants. However, these compounds are extremely sensitive to outside environmental factors including light,

heat treatment, pH changes, the presence of oxygen, ions, proteins, etc. due to their unique molecular and structural characteristics. (Milea et al., 2019).

Probiotics have long been recognized as important health boosters. Probiotics are well-known for their potential to help in the regulation of biological processes. Probiotics can activate, modify, and regulate the immunological response of the host, as well as alter gastrointestinal hormone secretion and affect brain function (Kerry et al., 2018). Intestinal mucosal tissue undergoes both acute and chronic inflammation, which can be controlled by probiotics. Specific growth circumstances apply to lactic acid bacteria, as they are unable to tolerate temperature and pH variations.

Whey proteins are widely utilized as functional food ingredients in the food industry because of their *in vivo* biological properties, such as benefits on the cardiovascular, digestive, endocrine, immunological, and neurological systems. Due to their unique structural characteristics, whey proteins can have bioactivities that are encoded in their original protein sequences, making it difficult for digestive enzymes to hydrolyze them (Pihlanto-Leppälä, 2000). Whey proteins may be hydrolyzed by enzymes to produce peptides that have beneficial features including lowering blood pressure, preventing dental cavities, blood clotting, antibacterial and antiviral activity, promoting relaxation and sleep, inhibiting the DPP-IV enzyme, and reducing inflammation.

Various microencapsulated delivery techniques have been suggested to overcome the processing instability and bioavailability of polyphenols and probiotics, therefore improving flavor, providing protection against processing conditions, and enhancing absorption through the digestive tract. Flavonoids and probiotic bacteria can be directly delivered to the intestine through encapsulation, which integrates them into gastro-resistant coating materials. Microencapsulation can be used to create novel food ingredients with improved flavonoid and probiotic functional applications. There are a variety of encapsulation materials that may be used, such as proteins with hydrophobic interactions and covalent disulfide crosslinking, lipids stabilized by van der Waals interactions, and polymers that form covalent crosslinking into hydrogel-like membranes. (Ye et al., 2018).

Choosing the topic for the PhD thesis, entitled "***Food-grade functional composites based on yellow onion skins, peptides, and probiotics***" was determined by the current need for scientific and technological alternatives to add value through sustainable reintegration of by-products bioactive into innovative foods and ingredients. Therefore, the main objectives of the thesis are focused on the development of sustainable valorization of the yellow onion skin bioactives, whey proteins and peptides, and lactic acid bacteria into ingredients and foods with a constant concentration of target compounds and stable sensory characteristics.

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

- Profiling different extracts obtained by conventional and assisted solid-liquid extractions (conventional solvent extraction, ultrasound-assisted extraction, enzymes-assisted extraction, and supercritical fluid extraction) from the perspectives of identifying the most suitable conditions to enhance the content in flavonoids and polyphenolic compounds and antioxidant activity;
- Attempts to obtain bioactive peptides from the whey proteins and testing the binding affinity of the derived peptides for yellow onion skins flavonoids as a prerequisite for efficient encapsulation;

- Scientific and technological approaches for biofunctional ingredients development, based on whey proteins peptide/hydrolysates and proteins, yellow onion skins extracts, biopolymeric coating materials, and lactic acid bacteria through microencapsulation and co-microencapsulation;
- Different approaches for testing the bioingredient's functional properties and health impacts (phytochemical profile, bioaccessibility, *in vitro* biological activity, biocompatibility, and storage stability);
- Development and characterization of value-added food products based on multifunctional microparticles.

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

I. **THE DOCUMENTARY STUDY** is divided into 4 chapters, focused on the state of the art and newest technology regarding the extraction of biologically active compounds (BAC) from fruit and vegetables by-products, selected theoretical informations about structure and health benefits, bioavailability and processing stability of BAC. Additionally, it was considered relevant to add some theoretical and practical aspects of encapsulation.

II. **ORIGINAL CONTRIBUTIONS** consists of 7 chapters that present the results of the experimental study, as follows:

Chapter 5, entitled **COMPARATIVE STUDY OF YELLOW ONION SKINS BIOACTIVE EXTRACTION USING DIFFERENT TECHNIQUES FROM A PHYTOCHEMICAL PERSPECTIVE** contains the relevant data obtained using different extraction methods, on the solid-liquid basis, phytochemical characterization of extracts using spectrophotometric methods, liquid chromatography and, gas chromatography techniques.

Chapter 6, entitled **PREREQUISITES FOR AN EFFICIENT ENCAPSULATION OF FLAVONOIDS FROM YELLOW ONION SKINS TO WHEY PROTEINS PEPTIDES** focused on gaining sufficient and relevant insights on the whey proteins hydrolysis and the binding mechanisms between flavonoids and bovine β-lactoglobulin peptides using complementary methods, such as fluorescence spectroscopy, thermodynamic analysis, and molecular dynamics simulations.

Chapter 7, entitled **WHEY PEPTIDES/PROTEINS BASED MULTIFUNCTIONAL MICROPARTICLES CONTAINING YELLOW ONION SKINS EXTRACT,** presents the results obtained by testing several coating materials for microparticles, from peptides to biopolymers from a phytochemical and biological point of view.

Chapter 8, entitled **DESIGNING A GREEN METHOD FOR MICROENCAPSULATION OF FLAVONOID EXTRACT FROM YELLOW ONION SKINS,** proposes a new, environmentally friendly strategy both for the extraction and microencapsulation techniques previously discussed, highlighting the phytochemical profile, biological activity, and processing stability of the obtained powders.

Chapter 9, entitled **CO-MICROENCAPSULATION OF FLAVONOIDS IN YELLOW ONION SKINS AND DIFFERENT STRAINS OF LACTIC ACID BACTERIA,** focuses on the results obtained by using different strategies to enhance the functional properties of the microparticles by adding probiotic bacteria.

Chapter 10, entitled **INCREASING THE MICROENCAPSULATION EFFICIENCY AND FUNCTIONALITY OF MICROPARTICLUES THROUGH GLYCATION** highlights the possibility to use whey protein-xylose conjugates as coating materials for flavonoids obtained from yellow onion skins extraction. Microparticles have been added to a food product with improved functional properties.

Chapter 11, FINAL CONCLUSIONS, displays the main conclusions derived from the experiments.

The doctoral thesis comprises 180 pages, which includes 43 figures and 42 tables. The documentary study represents 25%, and the experimental part 75%.

Finally, the personal contributions and perspectives for future studies are presented. The dissemination of the results obtained in the researched field is highlighted in a list of publication containing seven published articles from doctoral thesis and 2 patent applications. The results were also presented at over 40 international and national conferences.

The experiments carried out in this PhD thesis were possible due to the Center for Integrated Research, Expertise and Technology Transfer (BioAliment-TehnIA) (www.bioaliment.ugal.ro) infrastructure, within the Faculty of Food Science and Engineering ”Dunărea de Jos”, Galați. In addition, most of the results were obtained within the project PN-III-P1-1.2-PCCDI-2017-0569-PRO-SPER (10PCCI), entitled ” Closing the bioeconomy value chains by obtaining innovative bioproducts required by the market”. Also, The National Institute of Research and Development for Biological Sciences, București (www.www.incdsb.ro) is acknowledged for performing the cytocompatibility tests.

The doctoral thesis was carried out under the scientific coordination of the steering committee:

Professor Nicoleta STĂNCIUC – PhD supervisor

Professor Gabriela RÂPEANU – coordinator of spectrophotometric analysis of polyphenolic compounds and extraction methods

Professor Gabriela – Elena BAHRIM – coordinator of microbiological analysis and microencapsulation studies

Professor Iuliana APRODU – coordinator of molecular modeling and binding mechanisms.

CHAPTER 5. COMPARATIVE STUDY OF YELLOW ONION SKINS BIOACTIVE EXTRACTION USING DIFFERENT TECHNIQUES FROM A PHYTOCHEMICAL PERSPECTIVE

5.2. OBJECTIVES OF THE STUDY

Onion peels are a source of valuable components for human health, due to the content of phytochemicals. The recovery of the phytochemicals from by-products refers to the extraction of phenolic compounds and the experimental validation of their biological activities.

The aim of this study is to compare the phytochemical and biological profile of the extracts from yellow onion skins obtained by two different methods of extraction, namely: conventional solid-liquid extraction (using different solvents) and solid-liquid extraction assisted methods (ultrasound, enzyme, supercritical fluid extraction) for the perspective of obtaining high-quality extracts. Several extracts were obtained, whereas the profiling included the separation and quantification of polyphenolic compounds, and antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH), and volatile compounds. Therefore, different techniques were used for analysis, such as spectrophotometric methods for polyphenolic and flavonoid quantification, chromatographic (HPLC) analysis of flavonoids, and GC-MS compound identification.

5.4. RESULTS AND DISCUSSION

5.4.1. Comparative analysis of the phytochemical profile of extracts obtained by conventional and assisted methods

The choice of the extraction process as well as the extraction parameters are essential for obtaining the bioactive compounds. Solvent type, solvent concentration, extraction temperature, and extraction method are the parameters responsible for the final concentration of bioactive compounds in the extracts. In order to identify the most non-invasive and efficient protocol for the extraction of biologically active compounds from yellow onion peel, a phytochemical comparison of the extracts obtained by different techniques was performed. The polyphenolic compounds from the extracts are shown in Table 5.1.

Table 5.1. *Antioxidant compounds from onion peel extracts obtained by conventional extraction and ultrasound-assisted extraction*

Bioactives	Conventional extraction	Ultrasound-assisted extraction
TFC, mg QE/g	334.97±19.41^{a}	230.63±8.36^{b}
TPC, mg GAE/g	150.05±5.65^{a}	139.67±3.82^{b}
AA, mM Trolox/g	312.28±2.36^{a}	285.90±3.86^{b}

Average values that on the same row do not share a letter (a,b) are significantly different, based on the Tukey method and 95% confidence.

The onion peel extract performed in the first study was characterized and showed the following results: 334.97 ± 19.41 mg QE / g, 150.05 ± 5.65 mg GAE / g, and 312.28 ± 2.36 mM Trolox / g. The yellow onion peel extract from the second experiment expressed a TFC of 230.63 ± 8.36 mg QE / g DW, a TPC of 139.67 ± 3.82 mg GAE / g and values of 285.90 ± 3.86 mM Trolox / g, for antioxidant activity.

Following the enzyme-assisted extraction, it can be seen from Table 5.2 that after 24 hours of extraction, the highest concentration of total polyphenols and flavonoids was highlighted by extraction with Zymorouge and Xylanase.

Table 5.2. *Antioxidant compounds from onion peel extracts obtained by enzyme-assisted extraction*

Bioactives	**Enzyme assisted extraction**		
	Cellulase	**Pectinase (Zymorouge)**	**Xylanase**
TFC, mg QE/g FW	63.36±4.51^{b}	108.36±3.62^{a}	106.85±12.49^{a}
TPC, mg GAE/g FW	16.22±2.01^{b}	25.19±3.56^{a}	25.17±2.44^{a}
AA, mM Trolox/g FW	58.7±2.19^{a}	51.00±1.78^{b}	31.9±0.83^{c}

Means on the same row do not share a letter (a,b,c) are significantly different, based on Tukey method and 95% confidence.

The contribution of enzymes refers to the extraction of bioactive compounds from the yellow onion peel by breaking the integrity of the cell walls. Enzymes are responsible for the hydrolysis of components in cell walls, thus increasing cell permeability and extraction efficiency. After 24 hours of extraction, the biologically active compounds had a lower concentration compared to the other extraction techniques. A possible explanation would be to keep the extracts at too high a temperature necessary to inactivate the enzymes, and a decrease in the concentration of flavonoids may indicate their degradation, quercetin, and kaempferol being thermolabile components.

In table 5.3, the results of the total flavonoid content and total polyphenol content of various extracts are presented.

Table 5.3. *Antioxidant compounds from onion peel extracts obtained by extraction with supercritical fluids*

Bioactives	**Supercritical fluid extraction**			
	S40 I	**S45 I**	**S40 II**	**S45 II**
TFC, mg QE/g DW	295.52±7.62^{a}	81.07±5.81^{d}	211.51±0.99^{c}	282.80±4.08^{b}
TPC, mg GAE/g DW	269.52±46.81^{a}	74.75±0.56^{d}	212.56±1.18^{b}	202.31±11.56^{c}
AA, mM Trolox/g DW	420.78±37.08^{a}	303.07±0.67^{c}	285.53±0.74^{d}	404.93±1.39^{b}

Means that on the same row do not share a letter (a,b,c) are significantly different, based on Tukey method and 95% confidence.

According to the results obtained from the extraction of biologically active compounds from onion peels, the extraction with supercritical fluids recorded the highest concentration of polyphenols and flavonoids, of 269.52 ± 46.81 mg GAE/ g DW and 295.52 ± 7.62 mg QE/ g DW, respectively, as well as the antioxidant activity in the S40 I fraction compared to the other fractions. At the same time, fairly close values were obtained for the S45 II fraction, as follows: 282.80 ± 4.08 mg QE / g for TFC, 202.31 ± 11.56 mg GAE / g for TPC, and 404.93 ± 1.39 mM Trolox / g DW for antioxidant activity. The lowest values of these parameters were recorded for the S45I fraction. Extraction with supercritical fluids recorded the highest value of antioxidant activity, which is also confirmed by the highest concentration of total polyphenols.

Table 5.4. A*ntioxidant compounds from onion peel extracts obtained by hot water extraction*

Liquid extract	**Values**
TFC, mg QE/ g FW	50.21±0.09
TPC, mg GAE g FW	21.68±0.69
AA, mM Trolox/ g FW	250.81±6.76

Extraction in hot water resulted in a flavonoid liquid extract with a concentration of 250.81 ± 6.76 mM Trolox / g raw material regarding antioxidant activity (250.81 ± 6.76 mM Trolox / g raw material), justified by a high concentration of flavonoids (50.21 ± 0.09 mg QE / g raw material). The TPC was 21.68 ± 0.69 mg GAE / g raw material.

5.4.2. Identification and chromatographic separation of antioxidant compounds from yellow onion peel extract

The chromatographic profile of the extract obtained by the conventional solvent method is shown in Figure 5.6. A few peaks can be seen, which signify the presence of 5 major compounds, namely Peak (1) - quercetin 7,4-diglycoside; Peak (2) - quercetin 3,4-diglycoside; Peak (3) - quercetin 4-glucoside; Peak (4) - quercetin; Peak-ul (5) - kaempferol. Thus, of the total flavonoid concentration, the compound that recorded the highest value of 22.87% of the total flavonoid compounds was quercetin 3,4-diglycoside. The lowest concentration of quercetin derivatives, 2.98%, was determined for quercetin 7,4-diglycoside. Quercetin and kaempferol, two of the most important compounds responsible for the antioxidant activity of onion peel extracts, were 7.51% and 1.12%, respectively.

Compared with the results obtained for the extract realized by conventional technique, the chromatographic profile of ultrasound extract showed the same compounds (Peak (1) - quercetin 7,4-diglycoside; Peak (2) - quercetin 3,4 -diglycoside; Peak (3) - quercetin 4-glucoside; Peak (4) - quercetin; Peak (5) - kaempferol), but with a slightly lower concentration, also confirmed by the phytochemical characterization of the extracts. Thus, concerning quercetin 7,4-diglycoside, this compound recorded a concentration of 2.76% of the total polyphenolic compounds while the major compound in the studied extract was quercetin 3,4-diglycoside, with a concentration of 18,78%. Regarding quercetin and kaempferol, lower concentrations were also identified compared to the extract obtained by conventional techniques, respectively 6.3% and 0.89%.

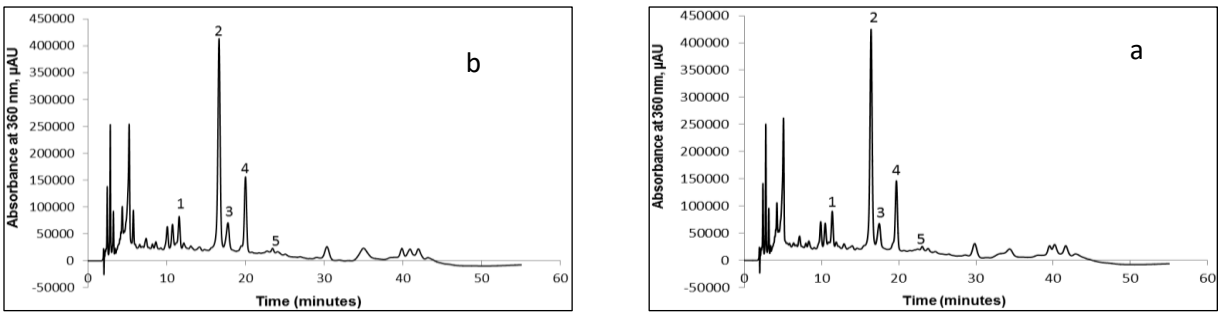


Figure 5.6. Chromatographic profile of onion peel extracts obtained after conventional extraction and ultrasound-assisted extraction (A - conventional extraction; B - ultrasound-assisted extraction) - Peak (1) - quercetin 7,4-diglycoside; Peak (2) - quercetin 3,4-diglycoside; Peak (3) - quercetin 4-glucoside; Peak (4) - quercetin; Peak-ul (5) - kaempferol;

Another method of extracting biologically active compounds studied in the literature is enzyme-assisted extraction, in this case, pectinase, xylanase, and cellulase. This type of extraction increases the efficiency of the recovery of polyphenolic compounds and is considered to be a method that has no adverse effects on the environment. The distinguishing feature of enzymes is that they can function efficiently under normal physiological conditions, at atmospheric pressure, and at a pH

in the range of 3.0-10.0. The chromatographic profile resulting from enzyme-assisted extraction revealed only the presence of quercetin. This compound showed the highest concentration in the extract obtained with xylanase at 9.88%, followed by the extract with cellulase at 4.63% and the one with pectinase at 4.42%.

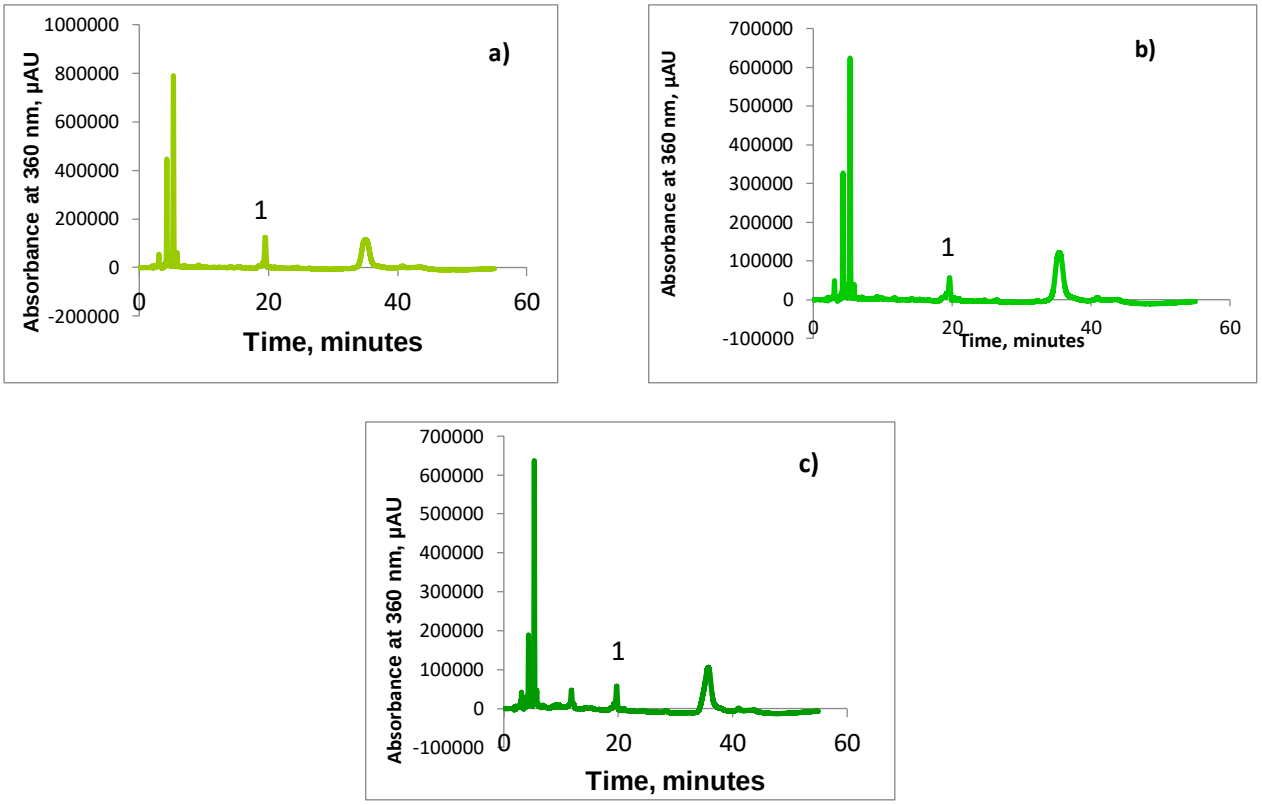


Figure 5.7. Chromatographic profile of onion peel extracts obtained after enzyme-assisted extraction (A - xylanase; B - pectinase; C - cellulase) - Peak (1) - quercetin

Extraction with supercritical fluids is considered an excellent alternative to the conventional method of extracting bioactive compounds, resulting in extracts free of organic solvents. The chromatographic profile of the extract obtained using supercritical fluids showed higher concentrations of bioactive compounds compared to those of previous extractions, and the values were comparable to those reported by conventional and ultrasound-assisted extraction. The chromatogram of the S40 fraction shows the presence of the previously identified compounds, but the highest concentration is noted in the case of quercetin 3,4-diglycoside with a percentage of 45.19%, while quercetin recorded a concentration of 2.41% and 1% kaempferol.

Thus, the extract S40 I (temperature of 40°C; pressure of 400 bar, extraction time 2.5h) showed a chromatographic profile from which 5 compounds were identified, respectively quercetin 7.4- diglycoside, quercetin 3,4-diglycoside, quercetin 4-glucoside, quercetin, kaempferol. The highest concentration was found for quercetin, about 40%, followed by quercetin 3,4-diglycoside with a concentration of 17.94% of the total extracted flavonoid compounds. Chromatogram of S40 II extract (temperature 55°C; pressure 400 bar, extraction time 2.5 h) showed the presence of the same compounds, but the highest concentration was identified for quercetin 3,4-diglycoside of 45.19%,

while quercetin had a concentration of 2.41%. Kaemperof showed concentrations around 1%. The extract from the S45 I separator fraction (40 C temperature; 400 bar pressure, 2.5 h extraction time) showed a much lower concentration of all identified compounds compared to the other extracts obtained by supercritical fluid extraction. Thus, the highest concentration was recorded for quercetin 3,4-diglycoside of 10.13% and quercetin showed a value of 2.02% of the total flavonoid compounds. The chromatographic profile of the extract obtained in cell S45 II (temperature of 55°C; pressure of 400 bar, extraction time 2.5 h) revealed the major compound quercetin, of about 35%.

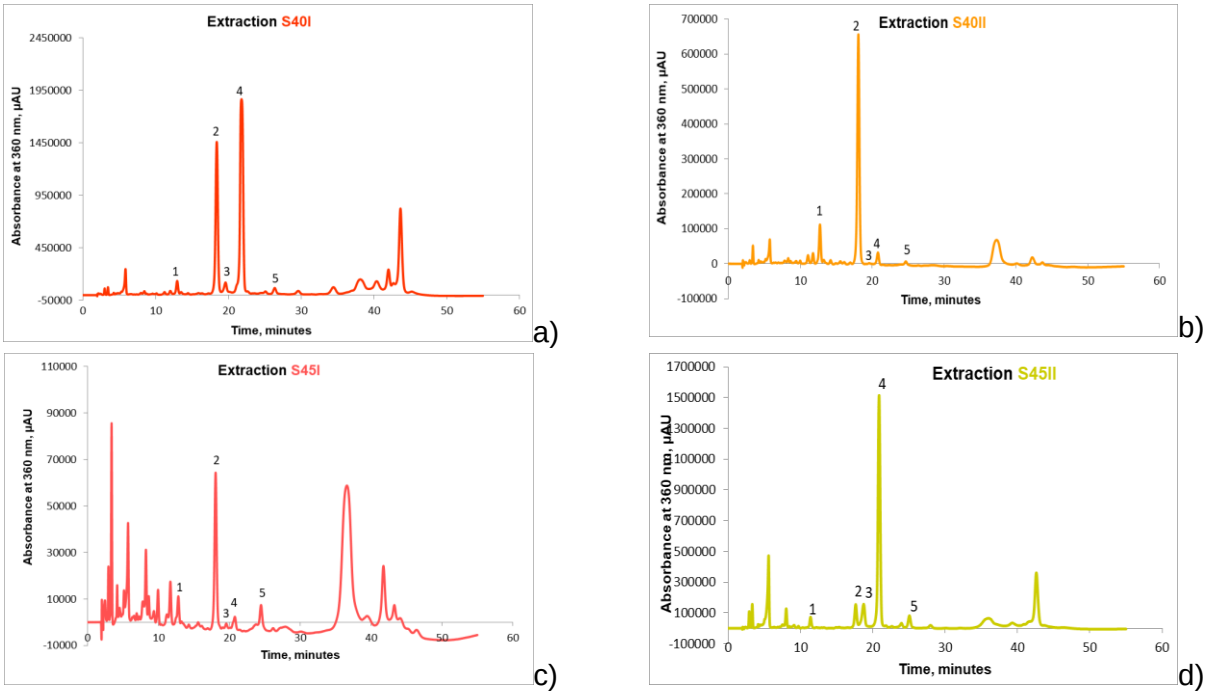


Figure 5.8. Chromatographic profile of onion peel extracts obtained by extraction with supercritical fluids (A - S40 I; B - S40 II; C - S45 I; D - S45 II) - Peak (1) - quercetin 7,4-diglycoside; Peak (2) - quercetin 3,4-diglycoside; Peak (3) - quercetin 4-glucoside; Peak (4) - quercetin; Peak-ul (5) - kaempferol

The chromatographic profile of the onion peel extract obtained by extraction with hot water at 70°C from the onion peels (Figure 5.9) revealed the presence of a single peak of high intensity, most likely compounds of the class of polyphenolic acids or quinones, colored compounds with a fairly high water solubility coefficient. The chromatogram resulted from this extract did not show at any wavelength the presence of compounds of the flavonoid class. This phenomenon can be explained by the low solubility of these compounds in water.

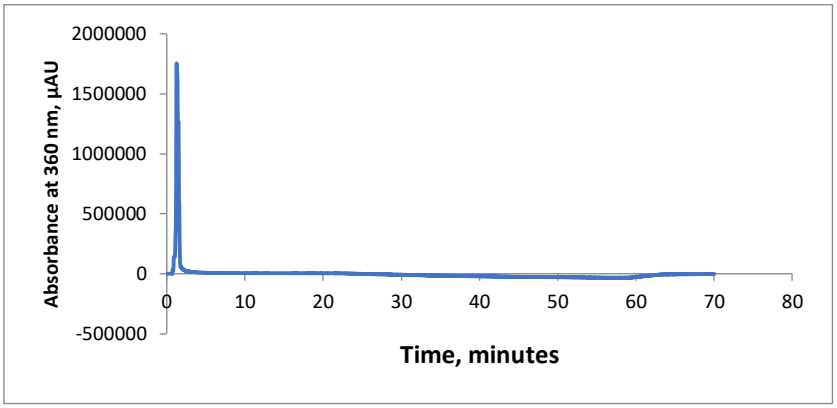


Figure 5.9. Chromatographic profile of onion peel extract obtained by extraction with water at 70°C

Identification and quantification of flavonoid compounds by chromatography can be correlated with the antioxidant activity of onion peel extract. Usually, the increased value of the antioxidant activity is due to the existence of flavonoid compounds (quercetin, kaempferol, myricetin, catechin), as well as secondary derivatives.

5.4.3. *In vitro* digestibility of yellow onion peel extract

After analyzing the phytochemical components of all the extracts, it can be concluded that the conventional extraction allowed to obtain satisfactory results. The method provides several advantages over other techniques, such as the short duration of the experiment, low difficulty, doesn`t require additional equipment, and last but not least, the very high extraction efficiency of the compounds of interest. Therefore, the extract obtained by the conventional method was selected to test the *in vitro* digestibility of flavonoids in the gastrointestinal tract. In Table 5.5 are given the TFC in the selected simulated environment.

Table 5.5. *In vitro digestibility of TFC from the onion peel extract*

Total flavonoids content		
Digestion time, min	**SGF V1**	**SIF V1**
0	0.1±0.001	0.1±0.001
30	0.114±0.004	0.12±0.004
60	0.115±0.006	0.159±0.021
90	0.123±0.001	0.144±0.001
120	0.095±0.002	0.078±0.001

SGF = simulated gastric fluid; SIF = simulated intestinal fluid

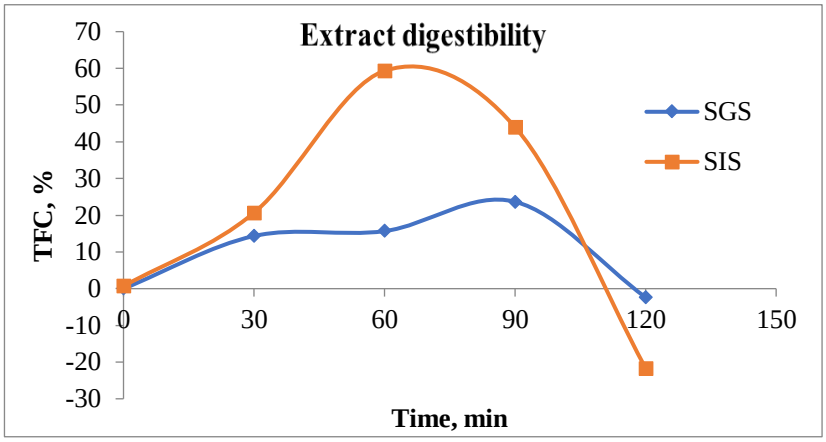


Figure 5.10. *In vitro* digestibility of flavonoids in yellow onion peel extract

In the first 90 minutes of simulated gastric digestion conditions, a very slight increase in flavonoid concentration can be observed from 0.1 ± 0.001 to 0.123 ± 0.001 QE / mL extract, followed by the same trend in the first 60 minutes of digestion in the intestine. Over the next 60 minutes, the concentration of flavonoids decreases, which means that they are degraded. Degradation of the compounds may be due to conditions in the intestinal tract, where the pH rises sharply and becomes unfavorable for flavonoids. The results highlited the need to improve the stability of phenolic compounds under various conditions, but also to ensure their controlled release.

5.4.4. GC-MS analysis

The extract's phytochemical profile highly depends on the method used for extraction. A large number of compounds have been identified in the case of enzyme-assisted extraction. The identified compounds belong to the following classes: alcohols, acids, aldehydes, ketones, alkanes, esters, and sulfur compounds are presented in Table 5.6.

Table 5.6. *Compounds identified in onion peel extract*

Compound Name	Retention Time (minutes)	Formula	Extraction method/ Peak area (%)
Catechol	26.59	$C_6H_6O_2$	CSE (5.44%) EAE (21.05%) SFE 40 (3.21%) SFE 45 (8.57%)
Vanillic acid	35.49	$C_8H_8O_4$	CSE (0.8%)
Tetradecanoic acid (myristic acid)	35.63	$C_{14}H_{28}O_2$	EAE (0.84%)
Hexadecanoic acid, methyl ester (methyl palmitate)	38.66	$C_{17}H_{34}O_2$	EAE (0.6%)

Compound Name	Retention Time (minutes)	Formula	Extraction method/ Peak area (%)
Hexadecanoic acid, ethyl ester (ethyl palmitate)	40.78	$C_{18}H_{36}O_2$	EAE (4.7%)
n-Hexadecanoic acid (Palmitic Acid)	41.15	$C_{16}H_{32}O_2$	CSE (3.58%) EAE (18.03%)
9,12-Octadecadienoic acid (Z, Z)-(linoleic acid)	43.68	$C_{18}H_{32}O_2$	CSE (25.87%) UAE (100%) EAE (25.81%) SFE 40 (20.46%) SFE 45 (58.61%)
l-(+)-Ascorbic acid 2,6-dihexadecanoate	42.59	$C_{38}H_{68}O_8$	EAE (5.65 SFE 45 (22.19%)

CSE – conventional solvent extraction, UAE – ultrasound-assisted extraction, EAE - enzyme assisted extraction, SFE – supercritical fluid extraction

Catechol (1,2-dihydroxybenzene), a compound found in plants such as onions, eucalyptus, and sugar beet, has also been identified in onion peel extracts, as follows: 5.44% in EC, 21.05% in the EAE, 3.21% in SFE 40 and 8.57% in SFE 45. In addition, the following phenols were identified in smaller amounts: 2-methoxy-4-vinyl phenol (p-vinyl guaiacol); 2-methyl-5- (1-methyl ethyl) -phenol (carvacrol), vanillic acid. (Z Z) -9.12 octadecadienoic acid (linoleic acid) was present in the analyzed samples. Linoleic acid is an essential fatty acid found in green beans, cloves, kumquat, and pecans. Thus, onion peels can be exploited in the food industry due to their physico-chemical properties and the presence of essential fatty acids and volatile components.

5.5. Partial conclusions

This study aimed to compare the phytochemical and biological profile of the yellow onion skins obtained using different extraction techniques. The results allowed to conclude the following:

A. The solid-liquid solvent conventional extraction recorded the highest value for total flavonoids (334.97±19.41 mg QE / g extract) when compared to assisted extraction techniques.

B. The use of supercritical fluids extraction allowed to obtain extracts with higher antioxidant activity, given probably by the concentration of total polyphenols.

C. Regarding the content of total polyphenols and total flavonoids in onion peels extracts obtained by enzyme assisted extraction method, it was shown that after 24 h, the extraction with pectinase and xylanase led to a higher concentration in flavonoids, leading to significant values of antioxidant activity.

D. The chromatographic profile of the extracts obtained from the yellow onion peels by different extraction techniques, such as conventional extraction, ultrasound-assisted extraction, and extraction with supercritical fluids revealed the presence of 5 major compounds such as quercetin 7,4-diglycoside, quercetin 3,4 -diglycoside, quercetin 4-glucoside, quercetin, and kaempferol.

E. Extracts obtained by enzyme-assisted extraction, following HPLC-DAD analysis, showed the presence of a single compound, quercetin, in deficient concentrations compared to other types of extraction.

F. Water extraction is not a suitable method for the extraction of flavonoid compounds from onion peels due to the low solubility of these compounds in water.

G. The results obtained after *in vitro* digestion experiment emphasized the need to improve the stability of phenolic compounds exposed to various conditions, but also to ensure their controlled release.

H. The fatty acid content of onion peels, which was revealed by GC-MS analysis to be rich in polyunsaturated fatty acids, making it nutritionally attractive. The polyunsaturated fatty acid composition of onion peels is a potential for nutritional recovery, but obtaining essential oils requires prior testing.

I. Based on the obtained results, it may be stated that all the used methods are appropriate for the extraction of polyphenols from yellow onion skins.

CHAPTER 6. PREREQUISITES FOR AN EFFICIENT ENCAPSULATION OF FLAVONOIDS FROM YELLOW ONION SKINS TO WHEY PROTEINS PEPTIDES

6.2. OBJECTIVES OF THE STUDY

This study aimed to investigate the possibility of using whey proteins, such as β-LG, and bioactive peptides resulted from the enzymatic hydrolysis of β-LG to bind flavonoids extracted from yellow onion peel. The interaction between peptides and flavonoids was evaluated using two complementary techniques: fluorescence and molecular modeling techniques. The flavonoids were extracted from the dried yellow onion peels by supercritical fluid extraction, and the extract was characterized in terms of total flavonoid content (TFC), total polyphenolic content (TPC), and antioxidant activity. Thermolysin-assisted enzymatic hydrolysis was used to produce β-LG hydrolysates. Fluorescence quenching experiments involved the use of hydrolysates with molecular weight less than 3 kDa, hereinafter referred to as small β-LG (β-SP) peptides. The results allowed the calculation of binding parameters such as Stern-Volmer and binding constants, as well as the number of binding sites, while based on thermodynamic analysis, the main forces involved in the formation of the complex were identified.

6.4. RESULTS AND DISCUSSION

6.4.1. Characterization in biologically active compounds of yellow onion peels extract

In this study, to increase the solubility of polar compounds and the extraction efficiency of flavonoids, the onion peels were mixed with a hydroalcoholic solution and continuously recirculated with supercritical CO_2. Yellow onion peel extract showed a TFC value of 202.31 ± 11.56 mg QE / g DW, 282.80 ± 4.08 mg GAE / g DW for TPC, and an antioxidant activity of 404.93 ± 1, 39 mM Trolox / g DW. The extraction yield was 0.65%, which demonstrates that the extraction yield increases with the extraction pressure. The chromatographic profile of the extract presented in this study is shown in Figure 6. Thus, five compounds were identified: quercetin 7,4-diglucoside, quercetin 3,4-diglucoside, quercetin 4-glucoside, quercetin and kaempferol. The highest concentration was found in the case of quercetin, 39.94%, followed by quercetin 4-glucoside with a concentration of 5.90%, quercetin 3,4-diglucoside with 3.99%, quercetin 7,4-diglucoside with 1.73%, and kaempferol with a concentration of only 1.27% of the total extract.

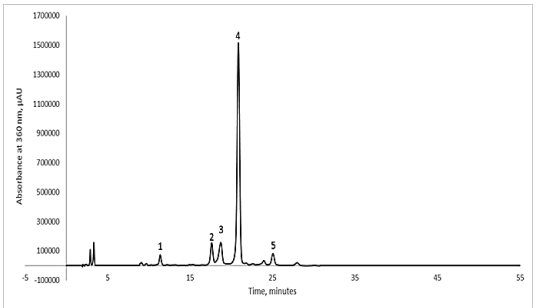


Figura 6 Chromatographic profile of supercritical CO2 extract from yellow onion peel: Peak (1) - quercetin 7,4'-diglycoside; Peak (2) - quercetin 3,4-diglucoside; Peak (3) - quercetin 4-glucoside; Peak 253 (4) - quercetin; Peak (5) - kaempferol.

6.4.3. Evaluation of the binding mechanisms between biologically active compounds in the extract and proteins/ peptides by fluorescence spectroscopy

Using ligand titration to quench the fluorescence of tryptophan in proteins is a quick and easy way to estimate the binding affinities of flavonoids to proteins and peptides. The interaction between β-lactoglobulin hydrolysates and flavonoids extracted from yellow onion peels was studied by increasing the concentration of flavonoids in the mixture, followed by the evaluation of the fluorescence intensity spectra of peptide solutions, pretreated at temperatures between 25°C and 100°C, for 15 minutes. The fluorescence quenching effect was observed in all peptide variants treated at different temperature ranges. Increasing the flavonoid concentration by more than 8.41×10^{-8} M resulted in an extinction of peptide fluorescence of approximately 46% (Figure 6.1, a), and heat treatment of up to 100 °C resulted in conformational changes with a quenching rate of about 42% (Figure 6.1, b).

Regardless of the temperature, the increase in flavonoid concentration did not cause red or blue shift deviations, which indicates that the addition of flavonoids did not lead to a loss of peptide structure, inducing an alteration at the hydrophobic subdomain to which tryptophan belongs.

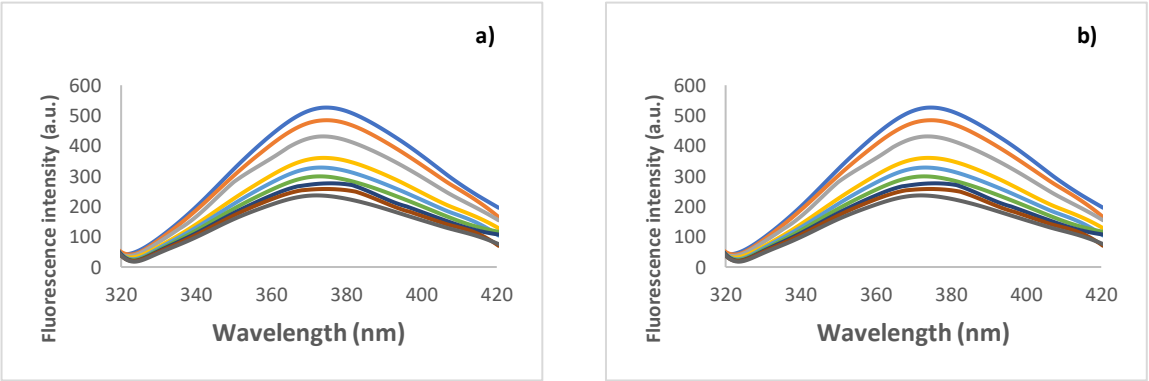


Figure 6.1. Fluorescence spectra of the interaction between β-LG hydrolysates and SFE flavonoids at 25°C (a) and 100°C (b)

Table 6.1. *Binding parameters between β-LG peptides and flavonoids at different temperatures*

Temperature(˚C)	*SFE extract*			*Quercetin*		
	$K_{SV}(10^{-10}$ Mol/L)	$K_b(10^{-8}$ Mol/L)	*n*	$K_{SV}(10^{-10}$ Mol/L)	$K_b(10^{-8}$ Mol/L)	*n*
25	12.51±1.10	2.37±0.04	1.07±0.16	6.45±0.62	2.12±0.18	0.88±0.19
70	12.09±1.49	2.71±0.08	1.11±0.08	5.35±0.91	3.34±0.06	1.00±0.01
80	11.73±1.69	2.42±0.20	1.09±0.04	5.05±0.21	2.77±0.43	1.28±0.25
90	10.75±1.67	2.14±0.13	0.92±0.16	5.37±0.91	2.11±0.09	0.83±0.10
100	8.19±0.12	2.09±0.29	1.09±0.10	8.30±1.41	1.84±0.77	0.77±0.02

The data shown in Table 6.1 indicates that the interactions between the flavonoids and peptides followed a static mechanism. The high linearity could be observed over the whole temperature range studied, which indicates that β-LG hydrolysates have unique binding modes. Increasing the temperature from 25°C to 100°C decreased the K_{SV} values from $12.51 \pm 1.10 \times 10^{10}$ L

/ Mol to 8.19 ± 0.12x10^{10} L / Mol. In the present study, flavonoids formed a nonfluorescent complex with β-LG hydrolysates.

From Table 6.1 it can be seen that the decrease of the K_b value with the increase of the temperature, and the *n* values of the flavonoid-peptide complex are approximately equal to 1, in the case of all the studied temperatures. These results suggest the existence of a single binding site for flavonoids. A slight decrease in the values of *n* can be observed as the temperature increases. However, in the temperature range of 80-100 ° C, there are no significant differences in the number of binding sites. Binding parameters indicate values of magnitude by two orders smaller, and values of *n* indicate the presence of a binding site for quercetin. The higher binding constant values for flavonoid extract compared to quercetin can be explained by the existence of a higher spectrum of compounds in the extract, with various affinities, which can practically compete for peptide binding. The increase in temperature did not have a significant effect on the structural conformation of the β-LG protein hydrolysate, except at 100°C, when a decrease in binding parameters could be observed. This could be justified by the folding and/or aggregation of peptide chains, which can lead to a block of quercetin binding sites.The linear relationship between ln K_b and 1/T suggests that the enthalpy sign remains constant, under heat treatment conditions. It can be seen that for the binding between flavonoids extracted from yellow onion peel and peptides, ΔH and ΔS were -1149.6 ± 18.11 J / mol and -2.36 ± 0.18 J / mol, respectively. These results indicate that the main forces that stabilize the system are hydrogen bonds and van der Waals interactions.

Table 6.2. *Thermodynamic parameters for the binding of β-LG hydrolysates to flavonoids extracted from yellow onion peels at different temperatures*

T(°K)	ΔH(J/Mol)	ΔS(J/Mol·K)	ΔG (J/Mol)	R
298	-1149.6±11.20	-2.36±0.47	-446.32±11.20	0.95
343			-340.12±10.21	
353			-316.52±1621	
363			-292.92±9.20	
373			-269.32±8.45	

6.4.4. Evaluation of the binding mechanisms between biologically active compounds from the extract and proteins/ peptides by molecular modeling

In silico approach was performed to identify details at the atomic level on the behavior of molecules, to complete the study of the fluorescence experiments. Whereas at the laboratory level, the preparation of the samples involved obtaining peptides by heat treatment of the mixture of β-LG and thermolysin, at 70°C temperature, the molecular modeling steps were performed respecting the same temperature conditions, for the β-LG optimized 3D model. This approach allowed the simulation of any folding/unfolding phenomenon that may occur on the β-LG structure and also on the exposure of sites susceptible to thermolysin hydrolysis. Therefore, in order to obtain a hydrolysis degree of approximately 21%, identical to that calculated in the laboratory experiment, the peptide bonds established between the amino acid residues with exposure above 3.2 Å2 (Figure 6.2) were cleaved, resulting in a mixture of 30 different peptides with sequences from 2 to 19 amino acid residues and molecular weights with values from 206.24 Da to 2132.43 Da (Table 6.3) and free residues of Leo, Ala, Ile.

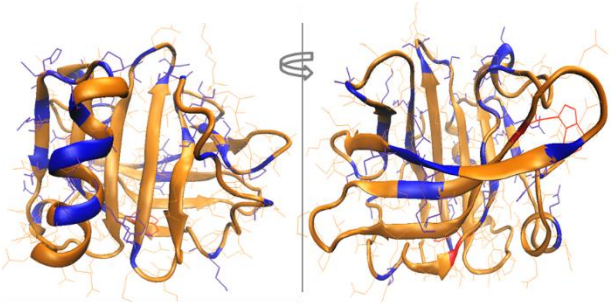


Figure 6.2. 3D model of heat-treated β-lactoglobulin at 70°C. The protein is represented in NewCartoon style using VMD software. Recognized binding sites by thermolysin are marked in blue and Trp residues are shown in red

As indicated by fluorescence quenching tests, the addition of flavonoid extract decreased the fluorescence of the β-LG hydrolysate. Regarding the experimental conditions used in fluorescence spectroscopy measurements (excitation at a wavelength of 295 nm), the fluorescent properties of the β-LG hydrolysate are attributed to the two Trp residues located at positions 19 and 61 of the initial protein structure (Figure 6.3). Verification of peptides resulting from β-LG hydrolysis with thermolysin indicated that Trp19 and Trp61 residues became peptides with a relatively high molecular weight of 782.85 Da and 1107.23 Da.

Table 6.3. *Details of the peptides resulting from the thermolysin hydrolysis of β-lactoglobulin (β-LG), the achievement of a degree of hydrolysis of 21%, and the interaction with the flavonoids in the extract of yellow onion peel, QDG, and QMG*

Peptides resulting by β-lactoglobulin hydrolysis with thermolysin			**Interaction energy, kcal/mol**	
Number of residues	Sequence	Molecular weight, Da	QDG	QMG
19	Leu^{117}-Lys^{135}	2132.43	>0	>0
14	Val^{43}-Ile^{56}	1569.75	-18.56±0.59	-15.44±0.15
9	**Leu^{58}-Cys^{66}**	1107.23	-128.22±8.92	-125.35±5.55
	Val^{94}-Tyr^{102}	1144.29	-17.50±4.67	-16.57±2.45
8	Leu^{103}-Ser^{110}	956.14	-15.30±2.66	-14.93±2.31
7	**Val^{15}-Ser^{21}**	782.85	-15.21±0.98	-14.75±0.75
	Met^{24}-Ser^{30}	693.77	-16.52±0.34	-14.43±1.17
	Leu^{32}-Pro^{38}	701.75	-10.15±1.46	-10.28±1.02
	Ala^{73}-Pro^{79}	786.95	-13.38±1.06	-15.15±1.55

Tryptophan peptides are bold. Cleavage sites were predicted with PeptideCutter on the ExPASy server.

Both peptides established higher interaction forces with QDG than with QMG (Table 6.3). Even though the Leu58-Cys66 peptide includes negatively charged amino acids, electrostatic contact with QDG and QMG may only be due to π-π and cation-π interactions because the ligands do not

have an electric charge. The molecular investigation did not indicate direct contact between Trp of the Leu58-Cys66 peptide and ligands, the indole ring is located on the other side of the peptide skeleton at the site of interaction with QDG and QMG (Figure 6.3). Considering that there were no changes in the environment of the Trp residue side chain at flavonoid binding, most likely the interaction between Leu58-Cys66 and QDG or QMG did not result in the change in fluorescence.

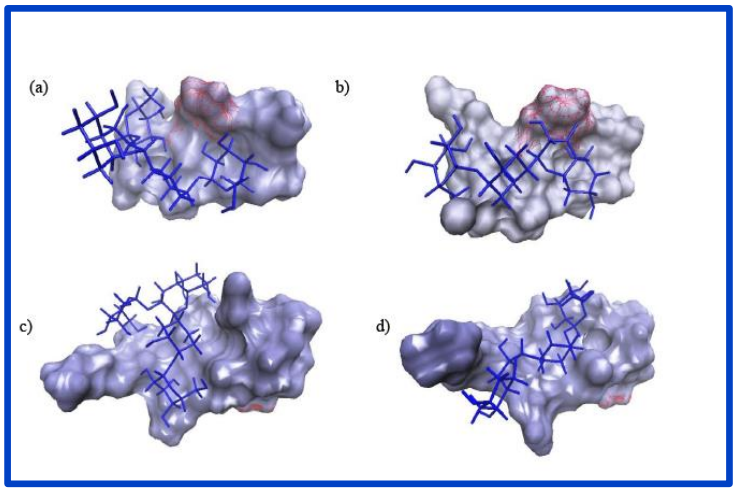


Figure 6.3. Details of the interaction between Trp peptides (Val^{15}-Ser^{21} (a and b) and Leu^{58}-Cys^{66} (c and d) resulting from β-LG hydrolysis with thermolysin and flavonoids extracted from yellow onion skins

6.5. Partial conclusions

A. Investigations about the interactions between proteins and various biologically active compounds are critical for the effective production of novel food-grade components, pharmaceuticals, and cosmetics. Flavonoid-protein complexes might be designed to boost flavonoids' functional uses and to overcome their fundamental drawbacks, such as bioavailability. β-lactoglobulin plays an important role in binding and transporting various ligands, such as steroids, fatty acids, retinoids, vitamin D, cholesterol, polyphenols, etc

B. This study looked at the interaction between peptides resulting from the hydrolysis of β-lactoglobulin with thermolysin and flavonoids extracted with supercritical fluids from a by-product resulting from onion processing. The CO_2 supercritical fluid method yielded a flavonoid content of 202.31±11.56 mg quercetin equivalents/g DW, a polyphenol content of 282.80±4.08 mg gallic acid equivalents/g DW, and an antioxidant activity of 404.9±31.39 mM Trolox/g DW for the yellow onion skins extract. The fluorescence intensity changes of protein hydrolysates allowed the estimation of the Stern-Volmer constant, the binding constants, and the number of binding sites. The binding mechanism between flavonoids and peptides was static, and the main forces involved were hydrogen bonds and van der Waals interactions. The number of binding sites was 1 for all temperatures tested. Molecular modeling has shown that the binding of quercetin-3,4'-O-diglucoside to quercetin-4'-O-monoglucoside and Val^{15}-Ser^{21} is primarily responsible for quenching fluorescence upon the gradual addition of yellow onion peel extract to β-lactoglobulin hydrolysate. Quercetin-3,4'-O-diglucoside and quercetin-4'-O-monoglucoside appear to interact with all the peptides with 7-14 amino acid residues released by thermolysin.

CHAPTER 7. WHEY PEPTIDES/PROTEINS BASED MULTIFUNCTIONAL MICROPARTICLES CONTAINING YELLOW ONION SKINS EXTRACT

7.1. OBJECTIVES OF THE STUDY

The major aim of this study was to test different technological variants for developing multifunctional ingredients, based on flavonoids, bioactive peptides, and different unique combinations of biopolymeric matrices, for further uses in high-functional foods or nutraceuticals, beneficial to human health. Additionally, our study may represent a basis for the suitable exploitation of by-products resulting from the industrial processing of vegetables, namely yellow onion skins.

Therefore, the objective of the study was to valorize the flavonoid-enriched extracts from yellow onion peels by microencapsulation in different biopolymers and/or peptides combinations. The enriched flavonoid extract from yellow onion peels was obtained by repeated ultrasound assisted extraction and characterised for phytochemical profile and antioxidant potential. Enzymatic hydrolysis involving two enzymes (thermolysin and Proteinase K) produced whey protein hydrolysates. The unique combinations between flavonoids and biopolymeric matrices were microencapsulated by complex coacervation and freeze-drying, whereas the resulting powders were tested for phytochemical profile (total flavonoids content, total polyphenols content), antioxidant activity, and stability storage. Additionally, selected powders were tested as probiotics lactic acid bacteria from co-microencapsulation perspectives.

The increase in the flavonoid bioavailability was tested by *in vitro* digestion experiments, whereas the *in vitro* biocompatibility was demonstrated by testing the viability and compatibility of fibroblast cells in the presence of microcapsules obtained. The morphological and structural characteristics of the powders were also evaluated by confocal laser scanning microscopy. The functionality of the selected ingredients was highlighted by incorporation into food, which was tested for sensorial acceptances.

7.3. MATERIALS AND METHODS

7.3.5. Variants for microencapsulation of yellow onion peels extract into different biopolymer matrices

Experiment 1: Two lyophilized powders were obtained as a result of two sets of microencapsulation experiments. Approximately 20 mL of the β-LG hydrolysates were combined with 0.5 g of the onion skins extract before being subjected to ultrasound at 40.0 ± 1.0 °C for 75 minutes. at. After that, the mixture was frozen at -70 ° C and lyophilized (CHRIST Alpha 1–4 LD plus, Germany) at -42 ° C under a pressure of 0.10 mBar for 48 hours. The corresponding powder, which included peptides with molecular weights under 10 kDa as encapsulating material, was labeled P1. The P2 sample was created by repeating the technique with native β-LG as shell material. Afterward, the powders (P1 and P2) were collected and packed in metal bags and stored in the refrigerator until further analysis.

Experiment 2: Two powder variants coded H and I were obtained as follows: for sample H, a quantity of 1.22 g extract of flavonoid from yellow onion peel brought to dryness was mixed with 50 mL 2% WPI solution hydrolyzed with transglutaminase and left on the ultrasonic bath to solubilize for

30 minutes. The sample was then thoroughly homogenized for 1 hour using a magnetic stirrer over which 0.5 g of *Lactobacillus casei* was added under sterile conditions. In the end, homogenization took place again for one hour followed by lyophilization. The same protocol was followed to obtain sample I, the only change being the replacement of the hydrolyzed protein solution with a 2% non-hydrolyzed WPI solution. The samples were then lyophilized and stored in the refrigerator until use.

Experiment 3: Microencapsulation was performed using the following maltodextrin: pectin ratios: (M: P): 1: 1 (variant V1), 2: 1 (variant V2), and 1: 2 (variant V3). Each variant was homogenized at 40°C and 450 rpm for 30 min, after which 15 mL of extract solution and 20 mL of protein hydrolysate (HP) were added and mixed again for 1 h at 40°C and 450 rpm. The samples were then frozen at -70 °C, and the ice particles were eliminated by lyophilization at -42 ° C for 48 hours at a pressure of 0.10 mBar. Afterwards, both powders were collected, packed in metal bags, and stored in the freezer at -20 ° C until further analysis. Each experiment was performed in duplicate.

Experiment 4: For the microencapsulation of the yellow onion peel extract, freeze-drying was used as the main technique; and mixtures of maltodextrin, pectin, and whey protein isolate as encapsulation matrices. Three experimental variants were obtained. Thus, variant A uses maltodextrin: pectin: whey protein isolate, in a ratio of 1: 1: 1, variant B uses maltodextrin: pectin: whey protein isolate: protein hydrolysate, in a ratio of 1: 1: 1: 0.5, and variant C uses maltodextrin: pectin: whey protein isolate: whey protein hydrolysate, in a ratio of 1: 0.5: 1: 0.5. Subsequently, each variant involved the addition of 1.6 g of ethanolic extract dissolved in 60 mL of acidified water at pH of 5.0, the mixture was stirred for one hour at 500 x g and a temperature of 40°C. Coacervation involved a decrease in the pH of the solution to 5.0, followed by lyophilization. The samples were lyophilized at a temperature of -42 ° C, under a pressure of 0,10 mBar, for 48 h. Subsequently, the powders were collected, packed in glass containers, and stored at 25 ° C until characterisation. Each experiment was performed in duplicate.

7.4. RESULTS AND DISCUSSION

7.4.1. Functional composites based on yellow onion skins in different molecular states of β-lactoglobulin: evidences on phytochemical profile, structure and morphology, bioaccessibility and prebiotic effects

Experiment 1: Microencapsulation of flavonoids from yellow onion peels into β-lactoglobulin and its thermolysin-derived hydrolysates

- **Preliminary *in silico* and experimental evaluation of the thermolysin-derived peptides from β-lactoglobulin**

The hydrolysis degree was 31.94 ± 0.49%. It is known that the main whey protein, β-LG, acts as a precursor for several functional peptides. For example, in trypsin-assisted digestion, the presence of five bioactive peptides in the total hydrolysate was identified. The sequences SAPLRVY f (36–42) and LIVTQTMKG f (1–9) were divided into dipeptides and tripeptides, respectively. On the other hand, the KPTPEG f (47–52) sequence proved to belong to the longest peptide (Val59-Ile72) obtained under the experimental conditions considered.i In our study, β-LG did not show antioxidant activity, while the hydrolysates <10 kDa had an antioxidant activity of 1.26 ± 0.16 mMol protein Trolox / mg.

Table 7.1. *Peptides released by complete thermolysin-assisted hydrolysis of β-lactoglobulin (β-LG)*

Residues number	***Sequences***
14	**Val^{59}-Ile^{72*}**
10	**Val^{139}-Ala^{148}**
9	Leu^{74}-Cys^{82}
8	**Leu^{111}-Tyr^{118}**
7	Leu^{48}-Pro^{54}; Ala^{89}-Pro^{95}
6	**Ala^{32}-Ser^{37}**; Ala^{127}-Ser^{132}; Leu^{172}-His^{177}
5	Leu^{26}-Lys^{30}; Ala^{42}-Ser^{46}; Leu^{103}-Lys^{107}; Phe^{167}-Gln^{171}
4	Val^{19}-Thr^{22}; Ala^{83}-Lys^{86}; Met^{123}-Ser^{126}; Ala^{134}-Cys^{137}
3	Met^{23}-Gly^{25}; Ile^{100}-Ala^{102}; Leu^{149}-Lys^{151}; Phe^{152}-Lys^{154}; Ala^{158}-Pro^{160}
2	Leu^{55}-Arg^{56}; Val^{57}-Tyr^{58}; Phe^{98}-Lys^{99}; Phe^{121}-Cys^{122}; Leu^{156}-Lys^{157}; Met^{161}-His^{162} Ile^{163}-Arg^{164}; Leu^{165}-Ser^{166}

* Sequence similarity with BioDADPep peptides

- **Extract characterization, microencapsulation efficiency, and phytochemical characterization of powders**

The yellow onion peel extract obtained by ultrasound assisted extraction exhibited a total flavonoid content of 228.68 ± 3.09 mg QE / g DW, a total polyphenolic compound content of 96.06 ± 2.70 mg gallic acid equivalents (GAE) / g DW, and an antioxidant activity of 495.89 ± 2.42 mM Trolox equivalents / g DW.

Furthermore, the chromatographic profile of the yellow onion peel extract was performed (Figure 7.2). The profile allowed the identification of 5 compounds, namely quercetin 7,4-diglucoside, quercetin 3,4-diglucoside, quercetin 4-glucoside, quercetin and kaempferol. The main compound found was quercetin 3,4-diglucoside with a percentage of 19.29%, followed by quercetin which revealed a content with a concentration of 7.30% of the total extracted flavonoids. The first compound identified was quercetin 7,4-diglucoside, which showed a content of 2.79% of the total flavonoid compounds extracted, while kaempferol showed a content of less than 1%.

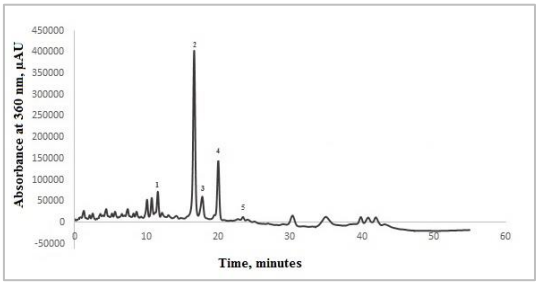


Figure 7.2. HPLC analysis of onion peel extract: Peak (1) - quercetin 7,4-diglucoside; Peak (2) - quercetin 3,4-diglucoside; Peak (3) - quercetin 4-glucoside; Peak (4) -quercetin; Peak (5) - kaempferol

The samples coded P1 and P2 containing peptides with a molecular weight of less than 10 kDa and native β-LG as encapsulation material were distinguished by different results regarding encapsulation efficiency and phytochemical constituents. The encapsulation efficiency values for the

flavonoids obtained in the first variants were 43.77 ± 0.78% for P1 and 61.07 ± 1.07% for P2. The highest ability to encapsulate flavonoids in onion peel extract was given by β-LG and decreased with the size of the polypeptide chain. In a previous study, pectin, maltodextrin, WPI, and HP were used to encapsulate flavonoids from yellow onion skins extract by lyophilization. The efficiency of the encapsulation depended on the composition of the biopolymers ranging from 55.95 ± 0.44% to 66.46 ± 0.18%, depending on the ratio of maltodextrin:pectin.

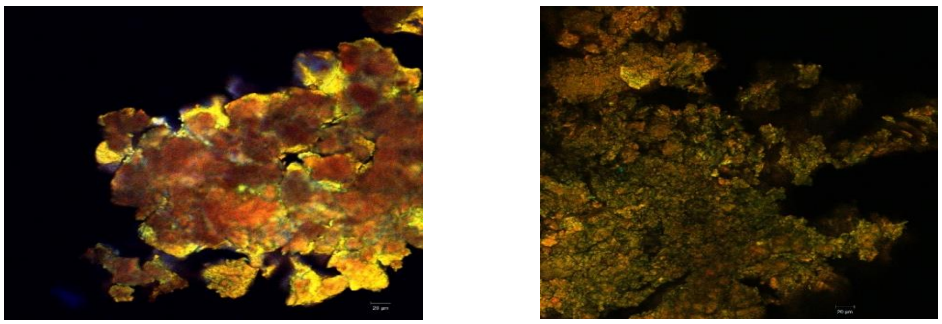
Table 7.2. *Phytochemical profile of extract and powders obtained by encapsulating flavonoids in different coating materials*

Characteristics	Extract	P1	P2
TFC, mg QE/g DW	228.68±3.05^{c}	339.64±2.70^{a}	329.57±4.10^{b}
TPC, mg GAE/g DW	96.06±2.70^{c}	188.26±7.13^{a}	174.66±1.29^{b}
AA, mM Trolox/g DW	495.89±2.42^{b}	503.54±2.89^{a}	495.32±0.99^{b}

The average values that do not have a letter in common in the same row (a,b,c) are significantly different. The differentiation is based on the Tukey method and the 95% confidence factor.

- **Microparticles structure and morphology**

Confocal microscopic analysis was used to analyze the microstructural design of microparticles in variants P1 and P2. Confocal powder images are shown in Figure 7.3. The P2 variant showed a uniform microscopic appearance, having small microspherosomes with diameters ranging from 1-2 μm, with emission predominantly in the green range (550 nm). The tendency of microspherosomes to aggregate into clusters can be seen in Figure 7.3. b. This is well correlated with the results obtained in terms of encapsulation efficiency. However, the use of β-LG hydrolysates has obviously changed the structure of microparticles. Peptides with a molecular weight of less than 10 kDa (shown in yellow) determined the agglutination of polyphenolic compounds and flavonoids, leading to coacervates larger than 20 μm, with the emission spectrum carried in the red range (630-650 nm) (Figure 7.3, a).



a) b)

Figure 7.3. Confocal images with laser scanning microscopy performed with ZEN 2012 SP1 software (Black Edition) on samples obtained by encapsulating flavonoids in various encapsulation materials: peptides obtained by hydrolysis of β-lactoglobulin by thermolysin and β-lactoglobulin

- **Antidiabetic potential**

In our study, the inhibition efficiency decreased in the following order P1> P2, and at the measured concentration (10 mg/mL), the powders were typically more effective against -glucosidase

than -amylase. According to Table 7.3, P1 had the greatest effect against α-glucosidase with a value of 44.80 ± 0.58%, followed by P2 with a value of 32.76 ± 2.08%. Additionally, P1 had the highest inhibitory action against α-amylase, 5.30 ± 0.31%, as compared to P2's 2.24 ± 0.80% inhibitory effects.

Table 7.3. *The effect of microencapsulated powders on enzymes associated with carbohydrate metabolism*

The results are reported in percent (%) as an inhibitory effect.

Enzymes	P1	P2
α-glucosidase	44.80±0.58^{a}	32.76±2.08^{b}
α-amylase	5.30±0.31^{a}	2.24±0.80^{b}

The average values that in a row do not share a letter (a,b) are significantly different, based on the Tukey method and 95% confidence.

Experiment 2: Microencapsulation of flavonoid extract in cross-linked whey protein isolate

Microencapsulation used protein hydrolysate (Variant H) and whey protein isolate (Variant I) as coating material. As can be seen from Table 7.4, a higher concentration of biologically active compounds, respectively flavonoids (158.16 ± 1.18 mg QE / g DW compared to 128.95 ± 1.42 mg QE / g DW) and polyphenols (82.14 ± 4.11 mg GAE / g DW compared to 70.51 ± 5.21 mg GAE / g DW), was identified in variant H. Probably the lower content of polyphenolic compounds in variant I am due to a higher encapsulation in the matrix given by the whey protein isolate and its release under controlled conditions. This hypothesis is also confirmed by the results obtained in the case of higher encapsulation efficiency for variant I, respectively 25.01 ± 2.11% compared to 16.20 ± 1.82%. The antioxidant activity showed a slightly higher value in the case of a variant I (157.48 ± 1.02 mMol Trolox / g DW compared to 154.51 ± 1.66 mMol Trolox / g DW) due to the existence of the protein hydrolysate in variant H, which could have antioxidant potential.

However, after obtaining the results of this experiment and comparing them with those reported previously, the powder was not used as a functional ingredient.

Table 7.4. *Phytochemical characteristics of the samples*

Phytochemicals	H	I
TPC (mg GAE/g DW)	82.14±4.11^{A}	70.51±5.21^{B}
TFC (mg QE/ g DW)	158.16±1.18^{A}	128.95±1.42^{B}
AA (mMOL TROLOX/g DW)	154.51±1.66^{A}	157.48±1.02^{A}
EE (%)	16.20±1.82^{B}	25.01±2.11^{A}

Based on the Tukey method and 95% confidence, average values on the same row that do not share a letter (A,B) are significantly different.

7.4.2. Functional composites based on yellow onion skins in whey protein hydrolysate and polyglucides: evidences on phytochemical profile, structure and morphology, bioaccessibility and prebiotic effects - Experiment 3

- **Enzymatic hydrolysis of whey protein isolate**

WPI hydrolysis by proteinase K was performed for 6 hours. Extending the period of hydrolysis has led to a very bitter hydrolysate, due to the breakdown of small peptides during the release of amino acids. Therefore, it is very important to know the degree of hydrolysis (DH), time, enzymes, etc. which make it possible to obtain a large number of small, less bitter peptides. In this study, after

6 hours of hydrolysis, the protein hydrolysate obtained had a HD of 31.20 ± 1.23%. The higher the HD, the lower the number of low molecular weight peptides. Peptides are known to have physiological effects on the human body, on the nervous system and the digestive system by improving digestion, antihypertensive activity, and the cholesterol-lowering effect.

Encapsulation efficiency and characterization of the resulting powders

Experimental encapsulated powders were obtained using each other protein hydrolysate and different ratios of maltodextrin: pectin, such as 1:1 for variant V1, 2:1 for variant V2 and 1:2 for variant V3.

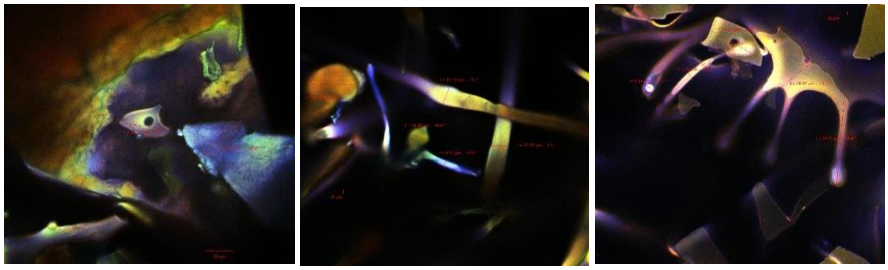
The encapsulation efficiency of flavonoids in onion skin extracts was 55.95 ± 0.44% in variant 1, 66.46 ± 0.18% in variant 2, and 57.99 ± 1.73% in variant 3. Microparticles obtained in this study had TFC of 98.12 ± 0.55 mg QE / g DW, TPC of 53.53 ± 1.71 mg GAE / g DW and antioxidant activity of 280.61 ± 3.08 mM Trolox / g DW in variant 1, while variant 2 had values of 103.75 ± 0.57 mg QE / g DW, 57.17 ± 0.23 mg GAE / g DW and 321.59 ± 1.77 mM Trolox / g DW for TFC, TPC, and antioxidant activity respectively.

Variant 3 had a TFC value of 101.11 ± 0.47 mg QE / g DW compared to the other experimental variants, while the total polyphenol content was significantly higher (69.26 ± 1.03 mg GAE / g DW). Consequently, the antioxidant activity of variant 3 (337.57 ± 0.89 mM Trolox / g DW) was higher for variants 1 and 2. It should be noted that experimental variants containing HP showed satisfactory antioxidant activity, suggesting that bioactive peptides in whey hydrolysates contributed to the increased functionality of microencapsulated powders. However, based on the encapsulation efficiency and the phytochemical profile of the powders, it is fair to conclude that a combination of HP, maltodextrin, and pectin, probably led to the formation of colloidal particles that successfully encapsulated the flavonoids in onion skin extracts.

- **Structure and morphology of microencapsulated powders**

Powders were compared and the presence of bioactive compounds in the form of asymmetric and thin scales with emission across the full spectral range due to complexity (small peptides, different types of polyphenols, and polysaccharides) was observed. In variant 2, the morphological appearance of the microparticles is predominantly filamentous with variable lengths and a diameter between 4.41 and 15.19 μm, ending in large globular conformations (12.44 - 36.31 μm) (Figure 7.5.B). In variant 3 (Figure 7.5.C) a mixed configuration is observed, both in scale (29.97 - 44.70 μm) and in the form of filaments (finger-shaped or needle-shaped expansions with a thickness of 5, 62 μm and a length of 73.71 μm) with globular ends (approximately 6.81 μm in diameter).

The bioactive compounds in the yellow onion skins extract formed large, compact clusters, as can be seen in Figure 7.5. (small red particles), distributed in the biopolymer fractions (colored blue). Quercetin forms microparticles with a diameter of 1-2 μm, rarely: 5-6 μm, which agglomerate in tight clusters together with spherosomes (10-11 μm diameter) formed from the polysaccharide matrix. Sometimes these spherosomes aggregated into larger vesicles (34.95 μm) (Figure 7.5.B and C).



a) b) c)

Figure 7.5. Confocal laser scanning microscopic images of hydrated colored microencapsulated powder: variant 1 (a), variant 2 (b), variant 3 (c)

- ***Prebiotic effect on Lactobacillus bifermentans***

The survival rate of *Lactobacillus bifermentans* in MRS broth supplemented with microencapsulated flavonoids was analyzed over 21 days of storage at 4°C (Table 7.6). In all samples, the viability of *Lactobacillus bifermentans* decreased after 14 days. The high stimulant effect regarding the viability of *Lactobacillus bifermentans* was observed after 14 days in variant 2. Thus, after 14 days, the viability of *Lactobacillus bifermentans* in the control samples in variant 1 and variant 3 decreased by 3 log CFU / mL compared to variant 2.

Table 7.6. *Lactobacillus bifermentans cell count (log CFU / g) during 21 days of storage*

Sample	**Time storage at 4°C (days)**			
	0	7	14	21
V1	8.47 ± 0.03^{aB}	6.61 ± 0.12^{bB}	3.01 ± 0.04^{cC}	1.00 ± 0.00^{dC}
V2	8.94 ± 0.05^{aA}	8.80 ± 0.009^{aA}	8.07 ± 0.19^{bA}	5.17 ± 0.01^{cA}
V3	8.91 ± 0.18^{aA}	8.73 ± 0.008^{aA}	5.75 ± 0.01^{bB}	4.54 ± 0.08^{cB}

Average values that do not have one letter in common on the same row (a, b, c) differ significantly ($p < 0.05$), based on the Tukey method and the 95% confidence level. Averages that do not have one letter in common on the same column (A, B, C) are significantly different $p < 0.05$, based on the Tukey method and the 95% confidence level.

- ***In vitro digestibility of microencapsulated powders***

The *in vitro* release of total flavonoids from encapsulating materials has been studied in simulated gastric fluids (SGF) and simulated intestinal fluids (SIF). Results of SGS *in vitro* digestibility assessment show that the selected encapsulation materials showed a protective effect on the release of flavonoids, depending on the experimental variant (Table 7.7), Variants 1 and 2 allowed the gradual release of flavonoids from capsules up to 15% (variant 1) and 6% (variant 2) after 120 minutes of digestion. Variant 3 showed a maximum protective effect, with a slight decrease of a maximum of 2% after 120 minutes of digestion. Therefore, it can be appreciated that the microparticles coated with maltodextrin, pectin, and whey proteins hydrolyzed in a ratio of 2: 1: 0.5 are resistant in the gastric environment, thus protecting the flavonoid compounds during crossing through the stomach. In the simulated intestinal juice, the results showed that the maximum total flavonoids are released after 120 minutes of simulated intestinal conditions in all variants, with a maximum value recorded for variant 2 at 27%, variant 3 at 25%, and variant 1 at 21%, respectively.

Table 7.7. *Total flavonoid content after in vitro digestion in simulated gastric and intestinal fluids*

	SGF			SIF		
Time (min)	**V1**	**V2**	**V3**	**V1**	**V2**	**V3**
0	79.96 ± 0.63^{c}	89.15 ± 0.57^{b}	93.99 ± 0.40^{a}	82.06 ± 1.89^{A}	67.42 ± 1.77^{C}	67.50 ± 1.37^{B}
30	85.35 ± 2.21^{b}	91.67 ± 1.72^{a}	92.26 ± 0.10^{a}	92.38 ± 1.95^{A}	80.56 ± 0.54^{B}	81.23 ± 1.61^{B}
60	87.81 ± 0.31^{b}	92.48 ± 0.75^{a}	92.58 ± 0.66^{a}	94.63 ± 1.10^{A}	82.79 ± 2.74^{B}	83.66 ± 1.15^{B}
90	89.61 ± 1.90^{b}	93.59 ± 0.36^{a}	92.65 ± 0.35^{a}	97.54 ± 1.78^{A}	87.28 ± 1.63^{B}	84.08 ± 1.81^{B}
120	91.52 ± 1.96^{a}	94.52 ± 1.20^{a}	92.40 ± 0.20^{a}	103.50 ± 2.20^{A}	92.04 ± 0.85^{B}	90.59 ± 1.47^{B}

The average values that on the same row do not share a letter (a,b,c – SGF and A,B,C - SIF) differ significantly, based on Tukey method and 95% confidence.

- ***Storage stability test***

All powders were stored at room temperature for 28 days and were characterized for TFC and antioxidant activity. For TFC, a release phenomenon was observed in variants 1 and 2, with approximately 19% and 18%, respectively. In variant 3, a decrease of approximately 18% was found after 28 days. However, in variants 1 and 2, an increase in TPC up to ~ 3% and ~ 15%, respectively, was observed, while in variant 3, TPC increases by 6% (Table 7.8). As a consequence of the increase in TFC content, variant 1 showed an increase in antioxidant activity (Table 7.8) by approximately 8%. Variant 3 showed a decrease in antioxidant activity, by approximately 8%.

Table 7.8 *Phytochemical content of microencapsulated variants during 28 days of storage at room temperature*

Bioactives	**Storage time (days)**					
	V1		V2		V3	
	0	**28**	**0**	**28**	**0**	**28**
TFC (mg QE)/g	53.53 ± 1.71^{bC}	63.52 ± 0.08^{aB}	57.17 ± 0.23^{bB}	67.30 ± 1.02^{aA}	69.26 ± 1.03^{aA}	57.12 ± 0.72^{bC}
TPC (mg GAE/g)	98.12 ± 0.55^{aC}	101.68 ± 4.84^{aC}	103.75 ± 0.57^{bA}	121.21 ± 7.97^{aA}	101.11 ± 0.47^{bB}	107.16 ± 1.09^{aB}
AA (mM Trolox/g)	280.61 ± 3.08^{bC}	304.92 ± 1.58^{aA}	321.59 ± 1.77^{aB}	316.95 ± 8.51^{aA}	337.57 ± 0.89^{aA}	311.74 ± 2.30^{bA}

Average values that do not have one letter in common on the same row – time-dependent (a, b) differ significantly, $p < 0.05$, based on the Tukey method and the 95% confidence level. Averages that do not have one letter in common on the same row – samples dependent (A, B, C) are significantly different $p < 0.05$, based on the Tukey method and the 95% confidence level.

7.4.3. Functional composites based on yellow onion skins in whey protein isolate, and polyglucides: evidences on phytochemical profile, structure and morphology, bioaccessibility and prebiotic effects - Experiment 4

Phytochemical and physico-chemical analysis of the formulated functional ingredients

The phytochemical analysis of the formulated functional ingredients involved the determination of encapsulation efficiency, total polyphenol content, total flavonoid content, and antioxidant activity. Variant A had a total polyphenol content of 41.09 ± 1.69 mg GAE / g DW, variant B 39.00 ± 1.42 mg GAE / g DW and variant C 43.97 ± 1.36 mg GAE/ g DW. Regarding the total

flavonoid content, the highest concentration was found in the mixture of variant B, 103.54 ± 15.09 mg equivalents quercetin / g powder, followed by variant C, 99.15 ± 8.39 mg equivalents quercetin / g powder, and variant A, 97.49 ± 3.19 mg equivalents quercetin / g powder. The highest antioxidant activity was presented by the formulation related to variant B (Table 7.10).

Table 7.9. *Characteristics of yellow onion peel extract*

Characteristics	Flavonoidic extract
Humidity (%)	1.74±0.21
Ash (%)	98.26±0.98
TPC (mg GAE/g DW)	55.27±2.47
TFC (mg QE/g DW)	97.28±3.01
AA (mMol Trolox/g DW)	344.97±2.68

Table 7.10. *Physico-chemical and phytochemical characteristics of the formulated functional ingredients*

Characteristics	A	B	C
Ash, %	$9.79±0.08^{C}$	$10.09±0.19^{B}$	$10.51±0.01^{A}$
Humidity, %	$4.53±0.03^{A}$	$4.54±0.09^{A}$	$4.55±0.06^{A}$
Protein substances, %	$22.6±0.001^{C}$	$27.9±0.012^{B}$	$28.4±0.17^{A}$
Carbohydrates, %	$63.08±0.12^{A}$	$54.47±0.9^{C}$	$56.54±0.24^{B}$
Energetic value, kcal/100 g	351.28	337.71	348.25
Encapsulation efficiency, %	$91.54±0.99^{A}$	$90.55±1.38^{A}$	$89.46±1.62^{A}$
TPC (mg GAE/g DW)	$41.09±1.69^{A}$	$39.00±1.42^{B}$	$43.97±1.36^{A}$
TFC (mg QE/g DW)	$97.49±3.19^{A}$	$103.54±15.09^{A}$	$99.15±8.39^{A}$
AA (mMol Trolox/g DW)	$130.94±4.57^{C}$	$147.75±4.33^{A}$	$141.04±1.64^{B}$

Superscript values in the same row with distinct letters (A,B,C) are significantly different at $p < 0.05$, based on the Tukey method.

It can be seen from Table 7.10 that the addition of bioactive peptides derived from whey protein isolate hydrolysis with a high content of bioactive peptides has led to an increase in protein content in formulations B and C, which has also contributed to increasing the overall antioxidant activity of related functional formulations. The stability of the formulated functional ingredients was verified by storage at 25 °C for 35 days concerning the variation of phytochemicals and antioxidant activity and is shown in Table 7.11.

Table 7.11. *Variation in phytochemicals content in formulated functional ingredients during storage at 25 ° C*

	Storage period (days)					
Phytochemicals	V1		V2		V3	
	0	35	0	35	0	35
TFC (mg QE)/g	$97.49±3.19^{aB}$	$94.52±2.46^{aA}$	$103.54±1.09^{aA}$	$87.14±0.68^{bB}$	$99.15±1.39^{aB}$	$87.65±2.98^{bB}$
TPC (mg GAE/g)	$41.09±1.69^{aB}$	$38.79±2.06^{aA}$	$39.00±1.42^{aB}$	$33.35±0.59^{bB}$	$43.97±1.36^{aA}$	$38.31±0.92^{bA}$
AA (mM Trolox/g)	$130.94±0.57^{aA}$	$127.75±1.75^{aA}$	$117.75±4.33^{aB}$	$114.79±0.70^{bC}$	$131.04±1.64^{aA}$	$120.94±1.79^{bB}$

Average values that do not have one letter in common on the same row – time dependent – 0 vs. 35 days (a, b) differ significantly, based on the Tukey method and the 95% confidence level. Average values that do not have one letter in common on the same row – samples dependent V1 vs. V2 vs. V3, at the same time (A, B, C) are significantly different, based on the Tukey method and the 95% confidence level.

A decrease in the content of total polyphenols in all experimental variants, between 5 and 15%, can be seen from Table 7.11, which indicates relatively high storage stability of polyphenolic compounds. Regarding the total flavonoid content, a higher stability of the total flavonoid content in variant V1, which does not contain protein hydrolysate, can be seen in Table 7.11. The decrease in flavonoid content is 11-15% for V2 and V3 variants. As expected, the reduction in the content of biologically active compounds resulted in a decrease in antioxidant activity when storing the formulated functional ingredients.

Prebiotic potential of powder

The experimental variants of the formulated functional ingredients were used to test the physiological effect on the metabolic activity of *Lactobacillus casei ssp. paracasei* cells, the powders being added to the specific culture medium, liquid MRS, in the proportion of 0.5% and 2%. Cultivation was performed at 37 °C for 48 hours under aerobic conditions. In the fermented medium stored for 14 days at a temperature of 4 °C, the viability of *L. casei* cells was determined by indirect counting (ISO 8261 IDF122: 2001), by culturing on MRS agar medium with 1% $CaCO_3$ for 48 days. hours, the temperature of 37 °C. The number of viable cells was expressed in log cfu / mL fermented medium.

Table 7.12. *Viability of L. casei 431® cells (log cfu / mL) in culture on MRS medium supplemented with 0.5% formulated functional ingredients*

Storage days	A	B	C
0	8.17 ± 0.18^{aA}	8.22 ± 0.07^{aA}	8.25 ± 0.15^{aA}
7	7.12 ± 0.11^{bB}	7.36 ± 0.01^{aB}	7.06 ± 0.07^{bB}
14	5.30 ± 0.14^{bC}	5.30 ± 0.11^{bC}	5.60 ± 0.13^{aC}

Average values that do not have one letter in common on the same row (a, b,c) and the same column (A,B,C) are significantly different p <0.05, based on the Tukey method and the 95% confidence level.

Table 7.13. *Viability of L. casei 431® cells (log cfu / mL) in culture on MRS medium supplemented with 2% formulated functional ingredients*

Storage days	A	B	C
0	7.68 ± 0.15^{aA}	7.35 ± 0.11^{bA}	7.61 ± 0.15^{aA}
7	7.04 ± 0.11^{aB}	6.48 ± 0.14^{cB}	6.70 ± 0.11^{bB}
14	5.63 ± 0.09^{cC}	6.00 ± 0.17^{bC}	6.24 ± 0.13^{aC}

Average values that do not have one letter in common on the same row (a, b,c) and the same column (A,B,C) are significantly different p <0.05, based on the Tukey method and the 95% confidence level.

The results demonstrate the effectiveness of the functional ingredients in maintaining the viability of lactic acid bacteria. A higher concentration of formulated functional ingredients (2%) has a beneficial effect compared to a lower concentration (0.5%), with reduced viability after 14 days of storage under refrigeration conditions of 1.36 (V1) and 1.22 (V2 and V3), respectively.

Applications of functional ingredients for obtaining appetizer biscuits

Due to rising consumer awareness and the development of a healthy lifestyle and diet, the market for bioactive components and foods has massively extended. During food preparation and storage, it is still difficult to guarantee that functional components survive and continue to be "active" and "biologically available. In this study, a recipe for value-added biscuits was developed in order to increase their functional and sensorial characteristics. The bioactives and organoleptic characteristics were studied, as follows.

Table 7.14. *Phytochemical characteristics of appetizer biscuits with the addition of formulated functional ingredients*

Characteristics	M1	A1	B1	C1
TPC (mg GAE/g DW)	0.00 ± 0.00^{B}	0.30 ± 0.03^{A}	0.25 ± 0.04^{A}	0.28 ± 0.11^{A}
TFC (mg QE/g DW)	0.69 ± 0.10^{C}	2.61 ± 0.78^{A}	2.41 ± 0.56^{A}	1.23 ± 0.32^{B}
AA (mM TROLOX/g DW)	7.67 ± 1.57^{C}	16.04 ± 0.12^{A}	15.50 ± 0.21^{B}	15.58 ± 0.17^{B}

Superscript values in the same row with distinct letters (A, B, C) differ significantly, based on the Tukey method ($p < 0.05$).

Appetizer biscuits with the addition of formulated functional ingredients showed added value in terms of the content of total flavonoids, polyphenolic compounds, and antioxidant activity The results of the sensory analysis are shown in Table 7.15.

The sensory analysis

The sensory analysis was performed with 9 panelists, aged between 25 and 44, and researchers from the Faculty of Food Science and Engineering ("Dunărea de Jos" University of Galați). The hedonic scale of appreciation by sensory attributes was used on a scale from 1 to 9 (1 - the minimum intensity of the attribute, 9 - the maximum intensity of the attribute). The results obtained for the phytochemical characteristics are presented in Table 7.15.

Table 7.15. *The results of the sensory analysis of the appetizer biscuits with the addition of the formulated functional ingredients*

Characteristics	M1	A1	B1	C1
Color intensity	3.00±2.17	7.89±1.26	6.66±1.58	7.22±1.39
Color homogeneity	3.67±2.39	7.33±1.50	6.66±1.58	6.78±1.48
Appearance	5.44±2.60	6.67±2.00	5.89±1.83	6.89±1.69
Spice flavour	1.67±1.65	3.67±2.44	4.67±2.59	4.56±2.78
Sweet taste	2.33±2.06	2.55±2.18	2.67±2.39	3.78±2.33
Butter taste	5.22±2.90	6.00±2.23	4.89±2.20	6.11±2.36
Salty taste	4.44±2.06	5.78±1.85	6.33±2.34	5.67±1.58
Mouthfeel	5.11±3.01	6.44±2.35	5.44±2.40	6.56±2.12
Hardness	3.00±2.17	3.22±2.43	2.67+2.17	3.44±2.92
Crunchy	2.77±2.04	3.00±2.54	2.56±2.24	3.33±2.95
Acceptability	5.22±1.71	7.22±1.20	6.68±1.39	7.67±1.32

All three variants with the addition of the formulated functional ingredients obtained a higher overall score compared to the control sample, variant 3 being the best appreciated by the panelists.

7.5. Partial conclusions

A. In the study associated with the *first experiment*, bioactive peptides derived from β-lactoglobulin are still utilized for the encapsulation of flavonoids obtained from yellow onion peels. The possibility for microencapsulating flavonoids from yellow onion peels was examined between β-lactoglobulin and its hydrolysates obtained using thermolysin. The influence of peptides on the control of antioxidant and anti-diabetic activities was discovered by a comparative examination of both types

of microcapsules. The powder containing native β-LG as a coating material was the finest powder, having small microspherosomes (1–2 µm), whilst the peptides influenced the flavonoids to agglutinate, resulting in coacervates bigger than 20 µm. This research's results may be consistent with the idea that β-lactoglobulin along with its peptides could potentially be utilized in innovative approaches for flavonoid encapsulation. This research's results may be consistent with the idea that β-lactoglobulin along with its peptides could potentially be utilized in innovative approaches for flavonoid encapsulation. These formulations could be useful to develop nutraceuticals as alternatives to synthetic additives, but further studies are needed to create and carefully characterize their functionalities before they can be used in foods. The microparticles based on the yellow onion skins extract encapsulated in whey protein and cross-linked whey protein (*the second experiment*) presented a high content of phytochemicals, but a lower entrapping of flavonoids, regarding the encapsulation efficiency. Due to the general low bioavailability of native polyphenolic compounds, the results were not further used for other tests. Onion peel flavonoids have been efficiently encapsulated in a complex matrix based on various proportions of whey protein hydrolysates, maltodextrin, and pectin (*experiment 3*). According to *in silico* analysis, some tetrapeptides resulting from the hydrolysis of α-lactalbumin and β-lactoglobulin may be particularly important due to their antimicrobial function and the inhibitory activity of the angiotensin-converting enzyme. All microencapsulated variants had an important content of flavonoids and antioxidant activity. The results indicated that the maltodextrin:pectin:whey proteins hydrolysates ratio of 2:1:0.4 showed a higher encapsulation efficiency of flavonoids of 66.46±0.18%. *In vitro* digestibility and storage stability confirm the importance of the microencapsulation technique on biologically active compounds. Flavonoids encapsulated from yellow onion peels have the potential to be used as a functional food ingredient with a beneficial effect on consumer health or have a prebiotic effect on lactic acid bacteria.

B. The ingredients obtained from maltodextrin, pectin, and whey protein isolate (*experiment 4*) as encapsulation materials for ethanolic extracts from yellow onion peels are rich in biologically active compounds, with a high concentration of flavonoids. In addition, the formulated functional ingredients, microencapsulated by freeze-drying, contain peptides obtained by enzymatic hydrolysis of whey proteins, recognized for their exceptional biological and functional activity, translated into beneficial effects on the immune, cardiovascular, nervous, and gastrointestinal systems. It can be appreciated that the proposed study is suitable both for maintaining the viability of some probiotic bacteria and for adding as an ingredient in various food formulations as a natural dye, adding functional value through the content of biologically active compounds such as flavonoids and polyphenolic compounds, with high antioxidant activity.

CHAPTER 8. DESIGNING A GREEN METHOD FOR MICROENCAPSULATION OF FLAVONOID ENRICHED EXTRACT FROM YELLOW ONION SKINS

8.2. OBJECTIVES OF THE STUDY

The objectives of this paper were to find out if a green and inexpensive solvent could be used for the extraction of flavonoids from yellow onion peels. The extract will be encapsulated in a combination of whey protein isolates, whey protein hydrolysates, maltodextrin, and pectin to evaluate the functional properties of the resulting powder. The experiments involve the study of flavonoids encapsulation efficiency, flavonoids content, polyphenols content, antioxidant activity, *in vitro* digestion, and the phytochemicals' stability in different temperature and pH ranges.

The powders' cytocompatibility was assessed using mouse fibroblasts in order to assess the release of potentially toxic compounds from microencapsulated samples as well as the proliferative impact. The findings of this study may have implications for maximizing the bioactivity of yellow onion skin flavonoids in the formulation of health-beneficial combinations with superior functional qualities.

8.4. RESULTS AND DISCUSSION

8.4.1. Yellow onion peels extract and powder characterization

Applying hot water as solvent for the phytochemicals` extraction from yellow onion peel, the extract recorded a total flavonoid content of 50,21 ± 0,09 mg QE/g DW, a total polyphenol content of 21,68 ± 0, 69 mg GAE / g DW leading to the antioxidant activity of 250.81 ± 6.76 mM Trolox / g DW.

Table 8.1. Phytochemical profile of microencapsulated powders

SAMPLES	Encapsulation efficiency, %	TFC, mg QE/g DW	TPC, mg GAE/g DW	AA, mM TROLOX/g DW
W1	76.55±2.62^{b}	26.91±2.93^{a}	21.24±0.57^{a}	102.76±0.82^{b}
W2	83.51±1.81^{a}	20.75±0.78^{b}	18.57±0.32^{b}	109.28±0.12^{a}

Values are presented as the mean ± standard deviation. According to the Tukey technique, superscript values for the same column that don't share the same letter (a and b) are statistically different at $p<0.05$.

According to the results shown in Table 8.1, it can be observed that the microencapsulation in WPI, MD, and P of the freeze-dried flavonoid extracts allowed a higher flavonoid encapsulation efficiency of about 84%, compared to the use of WPI, MD, WPH, and P. The biopolymer combination of Variant W1 allowed for better retention of polyphenols, while Variant W2 showed a higher concentration of flavonoids. Freeze-dried ingredients have an important phytochemical profile, antioxidant activity, and a good protective impact, especially during simulated digestion, permitting their incorporation into various food systems.

In order to create a powder that is more stable, versatile, and safe, the matrices were selected. A review of prior research has revealed that Arabic gum, protein, MD, or WPI are commonly used for the microencapsulation of polyphenols, either alone or in combination. The selection of whey protein was based on its high nutritional quality and significant functional properties. Because of its strong water solubility, low viscosity, and low sugar content, MD is frequently used as encapsulating

material. HP is considered a source of bioactive peptides with antibacterial, antiviral, and anti-inflammatory activities for functional foods.

The W2 sample was found to have a lower concentration of flavonoids and polyphenols and a stronger antioxidant activity. This could be possible due to the influence of bioactive peptides which could have higher antioxidant activity and also the encapsulation of other classes of polyphenols, which could also exhibit antioxidant character. The samples were tested for the stability of phytochemicals after 30 days at refrigeration temperature. The results can be observed in Table 8.2.

Table 8.2. *Phytochemical stability in microencapsulated powders during storage* (Milea et al., 2021b)

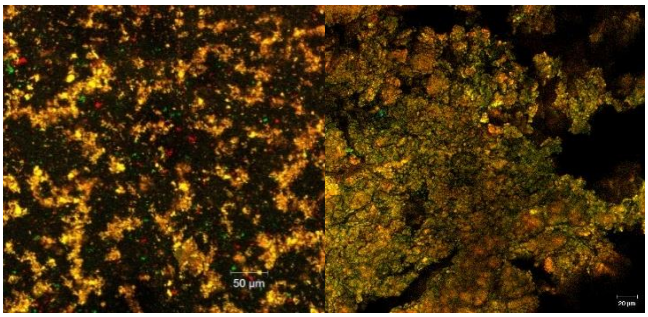
POWDERS	TFC, mg QE/g DW	TPC, mg GAE/g DW	AA, mM TROLOX/g DW
W1	22.35±0.60^{a}	19.56±0.05^{a}	82.23±0.92^{b}
W2	22.74±0.18^{a}	18.91±0.18^{b}	86.99±1.15^{a}

Average values ± standard errors are used to express the results. According to the Tukey method, superscript values in the same column that do not share the same letter (a and b) are statistically different at $p<0.05$.

Table 8.2 shows that variant W2 had improved phytochemical stability, resulting in a 10% increase in flavonoids, whereas variant W1 had a 17% loss in flavonoids. It was also observed that polyphenols are stable in variant W2, while they decreased by about 8% in variant W1. Antioxidant activity decreased by 20% in both versions.

8.4.2. Structural analysis of powders by confocal laser scanning microscopy

Important morphological and structural model properties were revealed by the CLSM analysis of the two powders. The images were collected after staining the samples and were produced utilizing point-by-point scanning at a high resolution in a wide field with the aid of digital focusing (Figure 8.2a; b).



a) b)

Figure 8.2. Confocal microscopic images gained with ZEN 2012 SP1 software (black edition) of the two powder samples obtained by encapsulating the flavonoids extracted from the yellow onion peels from different materials: Variant W1 - WPI: WHP: MD: P - 2: 0, 5: 1: 0.5 (a), Variant W2 - WPI: MD: P - 2: 1: 0.5 (b)

Flavonoids are a significantly useful category of polyphenolic compounds with a strong antioxidant capacity that is of vegetable origin, with over 4500 distinct compounds that offer a diverse absorption-emission spectrum that is dependent on the extraction source's biochemical profile. By confocal laser scanning microscopy, the microstructural design of the obtained powders was

evaluated. The fluorescence labeling of the two fluorescent-colored powders revealed a well-individualized matrix containing extremely small microspherosomes compacted with flavonoids and polyphenols, as well as the microencapsulation matrix. Regarding the analyzed powder, as can be seen from Figure 8.2, from a structural point of view, the W1 variant had the finest structure (Figure 8.2a), which showed a fairly uniform microscopic appearance in the form of very small microspherosomes, with diameters less than 2 µm, emitting around 550 nm, meanwhile for variant W2, the observed microspherosomes showed a tendency to agglomerate in larger groups.

8.4.3. *In vitro* digestibility of flavonoids

In this study, the *in vitro* behavior of simulated gastric (SGF) and intestinal (SIF) flavonoids from microparticles was carried out. Both variants' flavonoid levels were found to be significantly stable in SGS (Figure 8.3 a), W2 powder showing a minor increase in flavonoids of up to 1%. For that reason, it can be concluded that flavonoids have been accurately protected by selected coating materials.

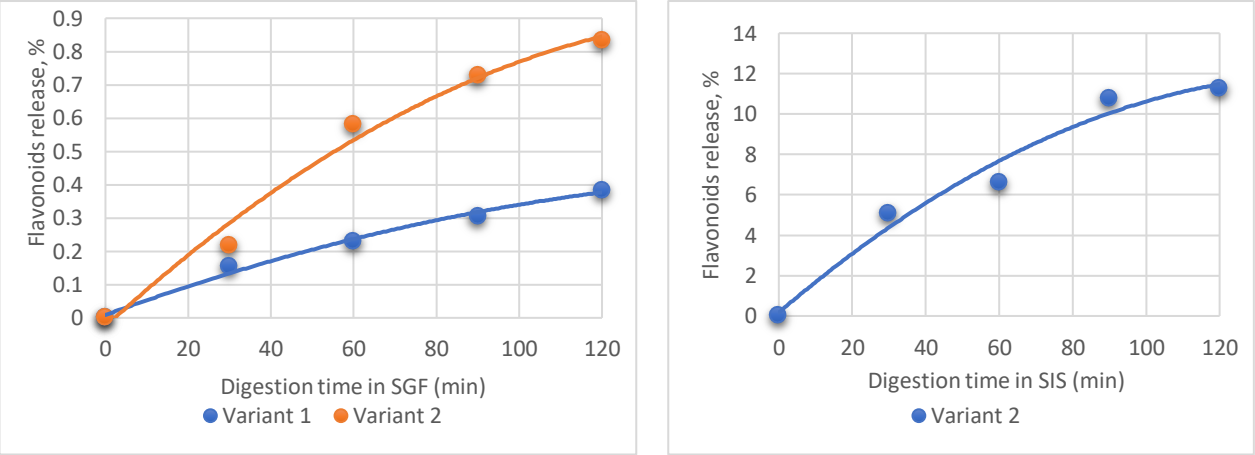


Figure 8.3. Release of flavonoids (%) after *in vitro* digestion in simulated gastric (a) and intestinal (b) juices

Regarding SIS, no flavonoids were found in sample W1, indicating a potential maximum absorption capacity. The total flavonoids were released over a period of 120 minutes under simulated intestinal conditions in sample W2, with a maximum recorded value of 11%.

8.4.4. Microencapsulated powders' *in vitro* cytotoxicity

The presence and release of any potentially toxic substances from powders, as well as cell viability, were investigated via NR testing in L929 fibroblast cell culture. After 24 and 48 hours of cultivation (> 80% cell viability), the results demonstrated that microencapsulated onion extracts had no cytotoxic impact when used in tested concentrations (Figure 8.4).

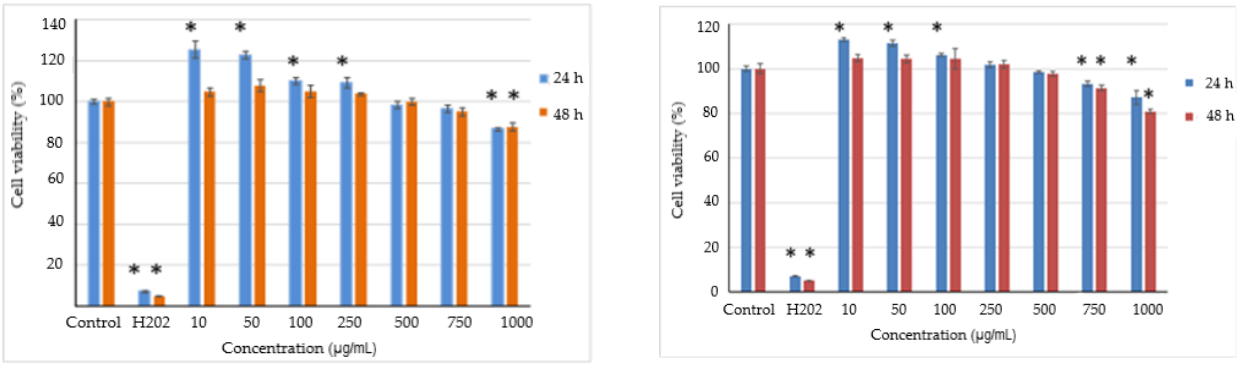


Figure 8.4. Cell viability of L929 fibroblasts cultured in the presence of microencapsulated yellow onion peel extracts (variant W1 (A) and variant W2 (B) for 24 h and 48 h, respectively, determined by NR test. Control (untreated) samples are considered to be 100% viable. Values represent mean ± SD (n = 3) * p <0.05 compared to control.

Cellular viability varied from 81% to 113% for W1 and from 87% to 125% for W2 and afterward decreased as powder concentration increased. According to these findings, the cytocompatibility of microencapsulated variations can reach up to 1 mg/mL. Furthermore, concentrations ranging from 10-100 µg/mL for sample W1 and 10-250 µg/mL for sample W2 promoted cell proliferation after 24 hours of cultivation, with the greatest values for sample W2. Concentrations higher than 750-1000 µg/mL for variation 1 and 1000 µg/mL for variant W2 resulted in a minor decrease in cell viability of up to 80% and 87%, respectively.

Cell morphology was also associated with NR quantitative data (Figure 8.5). Images revealed that the microencapsulated powder-treated cells preserved their typical fusiform phenotype, which is unique to fibroblast cells, in a manner comparable to untreated culture.

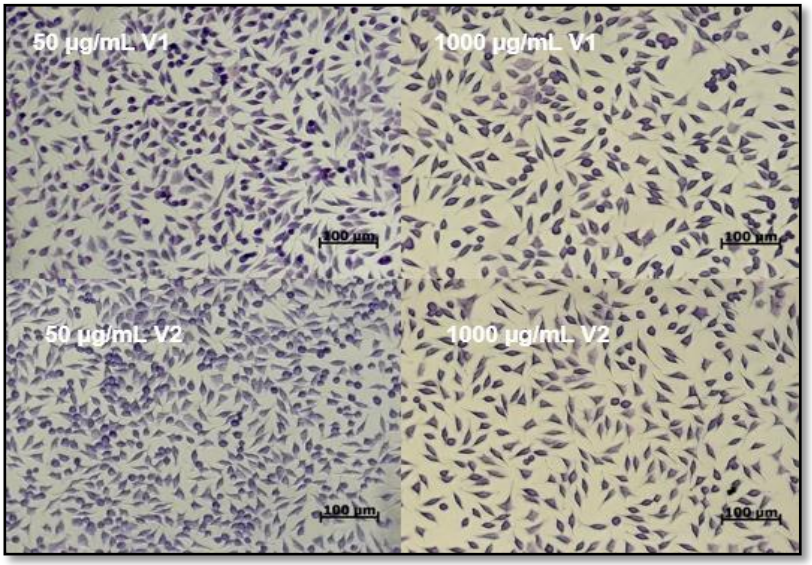
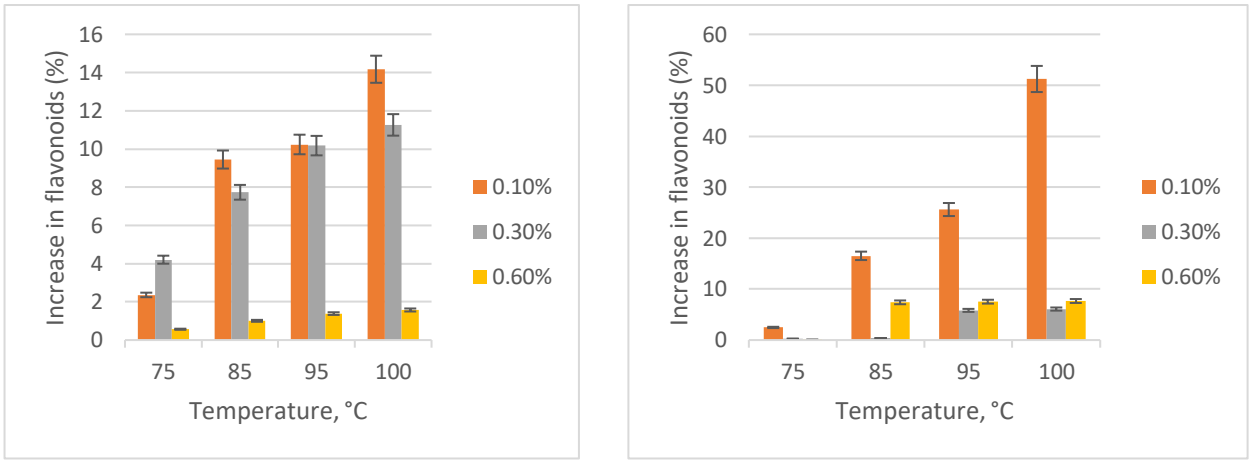


Figure 8.5. Micrographs of L929 cells treated for 48 hours with microencapsulated variants of yellow onion peel extracts

The density of the cells was equivalent to that of the untreated culture, and the dispersion of the cells on the culture plate was uniform. A higher cell density was detected at levels ranging from 10-100 µg/mL compared to the control sample. The cell density in the culture plates declined as particle concentrations increased.

8.4.5. Thermal and pH stability

Because many functional foods containing degradable bioactive compounds are typically subjected to heat treatments and pH variations, it is important to investigate the heat treatment and pH behavior of bioactive compounds in a replicated system before using these powders as functional ingredients. This led to the chosen powder concentration, which ranged from 0.1 to 0.6%, being dissolved in water and being exposed to a range of temperatures (25°C to 100°C) for 15 minutes. After heat treatment, the flavonoid concentration was determined. (Figure 8.6.). The release of the compounds and the heat breakdown of the microencapsulation matrices may have been what caused an increase in flavonoid concentration.



a b

Figure 8.6. Effect of heat treatment on flavonoids released from microencapsulated powders (a) variant W1, b) variant W2)

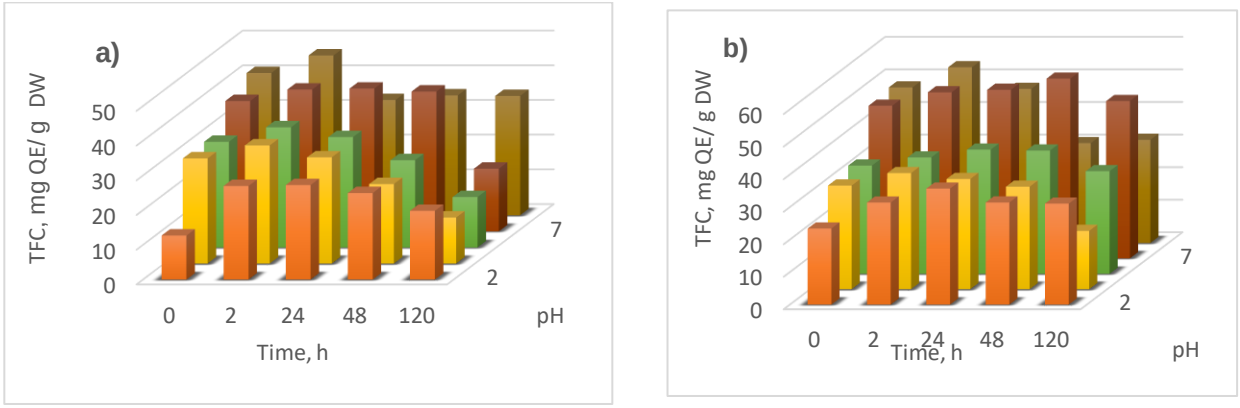


Figure 8.7. Effect of pH on the content of flavonoids in microencapsulated powders (a) variant W1, b) variant W2)

The release of flavonoids from microcapsules has been shown to be temperature and concentration-dependent. The maximum release of approximately 14 percent was recorded in variant W1 at 0.1% and 100 ° C, while a large release of more than 51% was detected in variant W2 under comparable conditions. As a result, when heat treatment was used, the W2 version exhibited less of a protective effect. In terms of stability at different pH values, the results are shown in Figure 8.7.

Various periods, ranging from 0 to 120 hours, were used to test the pH's stability as a constant value. Figure 8.7 shows that the flavonoid content grew with time, up to 24 hours, and then began to slowly decrease with each test, independent of the microencapsulated variant. After 2 hours, the highest increase in flavonoid content was recorded at pH 2.0, when variation W1 had a twofold increase in flavonoid content.

8.5. Partial conclusions

A. The extraction of flavonoid constituents from yellow onion peels was done effectively using hot water extraction, providing a safe extract enriched in flavonoids. The results showed that extracting flavonoids from yellow onion peel in hot water may be employed as an alternate solvent for the selective extraction of flavonoid components. A biopolymer matrix made of complex proteins, peptides, and carbohydrates was effectively used to microencapsulate the aqueous extract, with a success rate of over 76%. The chosen microencapsulation matrices offered a protective effect during simulated digestion, and the lyophilized powders exhibited considerable phytochemical content and antioxidant activity. CLSM analysis showed that the particle structure of the powders obtained by the two microencapsulation variants was evenly distributed. The powders had a wide range of cytocompatibility, up to 1 mg/mL, and encouraged cell growth at lower doses. They could possibly be added to many food matrices because of the powders' good temperature and pH stability.

B. The results of this research indicate that user-friendly approaches to maximize onion peel use can be used in the development of multifunctional components for culinary, biopharmaceutical, or nutraceutical.

CHAPTER 9. CO-MICROENCAPSULATION OF FLAVONOIDS FROM YELLOW ONION SKINS AND DIFFERENT STRAINS OF LACTIC BACTERIA

9.2. OBJECTIVES OF THE STUDY

In this study, a profile of co-microencapsulated flavonoids from yellow onion peel extract and LAB was determined. The microencapsulated powders were obtained by freeze-drying, using whey protein isolate, maltodextrin, and inulin as coating materials. The potency of phenolics against harmful free radicals and enzymes involved in hyperglycemia, dyslipidemia, oxidative stress, and the inflammatory process associated with metabolic syndrome were examined as well.

Total polyphenol (TPC) and total flavonoid (TFC) concentrations were measured in the powder to assess antioxidant activity. The powder's inhibitory effect on lipase, α-amylase, and lipoxygenase was also tested in order to predict biological activity. Cell viability, proliferation, and morphology were tested in the presence of co-microencapsulated powder to determine *in vitro* biocompatibility were tested in the presence of co-microencapsulated powder in fibroblast cells to determine *in vitro* biocompatibility. The powder's functional value was determined by incorporating it into appropriate food matrices, which were then evaluated for global phytochemical component stability in relation to antioxidant activity and bacterial cell viability during storage. These findings are relevant for using the positive benefits of flavonoids and probiotics in the development of novel useful components with enhanced functional qualities.

9.3. MATERIALS AND METHODS

9.3.1. Co-microencapsulation of flavonoids in yellow onion skins with two different strains of lactic bacteria

Table 9.1. *Samples codification*

Sample/codification	Inoculum/probiotic bacteria
B0	20 mL sterile ultrapure water
LB	20 mL *Lactobacillus bifermentans* suspension
LP	20 mL *Lactobacillus plantarum* suspension
BP	20 mL suspension of *Lactobacillus bifermentans* + *Lactobacillus plantarum* (1:1)

9.4. RESULTS AND DISCUSSION

9.4.1. Phytochemical characterization of yellow onion peel extract

Table 9.2 shows the phytochemical profile of the aqueous extract obtained from the yellow onion peels.

Table 9.2. *Phytochemical characteristics of flavonoid extract from yellow onion peels*

Characteristics	Extract
TFC (mg GAE/g)	50.21±0.10
TFC (mg QE/g)	21.68±0.69
AA (mMol Trolox/g)	250.81±6.76

It can be seen from Table 9.2 that the flavonoid extract from the yellow onion peels had a high content of polyphenols and flavonoids, which led to significant antioxidant activity.

9.4.2. Phytochemical and probiotic characterization of co-microencapsulated ingredients

Table 9.3. *Functional characteristics of the formulated ingredients*

Analyses	B0	LB	LP	BP
EE (%)	89.21±1.99^{a}	92.23±1.14^{a}	93.31±0.89^{a}	91.32±2.56^{a}
TPC (mg GAE/g DW)	16.42±0.06^{c}	17.40±0.83^{b}	17.73±1.59^{b}	18.40±0.68^{a}
TFC (mg QE/ g DW)	17.35±1.97^{a}	15.06±0.68^{b}	16.66±1.42^{a}	16.43±1.18^{a}
AA (mMol Trolox/g DW)	84.74±2.90^{b}	81.03±2.31^{c}	84.29±0.55^{b}	91.92±3.73^{a}
EE LAB (%)	-	84.18±2.12^{a}	71.2±2.17^{b}	76.63±3.11^{b}
LAB Viability (CFU/g DW)	-	1.94X10^{7c}	3.6X10^{7a}	2.57X10^{7b}

Superscript values in the same row with distinct letters (a, b, c) differ significantly at $p < 0.05$, based on the Tukey method.

As can be seen from Table 9.3, there are no significant differences in phytochemical profile and flavonoid encapsulation efficiency in the 4 experimental variants. It can be appreciated that the co-microencapsulation technique and the materials used allowed us to obtain variants of powders with a high content of biologically active compounds and lactic acid bacteria, with a high encapsulation efficiency. Therefore, all variants showed remarkable values of antioxidant activity. Significant differences were noted in the efficiency of the encapsulation of lactic acid bacteria, with a higher efficiency for the species *Lactobacillus bifermentans*.

Table 9.4. *Functional characteristics of ingredients formulated after 30 days of storage*

Phytochemicals	B0	LB	LP	BP
TPC (mg GAE/g DW)	15.52±0.60^{a}	12.89±0.38^{b}	15.03±0.53^{a}	14.33±0.51^{a}
TFC (mg QE/ g DW)	15.69±2.26^{a}	14.40±0.38^{a}	15.93±0.61^{a}	10.32±0.76^{b}
AA (mMol Trolox/g DW)	83.90±0.76^{b}	80.18±0.86^{c}	81.29±0.58^{c}	88.86±0.78^{a}
Viability (CFU/g DW)	-	1.90x10^{7b}	1.87x10^{6c}	2.56x10^{7a}

Superscript values in the same row with distinct letters (a,b,c) differ significantly at $p < 0.05$, based on the Tukey method.

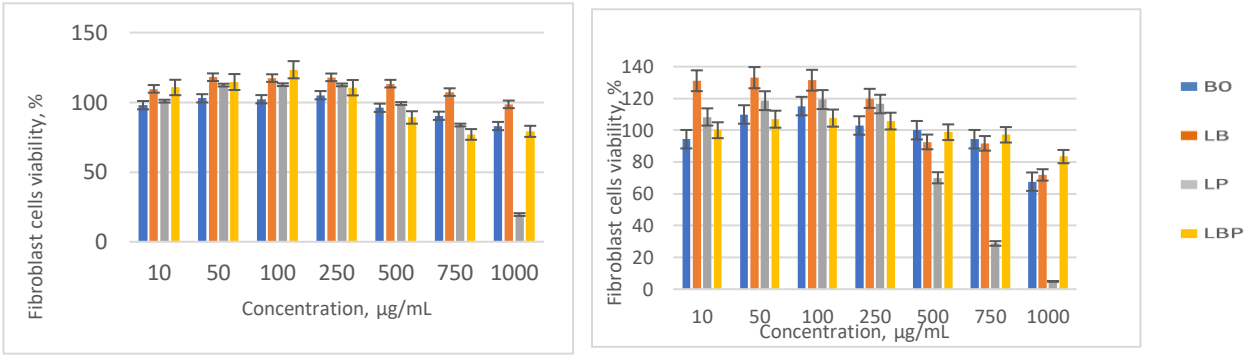
Storage at 4-6 ° C for 30 days resulted in a decrease in the content of biologically active compounds, especially in sample B0, for the total polyphenol content (by about 26%) and in sample BP, for total flavonoid content (about 37%). All samples retained antioxidant activity, over 80 mM Trolox / g DW P-coded sample showed a 1 log reduction in the number of lactic acid bacteria in storage, while samples LB and BP showed a 100% viability. The results obtained in the controlled storage for 30 days suggest that the multifunctional ingredients resulted from co-microencapsulation have a remarkable potential for use in food systems, both as antioxidant and probiotic, but also for the development of nutraceuticals.

9.4.3. *In vitro* cytotoxicity of microencapsulated samples

The results of the *in vitro* biocompatibility assessment (Figure 9.1) showed that all variants were biocompatible because, compared to the control sample (cells in culture medium, no products), cells cultured in the presence of functionalized ingredients showed high values of viability and

proliferation, and their morphology in culture was normal, characteristic of the fibroblast cell phenotype.

The BP sample was biocompatible in the range of 10-1000 µg / mL after 24 h of culture and in the range of 10-1000 ug / mL after 48 h of culture. The BP sample stimulated cell proliferation at concentrations of 10-250 ug / mL even after 24 h of culture, but also in the range of 10-250 µg / mL after 48 h of culture. Cell viability values were significantly higher compared to the untreated control, reaching up to 123% at 24 h of culture and 107% after 48 h of culture.



a b

Figure 9.1. Viability of fibroblast cells cultured in the presence of multifunctional ingredients for 24 hours and 48 hours, respectively

9.4.4. Testing co-microencapsulated ingredients in the food system

The four experimental variants of multifunctional ingredients were added, in the proportion of 1% in yogurt-based sauces, for which they were tested: initial phytochemical and lactic profile and storage stability, considering an average shelf life 21 of days. The sauces were stored in bottles with UV protection and stored for the mentioned period at 4-6°C. The derived sauces kept the coding system for the ingredients, adding the letter S to avoid confusion. The results obtained initially and after 21 days of storage are presented in Table 9.5 and Table 9.6.

Table 9.5. *Phytochemical and probiotic characteristics of value-added sauces*

Characteristics	SB0	SB	SP	SBP
TPC (mg GAE/g DW)	4.89 ± 0.08^{c}	5.72 ± 0.54^{b}	5.86 ± 0.36^{b}	7.22 ± 0.67^{a}
TFC (mg QE/ g DW)	1.52 ± 0.32^{b}	2.14 ± 0.22^{ab}	2.31 ± 0.73^{a}	2.29 ± 0.11^{a}
AA (mMol Trolox/g DW)	9.09 ± 0.25^{c}	14.91 ± 0.45^{b}	15.00 ± 0.35^{b}	17.42 ± 0.38^{a}
Viability of LAB (CFU/g DW)	-	$1.87x10^{7}$	$1.67x10^{6}$	$2.30x10^{7}$

Superscript values in the same row with distinct letters (a,b,c) differ significantly at $p < 0.05$, based on the Tukey method.

From Table 9.5, it is noted that samples with lactic acid bacteria showed a higher content of biologically active compounds and higher antioxidant activity.

Table 9.6. *Functional characteristics of value-added sauces after 21 days of storage*

Characteristics	SB0	SB	SP	SBP
TPC (mg GAE/g DW)	4.15 ± 0.31^{b}	6.26 ± 0.35^{a}	5.59 ± 0.50^{a}	6.10 ± 0.27^{a}
TFC (mg QE/ g DW)	2.54 ± 0.43^{a}	2.74 ± 0.25^{a}	2.78 ± 0.42^{a}	2.97 ± 0.46^{a}
AA (mMol Trolox/g DW)	6.32 ± 0.14^{b}	11.41 ± 1.11^{a}	12.79 ± 1.36^{a}	13.29 ± 0.35^{a}
Viability of lab (CFU/g DW)	-	$1.77x10^{6}$	$1.10x10^{5}$	$1.01x10^{6}$

Superscript values in the same row with distinct letters (a,b) differ significantly at $p < 0.05$, based on the Tukey method.

From Table 9.6, it can be seen that during storage, the multifunctional ingredients showed a stable profile, with insignificant release kinetics of flavonoids from microcapsules and a decrease of approximately 1 log of lactic acid bacteria.

9.4.5. Phytochemical characterization of the extract and powder co-microencapsulated with *Lactobacillus casei*

The extract and the overall phytochemical characterization of the powder are presented in Table 9.7. The TFC of the yellow onion peel extract was 229.14 ± 3.05 mg equivalents of quercetin (QE) /g of dry weight, the TPC was 96.06 ± 2.70 mg equivalents of gallic acid (GAE) /g of dry weight, and the antioxidant activity was 101.19 ± 0.53 mM Trolox /g of dry weight.

Table 9.7. *Phytochemical characterization of the extract and powder*

Phytochemicals	Extract	Powder
TFC, mg QE/ g DW	229.14 ± 3.05^{a}	89.49 ± 4.12^{b}
TPC, mg GAE/ g DW	96.06 ± 2.70^{a}	34.17 ± 1.79^{b}
AA, mM Trolox/ g DW	101.19 ± 0.53^{a}	39.27 ± 0.45^{b}
EE, %	-	84.82 ± 0.72
LAB EE, %	-	72.49 ± 0.11

Average values that do not share the same superscript letter (a, b) differ significantly.

The encapsulation efficiency of lactic acid bacteria in freeze-dried powder has reached values of 72.49 ± 0.11%.

The overall phytochemical profile of the powder exhibited high concentrations of flavonoids content of 89.49 ± 4.12 mg QE / g DW and a TPC of 34.17 ± 1.79 mg GAE/ g DW, with a DPPH radical removal capacity of 39 .27 ± 0.45 mM Trolox / g SU, reaching an inhibition rate of 87.40 ± 0.95% (Table 9.7)

9.4.6. Co-microencapsulated powder's solubility and hygroscopicity

In the present study, the co-microencapsulated powder showed a water solubility parameter of 65.07 ± 1.24%. This result can be attributed to the microencapsulation technique which consists of mixing for 20 hours and also to the complexity of the encapsulation coating material. Additionally, the particle size distribution and the recorded results are linked. The final solubility largely depends on the chemical composition of the final food product, which would eventually lead to a wider surface availability for the process of hydration.

9.4.7. Stability of flavonoids in simulated gastrointestinal conditions

The results related to *in vitro* digestibility tests in the presence of simulated gastric and intestinal fluids respectively demonstrate that the coating materials had a protective influence on the release of flavonoids. The sample showed a minor decrease of flavonoids in gastric fluids by about 3% after 120 minutes of gastric interaction, which means that almost all bioactive compounds are kept encapsulated (Figure 9.3).

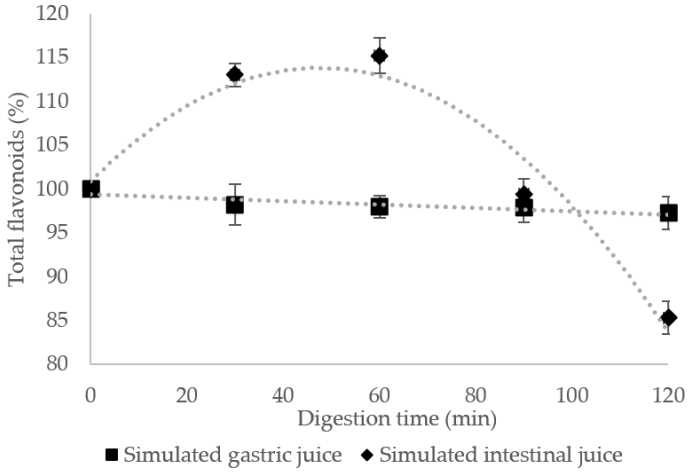


Figure 9.3. Total release of flavonoids from co-microencapsulated powder during *in vitro* digestion in simulated gastric and intestinal juices. The data shown represent the averages of the samples in triplicate. The error bars on the chart represent standard deviations.

On the other hand, the concentration of flavonoids increased by 15% in the intestinal digestion, highlighting a release from the encapsulated matrix, followed by a decrease of about 15% at the end of the reaction, suggesting that 85% of flavonoids are available after the entire digestion stage.

9.4.8 Antidiabetic and anti-inflammatory potential

α-amylase, lipase and lipoxygenase inhibition potential

The sample can be taken into consideration for its antidiabetic potential, as shown by the inhibition ratio of 76.40 ± 2.30% found in this research. Sources of natural antioxidants can be studied for lipase inhibition to replace drugs. The co-microencapsulated powder had an inhibitory activity of 82.58 ± 3.36% in this situation. The powder recorded an inhibitory activity of 49.01 ± 0.62% against lipoxygenase. According to these results, it can be specified that the functional ingredient can be used in order to achieve the desired beneficial effects on health.

9.4.*9. In vitro* cytotoxicity of microencapsulated powders

The release of possible toxic compounds from natural extracts and cell viability were evaluated in the L929 fibroblast cell culture by NR test. The results of the experiment (Figure 9.4) indicated that the yellow onion extract did not exhibit a cytotoxic impact until the tested doses of 500 g/mL after 24 hours and up to 250 µg/mL after 48 hours of culture (> 80% cell viability). Cell viability values ranged from 70.9% -116.7% for onion extract and declined with increasing concentration.

After 24 hours and 48 hours of treatment, concentrations more than 750–1000 µg/mL and 500–1000 µg/mL, respectively, were shown to moderately reduce cell viability. These results show that onion powder up to 50 µg/mL is cytocompatible. Additionally, compared to the control culture, cell proliferation was stimulated by onion extract concentrations in the range of 10-100 µg/mL after 24 and 48 hours of culture.

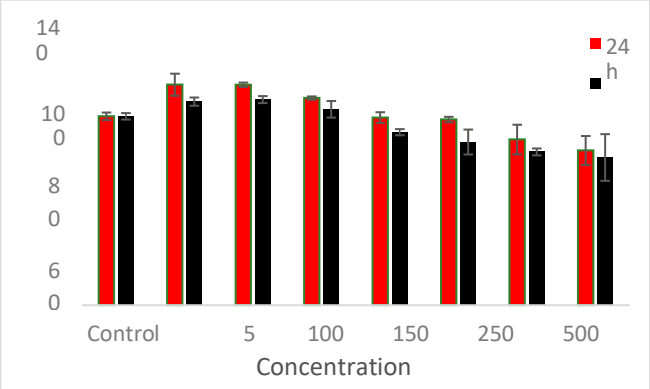


Figure 9.4. Cell viability of L929 fibroblasts cultured in the presence of onion plant extracts for 24 hours and 48 hours.

The results were expressed as a percentage of the control culture (untreated), considered 100% viable. Values represent mean ± SD (n = 3). p <0.05, compared to the control sample.

Observations related to cell morphology were consistent with quantitative NR data (Figure 9.5).

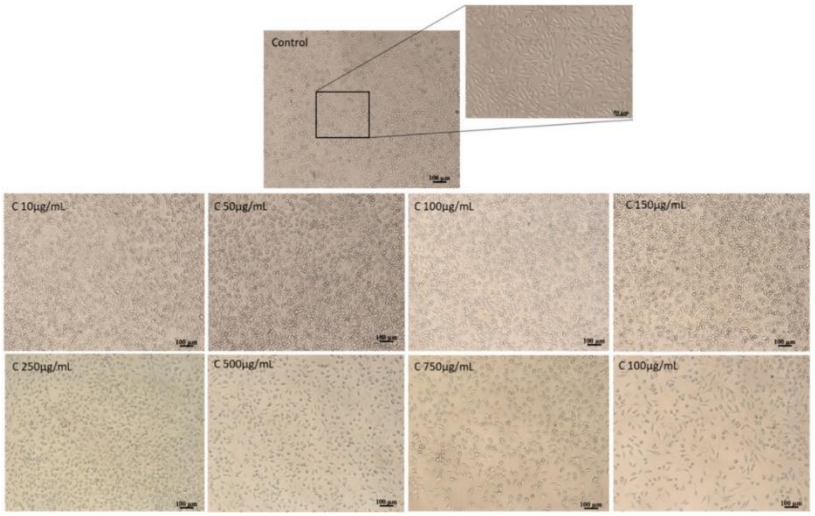


Figure 9.5. Images of L929 cells that have been exposed to onion extract for 48 hours

According to the images above, it can be seen that the co-microencapsulated powder-treated cells maintain their typical fusiform phenotype, which is typical of fibroblast cells, and are comparable to the control culture cells. In addition, a uniform distribution of cells with a density comparable to the control culture can be observed on the culture plate. In comparison to the control culture, the cell density was higher at doses of 10-100 g/mL for powder. Cell density in treated cultures was considerably lowered by greater levels of plant extracts > 750 g/mL. improvement in cytocompatibility terms.

9.4.10. Characterization of the newly formulated food product

The powder was integrated into a chosen matrix, in various proportions, in order to test its functional qualities. There were obtained two types of soft cheese: type C1 (1%) and type C2 (2%), as well as a blank sample free of powder, coded as sample C. The foods obtained were analyzed for the stability of bioactive compounds and the viability of lactic acid bacteria cells for 21 days during storage at refrigeration temperature (4-6°C). After 21 days of storage at 4°C, the C1 and C2 samples' co-microencapsulated LAB cell viability ranged from 6.66 to 7.51 log CFU / g DW. In comparison to samples C1 and C2, which had an increase of 1 log CFU/g DW, sample C showed a reduction in lactic acid bacteria viability of 0.5 log CFU/g DW after 21 days.

Table 9.8. *Phytochemical characteristics of the samples*

Sample	TFC, mg QE/g DW		TPC, mg GAE/g DW		AA, mM Trolox/g DW	
	0	21 days	0	21 days	0	21 days
C1	4.81 ± 0.32^{aB}	2.75 ± 0.23^{bA}	4.68 ± 0.24^{aA}	3.06 ± 0.24^{bB}	1.95 ± 0.01^{aB}	1.81 ± 0.01^{bA}
C2	5.87 ± 0.22^{aA}	3.16 ± 0.11^{bA}	5.15 ± 0.29^{aA}	3.60 ± 0.14^{bA}	2.01 ± 0.01^{aA}	1.83 ± 0.01^{bA}

Superscript values in the same row with distinct letters (a,b) are significantly different at $p < 0.05$, based on the Tukey method. Superscript values in the same column with distinct letters (A,B) are significantly different at $p < 0.05$, based on the Tukey method.

9.4.11. CIELAB analysis

Table 9.9. *Color parameters of microencapsulated powder and soft cheese*

Sample	Color parameters		
	L*	a*	b*
Powder	20.33 ± 0.89^{d}	15.85 ± 0.13^{b}	10.27 ± 0.13^{c}
Blank	102.39 ± 0.32^{a}	-2.24 ± 0.14^{c}	3.93 ± 0.45^{d}
C1	82.84 ± 5.02^{b}	15.70 ± 0.77^{b}	21.16 ± 1.74^{b}
C2	73.61 ± 0.09^{c}	20.02 ± 0.12^{a}	25.84 ± 0.05^{a}

Superscript values in the same column with distinct letters (a,b,c,d) differ significantly at $p < 0.05$, based on the Tukey method.

9.5. Partial conclusions

A. It can be appreciated that the co-microencapsulation process has been effective in protecting biologically active compounds and lactic acid bacteria, which has led to remarkable values for antioxidant activity and maintaining the viability of lactic acid bacteria, both in the formulated ingredients and in the sauce yogurt, after a long storage period (21 days).

B. The results presented in this study support the multifunctionality of co-microencapsulated ingredients, obtained by lyophilizing the ethanolic extract of yellow onion peel, rich in selected flavonoid compounds and lactic bacteria, in a combination of co-microencapsulation matrices, which exploits potentially technological and exploitative whey proteins, peptides and whey, maltodextrin and pectin. In conclusion, it can be stated that the ingredients showed high values of encapsulation efficiency, both for flavonoid compounds and for lactic acid bacteria, demonstrating the double functionality through the antioxidant and metabolic activity of lactic acid bacteria. The results of this experiment were included in patent request A/00471, 01.08.2019.

C. A biopolymer complex was used for encapsulation, including the use of freeze-dried whey protein isolate, maltodextrin, and inulin with the addition of *Lactobacillus casei*. The resulting powder

exhibited remarkable properties in terms of phytochemical profile, antioxidant, anti-diabetic, anti-inflammatory potential, and important cytocompatibility. Both flavonoids and lactic acid bacteria have achieved high encapsulation efficiency. The obtained *in vitro* digestibility results indicated that the coating material had a protective effect on flavonoid release. The physical properties of the microencapsulated powder have been investigated, and the result can be correlated with their storage stability. In order to test the functional potential of the food, the powder was added to a food system (cream cheese). A stimulating effect on the viability of *L. casei* was observed after 21 days in the variants containing microencapsulated ingredient. A synergistic effect between lactic acid bacteria and polyphenols was observed in this study.

D. These results confirmed that the powders obtained by encapsulating onion flavonoids and lactic acid bacteria have the potential to be used to obtain functional or nutraceutical foods.

CHAPTER 10. INCREASING THE MICROENCAPSULATION EFFICIENCY AND FUNCTIONALITY OF MICROPARTICLES THROUGH GLYCATION

10.2. OBJECTIVES OF THE STUDY

Many researchers have used whey protein as coating material, but none have employed whey protein isolate (WPI) in its glycosylate form containing xylo-oligosaccharides as an encapsulation material for flavonoids to our knowledge. The objective of the current research was to determine if WPI conjugates might be used as coating materials for flavonoids derived from yellow onion peels. Flavonoids were extracted using a combination of liquid-solid ethanolic and ultrasound-assisted extraction techniques. Freeze-drying was used to create the flavonoid microcapsules. The freeze-dried powders were assessed in terms of entrapping efficiency, phytochemical composition, antioxidant activity, and other factors using WPI and xylose with and without heating. Confocal laser electron microscopy was used to examine the structural and morphological characteristics of the materials. To determine the added value, the powders were mixed into a recipe for a food product (nachos), then characterized phytochemically and physicochemically. The findings of this study might have implications for both maximizing the bioactive potential of phytochemicals and designing formulae with enhanced functional qualities.

10.4. RESULTS AND DISCUSSION

10.4.1. Phytochemical characterization of yellow onion peel extract

The ultrasound-assisted method used in this study resulted in an extract enriched with bioactive compounds, containing total flavonoids of 228.68 ± 3.00 mg QE/ g DW, a total polyphenol content of 96.06 ± 2.70 mg GAE / g DW, producing an antioxidant activity of 495.89 ± 2.42 mM Trolox/ g DW.

10.4.2. Correlation between microencapsulation efficiency, grafting degree, and browning intensity

Using absorbance readings at 420 nm and 600 nm, respectively, the formation of brown pigment in microencapsulated particles was assessed. As expected, the browning index was higher (0.12 ± 0.01) for the heat-treated sample (M2) than for the untreated variant (0.09 ± 0.01) (M1).

The browning intensity and the powders' antioxidant activity increased proportionally, demonstrating the glycated variant's noticeable antioxidant capacity as a result of the heating processIn terms of antioxidant activity, the powders demonstrated significant differences, with readings of 179.7 ± 4.5 mMol TE / g DW for sample M1 and 184.4 0.7 mMol TE / g DW for sample M2. In this study, a correlation can be observed between the degree of glycation and the efficiency of encapsulation in both variants. Therefore, for sample M1, a grafting degree of 22.6 2.5% and an encapsulation effectiveness of 86.7 ± 1.4% were noted. Variant 2 was calculated to have significantly greater values ($p<0.05$), with an encapsulation efficiency of 90.5 ± 0.3% and a grafting degree of 30.4 ± 1.6%. As a result, it is clear that these Maillard reaction conjugates had a greater capacity to bind and encapsulate the flavonoids in the extract of yellow onion peels.

10.4.3. Molecular modeling

The *in silico* method was used to collect data on how protein glycation with xylose affects further flavonoid binding. By running molecular docking models, potential binding sites for X have been identified for both α-LA and β-LG molecules. Based on binding energy levels and interface area, the most suitable three matches with α-LA and β-LG as receptors were chosen and thoroughly examined (Table 10.1).

Table 10.1. *Molecular details of the interaction between the major whey proteins (α-lactalbumin (α-LA) and β-lactoglobulin monomer (β-LG)) tempered at 25 ° C, xylose (X) and major onion peel flavonoids (quercetin-4 ' -O-monoglycoside (QMG) and quercetin-3,4'-O-diglucoside (QDG)* (Milea et al., 2021c)

	α-lactalbumin - xylose			**α-lactalbumin - QMG**	**α-LA - QDG**
	Complex 1	**Complex 2**	**Complex 3**	**Horincar et al. (2019)**	
			Interaction descriptors		
Amino acids interacting with ligands	Thr^{33}, Glu^{49}, Phe^{53}, Gln^{54}, Tyr^{103}, Trp^{104}, Leu^{105}, Ala^{106}	Thr^{33}, Val^{42}, Asn^{44}, Glu^{49}, Phe^{53}, Gln^{54}, Tyr^{103}, Trp^{104}, Leu^{105}, Ala^{106}	Glu^{11}, Leu^{12}, Asp^{14}, Leu^{15}, Thr^{38}, Leu^{85}, Thr^{86}, Ile^{89}, Met^{90}, Lys^{93}	Leu^{3}, Glu^{11}, Leu^{12}, Lys^{13}, Asp^{14}, Thr^{38}, Leu^{52}, Leu^{85}, Thr^{86}, Asp^{88}, Ile^{89}, Met^{90}, Lys^{93}	Glu^{1}, Leu^{3}, Arg^{10}, Glu^{11}, Leu^{12}, Lys^{13}, Thr^{38}, Leu^{52}, Asp^{83}, Leu^{85}, Thr^{86}, Asp^{88}, Ile^{89}
Binding energy, kcal/mol	-13.00	-10.52	-7.48	-24.41	-32.01
Interface area, Å^2	153.5	147.5	159.8	625.20	541.20
			Pocket descriptors		
Volume, Å^3	339.58	339.58	381.50	435.84	
Depth, Å	15.72	15.72	13.18	13.94	
Enclosure	0.16	0.16	0.20	0.30	
	β-LG - Xyl			**β-LG - QMG**	**β-LG - QDG**
	Complex 1	**Complex 2**	**Complex 3**	**Horincar et al. (2019)**	
			Interaction descriptors		
Amino acids interacting with ligands	Tyr^{20}, Tyr^{42}, Val^{43}, Glu^{44}, Gln^{59}, Cys^{66}, Pro^{126}, Leu^{156}, Glu^{157}, Glu^{158}, Gln^{159}, Cys^{160}, His^{161}	Ala^{23}, Met^{24}, Ala^{25}, Leu^{133}, Phe^{136}, Asp^{137}, Leu^{140}, Arg^{148}, Leu^{149}, Ser^{150}	Ile^{2}, Leu^{93}, Glu^{108}, Ala^{111}, Gln^{115}, Leu^{117}	Ala^{37}, Pro^{38}, Leu^{39}, Val^{41}, Leu^{58}, Lys^{60}, Ile^{71}, Ile^{84}, Asp^{85}, Ala^{86}, Leu^{87}, Asn^{88}, Asn^{90}, Met^{107}, Glu^{108}, Asn^{109}	Pro^{38}, Leu^{39}, Val^{41}, Leu^{58}, Lys^{60}, Glu^{62}, Ala^{67}, Lys^{69}, Ile^{71}, Asn^{90}, Met^{107}, Glu^{108}, Asn^{109}
Binding energy, kcal/mol	-13.37	-16.41	-13.00	-35.05	-34.37
Interface area, Å^2	169.1	152.8	157.6	502.10	585.90
			Pocket descriptors		
Volume, Å^3	217.15	141.31	137.86	229.70	
Depth, Å	9.42	7.68	10.16	13.08	
Enclosure	0.12	0.19	0.19	0.33	

Moreover, it appears that this expansive region, which has a volume of 435.8 Å3, can host an X molecule that covers QMG without influencing QDG binding. However, it should be observed that α-LA has a higher affinity for QMG and QDG (binding energies of -24.21 kcal/mol and -32.01 kcal/mol, respectively) than X (binding energy of - 7.48 kcal/mol).

However, the X molecule can be handled by the β-LG molecule in three distinct areas with sizes ranging from 137.86 to 217.15 (Table 10.1) without affecting QMG or QDG binding (Figure 10.3.).

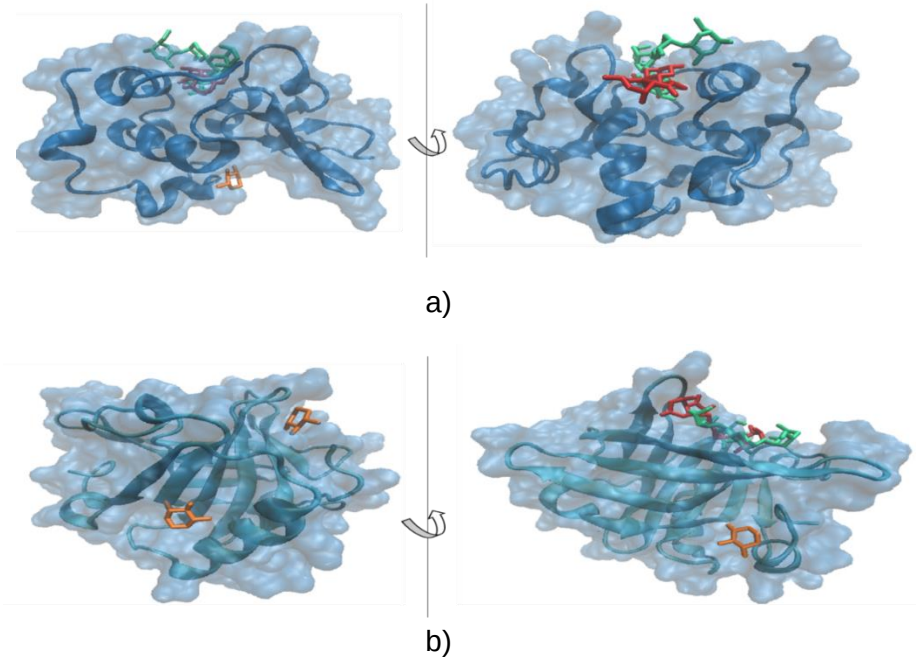


Figure 10.3. Overlapping models that show the most likely complexes between (a) α-lactalbumin and (b) β-lactoglobulin (represented in blue) and xylose (represented in orange - models 1 and 2), quercetin- 4′-O-monoglucoside (represented in red) and quercetin-3,4′-O-diglucoside (represented in green). VMD software was used to record the images

Compared to α-LA, β-LG bonds X molecules more closely; the β-LG monomer's binding energy to X ranges between -16.41 and -13.00 kcal/mol. As shown in Table 10.1, hydrogen interactions with Glu158 in complex 1 and Met24, Asp137, and Leu149 in complex 2 are also essential to the attachment of two X molecules to β-LG pockets.

10.4.4. Phytochemical profile of powders

The two powders had different phytochemical content, consisting of a total flavonoid content of 97.65 ± 3.74 mg QE / g DW in M1 and 119.99 ± 1.57 mg QE / g DW in M2. The total polyphenolic content reported no significant differences, respectively 45.11 ± 0.65 mg GAE / g DW and 46.98 ± 1.57 mg GAE / g DW in M1 and M2, producing an appropriate antioxidant activity of 179.74 ± 4.51 mMol Trolox / g DW and 184.38 mMol / g DW respectively.

10.4.5. Powder structure and morphology

Using a Carl Zeiss 710 scanning microscope with ZEN 2012 SP1 (Black Edition) software, images of M1 and M2 powders (Figure 10.4.) were captured, both in their native state without any

additional dye added (Figure 10.4. a and c) and colored Congo red (Figure 10.4., b and d, respectively).

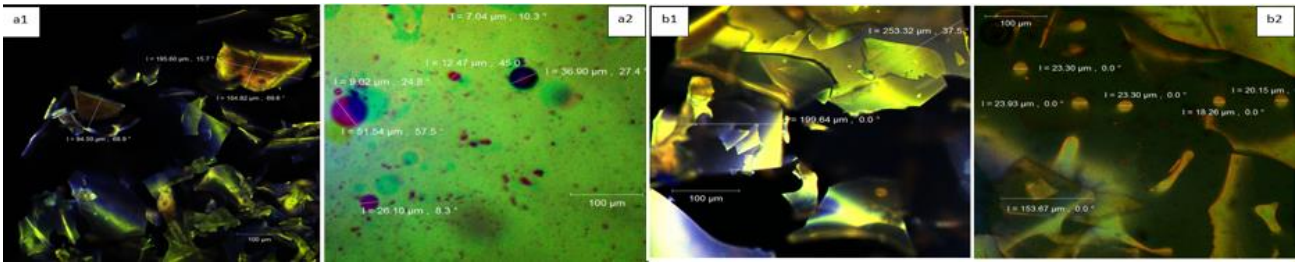


Figure 10.4. Images of sample M1 (a - native state and b - stained with fluorophores) and sample M2 (c - native state and d - stained with fluorophores) captured by CLSM

Biologically active compounds from the onion peels were better incorporated into finer spherosomes (about 20 μm) or in the shape of scales with digit-like extensions, as a result of WPI's connections with low carbohydrates during the Maillard reaction. A fine, orange wall with a fluorescent emission ranging from 600 to 620 nm has appeared around the flavonoids as a result of the fluorophore's binding to the conjugated proteins.

10.4.6. Characterization of a newly formulated food product

The powders were incorporated into a nachos recipe at 3% to evaluate the chosen applicability. In order to determine the long-term storage stability of its constituents for 28 days at 25 ° C, two variants of nachos were developed, each coded as N1 (3% of variant M1) and N2 (3% of variant M2) and a powder-free control, coded sample C. As expected, the differences in polyphenolic compounds and the antioxidant activity between the samples correlate with the amount of added powder (Table 10.2). The flavonoids in N1 did not significantly decrease, as opposed to N2, where a substantial reduction was seen. Both versions presented a substantial increase in antioxidant activity values during storage, which was likely caused by the release of other antioxidant molecules s from microcapsules, like phenolic acids. As a result, antioxidant activity in both samples increased by about 26%.

Table 10.2. *Nachos' value-added phytochemical composition and stability over a 28-day storage period*

Compounds	Blank		N1		N2	
	0	**28**	**0**	**28**	**0**	**28**
TFC, mg QE/g DW	0.66±0.02^{a}	0.63±0.02^{a}	1.04±0.05^{a}	0.99±0.01^{a}	1.08±0.05^{a}	0.98±0.04^{b}
TPC, mg GAE/g DW	0.73±0.04^{a}	0.75±0.02^{2}	1.24±0.03^{a}	1.22±0.05^{a}	1.37±0.19^{a}	1.08±0.019^{a}
AA, mMol Trolox/g DW	156.07±2.57^{b}	197.97±1.74^{a}	157.89±1.41^{b}	199.49±0.81^{a}	158.19 ±0.48^{b}	198.57±0.35^{a}

Values are represented as mean ± standard errors. Superscript values in the same row with distinct letters (a, b) are significantly different at $p < 0.05$, based on the Tukey method.

10.5. Partial conclusions

A. In this research, xylose glycated whey protein isolates were successfully used to create flavonoid-loaded microcapsules through the Maillard reaction. An important range of flavonoids was present in the initial extract, and its antioxidant activity was also remarkable. In order to

microencapsulate flavonoid-rich onion skin preparations by freeze-drying, whey protein isolates and xylose were used, in both nonglycated and glycated forms. The browning index and antioxidant activity were found to be positively correlated, and as a result, the grafting degree and microencapsulation efficiency were also found to be strongly related. A greater capacity of xylose-conjugated whey protein isolates to capture flavonoids has also been proven by confocal laser scanning microscopy.

B. Molecular docking studies have identified potential areas on the surfaces of α-lactalbumin and β-lactoglobulin employed in the interaction with the xylose molecule. Following the addition of the powder to the nachos, a small reduction in phytochemicals was observed during storage. The antioxidant activity of value-added products, on the other hand, has grown, most probably as a result of the release of other bioactive molecules from the microcapsules.

C. According to the results of the investigation, Maillard protein-monosaccharide conjugates are a viable option for food ingredient carriers and attractive promising delivery methods.

CHAPTER 11. FINAL CONCLUSIONS

It has become increasingly prevalent in recent years to adopt a healthy lifestyle based on consuming value-added products such as food and drinks that may reduce the risk of developing a specific illness. This study is based on the exploitation of by-products from the industrial processing of vegetables, namely yellow onion peels, sources rich in natural antioxidants.

The present study aimed to diversify and compare the different extraction techniques concerning the content of biologically active compounds in onion peel. The results obtained revealed that all the procedures (conventional and assisted methods) represent suitable techniques for the extraction of biologically active compounds. The chromatographic profile of yellow onion skins extracts obtained through various extraction techniques revealed the presence of five essential compounds such as quercetin 7,4-diglycoside, quercetin 3,4-diglycoside, quercetin 4-glucoside, quercetin, and kaempferol. The *in vitro* digestibility of the extract revealed that the concentration of flavonoids decreases after gastric digestion suggesting the need for improvement. Onion peels can be exploited also due to their physicochemical properties and the presence of essential fatty acids and volatile components.

The interaction of peptides resulting from β-lactoglobulin hydrolysis with thermolysin and flavonoids isolated from yellow onion peels was examined in this work. The Stern–Volmer, binding constants, and a number of binding sites could all be estimated by quenching the fluorescence of the protein hydrolysates. The binding process between peptides and flavonoids was determined to be static, with hydrogen bonds and van der Waals interactions acting as the major forces in the interactions. Molecular docking tests suggested that all 7 to 14 amino acid-long peptides generated by thermolysin appeared to interact with the primary flavonoids found in onion peels. These study results could contribute to the identification of novel bioactive ligand binding molecules derived from β-lactoglobulin, which would have significant biotechnological and pharmacological implications.

Encapsulation by freeze-drying was the method used for obtaining functional ingredients. Encapsulation is a well-established technique for improving cell stability and protecting biologically active compounds and by-products. Flavonoids extracted from onion peel have been successfully encapsulated in complex matrices based on whey protein hydrolysate, whey protein isolate, maltodextrin, pectin, inulin, and whey protein hydrolysates in varying proportions, with or without the addition of lactic acid bacteria. Bioactive peptides sourced from β-lactoglobulin were then used to microencapsulate flavonoids derived from yellow onion peels. The findings validate the potential of β-lactoglobulin and its peptides to be used as a novel method of flavonoid encapsulation.

Maltodextrin, pectin, whey protein hydrolysates, and whey protein were used in a complex matrix to successfully encapsulate flavonoids extracted from onion skins. *In vitro* digestion has shown a protective effect of flavonoids in simulated gastric fluids, with a controlled release in the intestinal part. The results also demonstrated the effectiveness of the functional ingredients on maintaining the viability of lactic acid bacteria *Lactobacillus bifermentans* and *Lactobacillus casei.*

Another research tested the extraction of flavonoids from yellow onion peels using a green, low-cost solvent. In order to create a more stable, functional, and safe powder with a wide range of applications, a special mix of biopolymeric matrices—including whey protein isolate, whey protein hydrolysates, maltodextrin, and pectin—was dissolved in the flavonoid-enriched aqueous extract.

The achieved results concluded that an alternative solution for the precise extraction of the targeted compounds is a heated aqueous extraction of the flavonoids from onion peels.

Flavonoids and probiotics were as well co-encapsulated by freeze-drying using whey protein isolate, maltodextrin, and inulin with the objective to determine a profile of co-microencapsulated flavonoids from yellow onion skins extract and lactic acid bacteria. The resulting powders showed remarkable properties in terms of phytochemical, antioxidant, antidiabetic, anti-inflammatory profile, and also significant biocompatibility. Important results have been obtained regarding the efficiency of encapsulation for both flavonoids and lactic acid bacteria.

Whey protein isolate and xylose was tested, in unglycated and glycated form, as possible candidates for microencapsulation of flavonoid-enriched onion peel extract by lyophilization. Maillard conjugates are a good alternative to encapsulate food ingredients and promise attractive delivery methods. The *in vitro* digestibility findings revealed that the coating materials had a protective impact on the release of flavonoids. Following hot water extraction, it was concluded that this technique is an alternative method for the selective extraction of flavonoid compounds. The confocal analysis revealed that the powders' structure was uniformly dispersed and displayed a structural pattern. The physical properties have been investigated, and the result can be correlated with their storage stability. To test the functionality of the ingredients, the powders were added to food systems, which were then analyzed for phytochemistry and the viability of lactic acid bacteria. These results confirmed that flavonoids encapsulated in onion peels have the potential to be used as a functional food ingredient with a beneficial effect on consumer health or as having a prebiotic effect on lactic acid bacteria. The ingredients obtained represent a viable alternative to the variants of antioxidants and synthetic dyes and can have multiple destinations in several industries.

CHAPTER 12. PERSONAL CONTRIBUTIONS AND PERSPECTIVES FOR FUTURE STUDIES

The choice of the doctoral topic "***Food-grade functional composites based on yellow onion skins, peptides and probiotics***" was based on the need to close value chains in the bioeconomy by obtaining innovative bioproducts required by the market. The main objectives of the study were the sustainable use of onion waste as by-products and the development of functional ingredients in order to obtain value-added food products (biscuits, dressing salad, soft cheese and nachos) with a stable concentration of target compounds and sensory characteristics.

The original contributions of this paper include the following:

- ✓ Extraction, identification, and characterization of the biologically active compounds and antioxidant activity and the identification of volatile compounds from yellow onion skins, a valuable, underutilized source of polyphenols;
- ✓ The study of the binding mechanism between flavonoids extracted from yellow onion skins and peptides derived from whey protein hydrolysis by fluorescence spectroscopy;
- ✓ Study of the phenolics behavior from yellow onion skins during simulated *in vitro* digestion in order to increase bioaccessibility;
- ✓ Whey protein isolate and β-lactoglobuline hydrolysis in order to obtain bioactive peptides with biological functions and for use as coating material;
- ✓ Microencapsulation of flavonoids from yellow onion skins extract in biopolymeric complex matrices based on protein and polyglucides in order to increase the stability of target compounds;
- ✓ Co-microencapsulation of flavonoids extracted from yellow onion skins and lactic acid bacteria for obtaining multi-functional ingredients characterized in terms of phytochemical content, biological activities, *in vitro* digestibility and *in vitro* biocompatibility, and prebiotic potential;
- ✓ Design a green method to extract and encapsulate an aqueous flavonoid extract;
- ✓ Increase the encapsulation efficiency of flavonoids from yellow onion skins by Maillard reaction between whey protein isolate and xylose;
- ✓ Development of multi-functional ingredients based on biologically active compounds from yellow onion skins, enriched with peptides and lactic acid bacteria tested in different food matrices for their functionality;
- ✓ Development of value-added food products containing a high and stable concentration of biologically active compounds extracted from yellow onion skins, probiotics, proteins, and polyglucides.

The **perspectives for further studies** derive from the need to capitalize on the many insufficiently known and non-utilized by-products that represent rich sources of compounds with beneficial health effects. Therefore, the following may be considered:

- Hydrophobic profile analysis of the yellow onion skins extract;
- Extending the shelf life of powders in order to achieve the best stability of the biologically active compounds;
- Determination of phytochemical profile, cell viability, and colorimetric profile after more than one month for microcapsules and value-added food products, respectively;
- Testing another enzymes inhibition potential;
- Development of new technologies of value-added food products in order to scale up the industry;
- Valorization of other new wastes derived from industrial processing;

CHAPTER 13. RESULTS DISSEMINATION

13.1. List of publications

1. **Milea, Ș. A.,** Aprodu, I., Enachi, E., Barbu, V., Râpeanu, G., Bahrim, G. E., Stănciuc, N. (2021). Whey protein isolate-xylose Maillard-based conjugates with tailored microencapsulation capacity of flavonoids from yellow onions skins. *Antioxidants*, *10*(11), 1708. https://doi.org/10.3390/antiox10111708.

2. **Milea, Ș. A**., Vasile, M. A., Crăciunescu, O., Prelipcean, A. M., Bahrim, G. E., Râpeanu, G., Oancea, A., Stănciuc, N. (2020). Co-microencapsulation of flavonoids from yellow onion skins and lactic acid bacteria lead to a multifunctional ingredient for foods and pharmaceutics applications. *Pharmaceutics, 12*(11), 1053 DOI: 10.3390/pharmaceutics12111053.

3. **Milea, Ș. A**., Aprodu, I., Mihalcea, L., Enachi, E., Bolea, C. A., Râpeanu, G., Bahrim, G. E., Stănciuc, N. (2020). Bovine β-lactoglobulin peptides as novel carriers for flavonoids extracted with supercritical fluids from yellow onion skins. *Journal of Food Science, 85*(12), 4290–4299**.** https://doi.org/10.1111/1750-3841.15513.

4. **Milea, Ș. A**., Aprodu, I., Enachi, E., Barbu, V., Râpeanu, G., Bahrim, G. E., Stănciuc, N. (2021). B-lactoglobulin and its thermolysin derived hydrolysates on regulating selected biological functions of onion skin flavonoids through microencapsulation. *CyTA - Journal of Food, 19*(1), 127–136. https://doi.org/10.1080/19476337.2020.1864020

5. **Milea, Ș. A**., Aprodu, I., Vasile, A. M., Barbu, V., Râpeanu, G., Bahrim, G. E., Stănciuc, N. (2019). Widen the functionality of flavonoids from yellow onion skins through extraction and microencapsulation in whey proteins hydrolysates and different polymers. *Journal of Food Engineering,* 251, 29–35. https://doi.org/10.1016/j.jfoodeng.2019.02.003.

6. Constantin, O. E., **Milea, A. Ș.**, Bolea, C., Mihalcea, L., Enachi, E., Copolovici, D. M., Copolovici, L., Munteanu, F., Bahrim, G. E., Râpeanu, G. (2021). Onion (Allium cepa L.) peel extracts characterization by conventional and modern methods. *International Journal of Food Engineering, 17*(6), 485–493. https://doi.org/10.1515/ijfe-2020-0310.

7. **Milea, Ș. A.,** Crăciunescu, O., Râpeanu, G., Oancea, A., Enachi, E., Bahrim, G. E., Stănciuc, N. (2021). Multifunctional ingredient from aqueous flavonoidic extract of yellow onion skins with cytocompatibility and cell proliferation properties. *Applied Sciences, 11*(16), 7243. https://doi.org/10.3390/app11167243.

13.2. Patent requests:

1. **Milea Ștefania-Adelina**, Mihalcea Liliana, Bahrim Gabriela Elena, Vasile Aida Mihaela, Râpeanu Gabriela, Stănciuc Nicoleta, ”Dunărea de Jos” University of Galati, Ingrediente funcționale formulate pe bază de extracte flavonoidice din coji de ceapă galbenă și peptide bioactive din zer pentru utilizări în industria alimentară; 30564/27.11.2018
2. **Milea Ștefania-Adelina**, Bahrim Gabriela Elena, Râpeanu Gabriela, Stănciuc Nicoleta. Ingrediente multifuncționale pe bază de extracte flavonoidice din coji de ceapă galbenă și bacterii lactice co-microîncapsulate și aplicații ale acestora ; A/00471 din 01.08.2019

13.3. Participations and awards at international and national conferences

1. Student Scientific Session XVIIIth Edition, November 25-2, 2022, Arad, Romania, SUSTAINABLE USE OF ONION SKINS FOR THE DEVELOPMENT OF INNOVATIVE FUNCTIONAL INGREDIENTS, **Ștefania-Adelina Milea**, Nicoleta Stănciuc, Faculty of Food Science and Engineering, Dunărea de Jos University, Galati, Romania – premiul al III-lea;
2. SCDS-UDJG 2022 - The Tenth Edition, GALAŢI, Yellow onion skins: from food waste to valuable resource, **Ștefania-Adelina Milea**, Iuliana Aprodu, Gabriela Râpeanu, Gabriela Elena Bahrim, Nicoleta Stănciuc, poster.
3. SCDS-UDJG 2022 - The Tenth Edition, GALAŢI, Exploring the health benefits of multifunctional ingredients based on flavonoids from yellow onion skins and lactic acid bacteria through comicroencapsulation, **Ștefania-Adelina Milea**, Iuliana Aprodu Gabriela Râpeanu, Gabriela Elena Bahrim, Nicoleta Stănciuc, poster – mențiune.
4. CEREX UDJG 2021 Award for outstanding results in research, development, innovation, technology transfer, artistic creation, and sports performance - Research Gala at the University "Dunărea de Jos" in Galați - Research Assistant **PhD. eng. MILEA Ștefania-Adelina**.
5. The competition for the award of the research results of the doctoral students from IOSUD-UDJG for the year 2021, "Whey Protein Isolate-Xylose Maillard-Based Conjugates with Tailored Microencapsulation Capacity of Flavonoids from Yellow Onions Skins", published in Antioxidants - **first prize.**
6. Inovaliment 2021- Sustainable valorification of onion peels for the development of innovative functional ingredients - authors: Dermengiu Nicoleta, **Milea Ștefania Adelina**, Nicoleta Stănciuc, Mihalcea Liliana - Biotechnologies category – **first prize**.
7. Inovaliment 2021- Sustainable valorification of onion peels for the development of innovative functional ingredients - authors: **Milea Ștefania Adelina**, Nicoleta Stănciuc - Innovation category - **3rd prize**.
8. EuroAliment, Galati, 7-8 October, 2021, *Functional potential of natural co-microencapsualted ingredients based on flavonoids from yellow onion skins, whey bioactive peptides and lactic acid bacteria,* **Ștefania-Adelina Milea**, Gabriela Elena Bahrim, Gabriela Râpeanu, Iuliana Aprodu, Nicoleta Stănciuc, "Dunărea de Jos" University of Galati.
9. 9th edition of Scientific Conference of Doctoral Schools SCDS-UDJG, "Dunarea de Jos" University of Galati, 10-11 of June 2021*, Effect of glycosylation with xylose on the ability of whey proteins to encapsulate the flavonoids from yellow onions skins,* **Ștefania Adelina Milea**, Iuliana Aprodu, Elena Enachi, Gabriela Râpeanu, Gabriela Elena Bahrim, Nicoleta Stănciuc. – ***2nd prize.***
10. 9th edition of Scientific Conference of Doctoral Schools SCDS-UDJG, "Dunarea de Jos" University of Galati, 10-11 of June 2021*, Effect of glycosylation with xylose on the ability of whey proteins to encapsulate the flavonoids from yellow onions skins,* **Ștefania Adelina Milea**, Iuliana Aprodu, Elena Enachi, Gabriela Râpeanu, Gabriela Elena Bahrim, Nicoleta Stănciuc. – ***special prize " Profesor G.M. Costin".***
11. 9th edition of Scientific Conference of Doctoral Schools SCDS-UDJG, "Dunarea de Jos" University of Galati, 10-11 of June 2021, *Functional ingredients based on yellow onion skins extract and whey bioactive peptides for use in the food industry*, **Ștefania-Adelina Milea**, Liliana Mihalcea, Aida Mihaela Vasile, Nicoleta Stănciuc, Gabriela Râpeanu, Gabriela Elena Bahrim.

12. Ingredients Show by Ro.aliment, On-line, May 2021, *Ingrediente naturale inovatoare cu funcționalitate multiplă și aplicații în domeniile alimentar și farmaceutic,* Nina-Nicoleta Condurache (Lazăr), **Ștefania-Adelina Milea**, Gabriela Râpeanu, Gabriela Bahrim, Nicoleta Stănciuc. Universitatea Dunărea de Jos din Galați, Facultatea Știința și Ingineria Alimentelor.

13. Scientific Communications Session, Galati, 28 Mai 2021, A simplified, green extraction method of flavonoids from yellow onion from microencapsulation perspective – A preliminary study Valache Alondra, Professor Nicoleta Stănciuc, PhD student **Ștefania Adelina Milea – 3rd prize.**

14. Student Scientific Session XVIIIth Edition, November 26-27, 2020, Arad, Romania, *β-lactoglobulin peptides enhanced the health-benefits of flavonoids from yellow onion skins extract through microencapsulation,* **Ștefania-Adelina Milea**, Iuliana Aprodu, Gabriela Elena Bahrim, Gabriela Râpeanu, Nicoleta Stănciuc, Faculty of Food Science and Engineering, Dunărea de Jos University, Galati, Romania – ***1st prize;***

15. The IOSUD-UDJG PhD research award competition for 2020, ”Widen the functionality of flavonoids from yellow onion skins through extraction and microencapsulation in whey proteins hydrolysates and different polymers ”, published in the Journal of Food Engineering -**3rd prize*;***

16. Inovaliment - 23-27 November 2020, *Ingrediente naturale multifuncționale pe bază de flavonoide din coajă de ceapă și bacterii lactice co-microîncapsulate*, **Ștefania-Adelina Milea**, Gabriela Elena Bahrim, Gabriela Râpeanu, Nicoleta Stănciuc, Faculty of Food Science and Engineering, Dunărea de Jos University, Galati, Romania.

17. 8th edition of Scientific Conference of Doctoral Schools SCDS-UDJG, “Dunarea de Jos” University of Galati, 18-19 of June 2020*. Boosting the health-benefits of flavonoids from yellow onion skins and β-lactoglobulin peptides microencapsulation,* **Adelina Ștefania-Milea**, Iuliana Aprodu, Gabriela Râpeanu, Gabriela Elena Bahrim, Nicoleta Stănciuc, Faculty of Food Science and Engineering, Dunărea de Jos University, Galati, Romania – **1st prize;**

18. 8th edition of Scientific Conference of Doctoral Schools SCDS-UDJG, “Dunarea de Jos” University of Galati, 18-19 of June 2020, *Evidences of binding mechanism between flavonoids supercritical fluids extracted from yellow onion skins and bovine β-lactoglobulin peptides,* **Adelina Ștefania-Milea**, Iuliana Aprodu, Gabriela Râpeanu, Gabriela Elena Bahrim, Nicoleta Stănciuc, Faculty of Food Science and Engineering, Dunărea de Jos University, Galati, Romania;

19. Infoinvent, Chișinău, 20-23 November 2019, *Ingrediente multifuncționale pe bază de extracte flavonoidice din coji de ceapă galbenă și bacterii lactice co-microîncapsulate și aplicații ale acestora*, **Ștefania-Adelina Milea**, Gabriela Elena Bahrim, Gabriela Râpeanu, Nicoleta Stănciuc, Faculty of Food Science and Engineering, Dunărea de Jos University, Galati, Romania – ***Gold;***

20. Infoinvent, Chișinău, 20-23 November 2019, *Ingrediente naturale pe bază de antociani din struguri microîncapsulate în hidrogeluri din proteine din zer pentru utilizări în industria alimentară*, Carmen BOLEA, Elena ENACHI**, Ștefania-Adelina MILEA**, Gabriela RÂPEANU, Gabriela BAHRIM, Nicoleta STĂNCIUC, Faculty of Food Science and Engineering, Dunărea de Jos University, Galati, Romania – ***Gold;***

21. UGAL INVENT Innovation and Research Show, 16-18 October 2019, *Ingrediente multifuncționale pe bază de extracte flavonoidice din coji de ceapă galbenă și bacterii lactice co-microîncapsulate și aplicații ale acestora,* **Ștefania-Adelina Milea**, Gabriela Elena Bahrim, Gabriela Râpeanu, Nicoleta Stănciuc, Faculty of Food Science and Engineering, Dunărea de Jos University, Galati, Romania – ***Silver;***

22. International Expo-Conference Ingredients Show, 3rd Edition, 17-18 octombrie 2019, Sinaia, *Ingrediente co-încapsulate cu bacterii lactice și compuși bioactivi*, **Ștefania Adelina Milea**, Nina Nicoleta Condurache, Gabriela Râpeanu, Gabriela Elena Bahrim, Nicoleta Stănciuc, Faculty of Food Science and Engineering, Dunărea de Jos University, Galati, Romania – ***2nd prize;***

23. EuroAliment, Galati, 5-6 September, 2019, *Development of highly functional ingredient based on yellow onion skin flavonoids microencapsulation,* **Ștefania-Adelina Milea**, Gabriela Elena Bahrim, Gabriela Râpeanu, Nicoleta Stănciuc, Faculty of Food Science and Engineering, Dunărea de Jos University, Galati, Romania.

24. 2nd European One Health Conference, Bucharest, 21-22 June 2019, **Ștefania-Adelina Milea**, Gabriela Râpeanu, Gabriela Elena Bahrim, Nicoleta Stănciuc, *Yellow onion skins: a possible source for positive health effects;*

25. The 23rd International Exhibition of Inventics "INVENTICA 2019" Iași, Romania, *Functional ingredients based on flavonoid extract from yellow onion skins and whey bioactive peptides for use in the food industry***, Ștefania-Adelina Milea**, Liliana Mihalcea, Aida Mihaela Vasile, Nicoleta Stănciuc, Gabriela Râpeanu, Gabriela Elena Bahrim;

26. Seventh Edition of the Scientific Conference of the Doctoral Schools of Dunărea de Jos University, Galați, 13-14 June 2019, **Milea Ștefania-Adelina**, Bahrim Gabriela Elena, Râpeanu Gabriela, Stănciuc Nicoleta, *Onion (Allium cepa L.) skin extracts characterization by conventional and modern methods* ***–mention;***

13.4. Research projects:

1. **Research assistant in the project** "Complex system for the utilization of fruit by-products to obtain bioactive powders" - BIOPOWDER. PNDR financing contract C161A000011884200010/18.03.2021.
2. **Research assistant in the 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.
3. **Research assistant in the 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.
4. **Research assistant in the project** *„Innovative and emerging solutions for the design of natural co-microcomposites to improve food functionality*", financing contract no. RF 3637/30.09.2021.
5. **Research assistant in the 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.

Priority axis 6- Education and competences

Project title: "Program for increasing performance and innovation in excellence doctoral and postdoctoral research - PROINVENT"

Contract no: 62487/03.06.2022 POCU/993/6/13 - SMIS code: 153299