"Dunărea de Jos" University of Galaţi
Doctoral School of Fundamental Sciences and Engineering

PhD THESIS

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PhD student,
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Series I.7: FOOD ENGINEERING Nr. 17
GALAŢI
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Series F: **Pharmacy**

Content

Keywords: red onion skins, biologically active compounds, anthocyanins, microencapsulation, natural ingredients, value-added products

Introduction

The global population is expected to increase from 7.7 billion (2019) to 9.7 billion in 2050, for this reason, there are more concerns about the use of by-products from plant processing (Chiocchio et al., 2021). Undoubtedly, these premises challenge society's shift toward more sustainable development. In this scenario, wastes and by-products from food and agricultural industries are gaining international attention because of the increasing number generated in developed countries and the problems associated with pollution. A decisive change in the agri-food system is needed to properly manage these by-products. The circular economy promises to be an effective long-term option for preventing, reusing, or recovering natural resources and by-products. The aim is to reintroduce by-products to the production line as raw materials for new products with important health benefits and added value in the industry through sustainable technology to extract nutrient components (Osorio et al., 2021).

Red onion skins are obtained as a by-product in the red onion processing and manufacturing industry, with huge amounts of by-products generated annually worldwide. Much of the by-product quantity is directed to landfill sites unsuitable for animal feed or as fertilizer due to the rapid growth of plant pathogens. In terms of the overall phytochemical profile, red onion skins offer an opportunity for the valorization of biologically active compounds with well-defined biological functions for the human body. Given the antioxidant characteristics of onion skins, these materials may be helpful in many aspects of the food industry involving the avoidance of oxidative damage or free radical formation. Therefore, food quality, shelf life extension, and sensory properties can be improved or maintained by enhancing these characteristics. Thus, these onion by-products can be used as a natural, cheap, and readily available source for obtaining valuable ingredients, especially antioxidants (anthocyanins) (Chadorshabi et al., 2022).

In the food industry, anthocyanins have important applications as antioxidants and natural colorants. The main justification for their use is their presence in the functional food ingredients market. In 2017, this market was valued at over $64 billion and has a projected growth of 6.6% for 2023 when the market value will exceed $64 billion (Echegaray et al., 2020). However, the use of anthocyanins as natural food additives is contingent on the prior extraction and stabilization of these active compounds. From this perspective, the topic of this PhD thesis is aimed at the extraction, quantification, and stabilization of anthocyanins to improve the handling of these compounds in the food industry. In addition, this work also explores the potential of these biomolecules as natural ingredients in the formulation of value-added foods.

The PhD thesis entitled "**Development of ingredients based on biologically active compounds from red onion skins for use in the food industry**" aimed to study the biochemical and functional behaviour of biologically active compounds from red onion skins, mainly anthocyanins, through extraction, quantification and evaluation of the biologically active potential in order to establish innovative directions for the valorisation of biologically active compounds from red onion (*Allium cepa L*.) skins through the development of technologies to obtain value added products with health benefits for consumers.

The research carried out during the doctoral studies aimed at the following **scientific objectives**:

- Testing different extraction techniques to obtain anthocyanin extracts enriched in biologically active compounds with defined functions, such as antioxidant activity;
- Establishing the phytochemical profile of extracts from red onion (*Allium cepa L*.) skins by optimizing extraction conditions, identifying and quantifying bioactive compounds in red onion skins in correlation with antioxidant activity;
- Evaluation of the thermal treatment behaviour of polyphenolic compounds in extracts from red onion skins at different thermal regimes (from 75 to 155 °C) with the aim of highlighting degradation kinetics and establishing optimal conditions for obtaining, processing and storing products rich in biologically active compounds;
- *In vitro* evaluation of the biological activity of red onion skins extract by calculating the inhibition potential of some enzymes involved in the metabolic syndrome;
- Development of procedures for the valorisation of anthocyanins from red onion skins by combined microencapsulation techniques, obtaining natural ingredients with high functionality from the perspective of integration into value-added foods;
- Development of technological variants to obtain value-added food products by exploiting microencapsulated ingredients with red onion skins extracts and characterisation of the products obtained from phytochemical, storage stability, physicochemical and sensory points of view.

The PhD thesis is structured in two parts, and 9 chapters, as follows:

I. **The DOCUMENTARY STUDY**, entitled "THEORETICAL CONSIDERATIONS ON THE FUNCTIONALITY OF BIOLOGICALLY ACTIVE COMPOUNDS IN RED ONION SKINS" comprises three chapters and presents a summary of recent literature data on the characteristics of bioactive compounds (mainly anthocyanins) present in red onions and their impact in the food industry, highlighting their beneficial effects on health. Theoretical and practical data on extraction and microencapsulation processes are also included.

II. **THE EXPERIMENTAL STUDY** entitled "RESEARCH ON THE VALORIFICATION OF BIOACTIVE COMPOUNDS FROM RED ONION SKINS: EXTRACTION, ADVANCED PHYTOCHEMICAL CHARACTERISATION AND PERSPECTIVE FOR USE IN THE FOOD INDUSTRY" contains the results of the research studies carried out throughout the doctoral studies, and is composed of four chapters briefly presented below:

CHAPTER 4, entitled "OPTIMIZATION OF THE EXTRACTION OF BIOACTIVE COMPOUNDS FROM RED ONION SKINS", presents the results obtained in the extraction experiments and phytochemical characterization of biologically active compounds (anthocyanins, flavonoids, polyphenols) and antioxidant activity using spectrophotometric techniques; optimization and validation of the extraction of anthocyanins and antioxidant activity from extracts obtained from red onion skins.

CHAPTER 5, entitled "ADVANCED PHYTOCHEMICAL CHARACTERIZATION OF OPTIMIZED EXTRACT FROM RED ONION SKINS" presents the results obtained from the extraction, separation, identification and quantification of biologically active compounds (anthocyanins, flavonoids) using high performance liquid chromatography (HPLC) techniques; data from the evaluation of the impact of thermal treatment on biologically active compounds and antioxidant activity at different temperature-time combinations; data on the modelling of degradation kinetics using degradation kinetic models, estimated kinetic and thermodynamic parameters in view of the use of red onion skins extract in different applications involving the use of thermal treatment. The chapter also includes the evaluation of the inhibition potential of red onion skins extract on some enzymes involved in metabolic syndrome.

CHAPTER 6, entitled "OBTAINING BIOACTIVE COMPOUND-BASED INGREDIENTS FROM RED ONION SKINS FOR USE IN THE FOOD INDUSTRY" presents the results obtained in the microencapsulation steps of anthocyanins from red onion skins extract and the development of natural ingredients exploiting the functional potential of the microencapsulated extract, as well as the characterisation of the resulting powders from phytochemical, antioxidant activity, colorimetric, structural and morphological, *in vitro* digestibility and storage stability points of view.

CHAPTER 7, entitled "DEVELOPMENT OF VALUE-ADDED FOOD PRODUCTS BY INCORPORATING THE POWDERS" outlines the results obtained which contributed to the development of technologies for the manufacture of products with added value by the use of microencapsulated powders with red onion skins extract, namely the formulation of a tahini dressing and a yogurt dressing for salads, likewise a technology for the production of value-added aperitif biscuits.

Each chapter of the experimental study is structured as follows: Introduction, Objectives of the study, Materials and methods, Results and discussion, Partial conclusions and Bibliography.

CHAPTER 8, FINAL CONCLUSIONS, presents the main conclusions resulting from the experiments.

CHAPTER 9, ORIGINAL CONTRIBUTIONS AND PERSPECTIVES FOR FUTURE STUDIES, describes the main contributions with an impact on the development of knowledge in the topic addressed and the proposed perspectives for further studies.

CHAPTER 10, EXPLOITATION OF RESULTS, describes the dissemination of the results obtained in the PhD thesis.

The PhD thesis comprises 191 pages, including 30 figures and 43 tables. The documentary study represents 25% and the experimental part 75%.

Finally, the original contributions of the PhD thesis and how the dissemination of the results obtained in the PhD thesis was carried out are reported. These original contributions have had a remarkable impact on the development of knowledge in the field, as well as on the prospects for further study and research. Thus, the results of the research have been capitalized through the elaboration of **3 scientific articles** published in ISI rated journals (Food Chemistry: X, Antioxidants, Plants), **2 scientific articles** published in ISI indexed journals (Inventions, The Annals of the University Dunarea de Jos of Galati Fascicle VI-Food Technology), as well as **15 communications** at scientific events representative for the field of food engineering, abroad and in the country.

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

The thesis was carried out under the scientific coordination of Prof.dr.eng. Gabriela RÂPEANU, as PhD supervisor, and of the scientific committee composed of Prof.dr.eng. Gabriela Elena BAHRIM, Prof.dr.eng. Iuliana APRODU and Prof.dr.eng. Nicoleta STĂNCIUC.

CHAPTER 4. Optimization of extraction of bioactive compounds from red onion skins

4.1. Introduction

Onion is the most widely grown and consumed vegetable in the world and is recognized as an important source of valuable phytonutrients such as phenolic acids, flavonoids, fructooligosaccharides, thiosulphates, and other sulfur compounds (Slimestad et al., 2007). Extraction is an important step in the separation and purification of bioactive components from plant material. In general, extraction efficiency is influenced by several factors such as particle size of the plant matrix, type and concentration of solvent, solid-solvent ratio, time, temperature, pH, etc. (Sagar et al., 2018). Different extraction techniques can be applied to recover bioactive compounds from plant-derived by-products.

4.2. Objectives of the study

In this first experimental chapter, the following scientific objectives were pursued:

- To test four extraction techniques (conventional, ultrasound-assisted, microwave-assisted, and the use of enzymatic preparations) and four extraction parameters (time, temperature, concentration of ethanol and acids) on bioactive compounds in red onion skins to obtain extracts rich in biologically active compounds with outstanding antioxidant activity;
- Comparative evaluation of the phytochemical (total anthocyanins (AT), total flavonoids (FT), total polyphenols (PT), and antioxidant profile of extracts obtained from red onion skins using spectrophotometric evaluation techniques;
- Optimization and validation of extraction conditions for AT content and antioxidant activity of red onion skin extract obtained from conventional solvent extraction by testing the influence of four factors namely: ethanol and citric acid concentration, extraction time, and extraction temperature. Four-factor central composite design (CCD) matrix with three replicates at the midpoints and 21 experimental variants was used. Subsequently, the validated extraction model was analyzed and could be a reference for large-scale application of anthocyanin extraction from red onion skins.

4.3. Materials and methods

Extraction of biologically active compounds from red onion skins was achieved by conventional ultrasound-assisted ethanol extraction, microwave-assisted ethanol extraction, and extraction with the addition of enzyme preparations. For the phytochemical characterization of the extracts, the AT content was determined by the differential pH spectrophotometric method, the results were expressed as mg cyanidin-3-O-glucoside equivalent (C3G)/g d.w. The colorimetric method, based on $AlCl_3$, was used to determine the FT content, and the results were reported as mg quercetin equivalent (EQ)/g d. w. PT content was determined by the Folin-Ciocalteu colorimetric method and the results were expressed as mg gallic acid equivalent (GAE)/g d.w. and for the determination of the antioxidant activity of the extracts the method using DPPH was used and the results were expressed as mM Trolox/g d.w.

4.9. RESULTS AND DISCUSSIONS

4.10. Analysis of the content of biologically active compounds in extracts obtained by different extraction methods

In red onion skins, anthocyanins are the main class of compounds with high antioxidant activity. The highest amount of AT of all extraction methods tested was obtained with conventional extraction with 70% ethanol. Thus the combination with 0.1N HCl after 2 h of extraction at 25 °C at pH=2.16 extracts the highest AT content of 1.75±0.04 mg C3G/g d.w. Regarding pH, a low pH value of solvent helps extraction efficiency, also low pH value of extraction solvent can prevent oxidation of anthocyanins. In the case of FT content and antioxidant activity, extraction with the addition of enzyme preparations obtained remarkable results. The enzyme preparation with xylanase activity extracted the highest concentration of FT of 142.09±10.87 mg EQ/g d.w. after 2 h of extraction. After only 1 h of extraction, the enzyme preparation with cellulase activity resulted in an extract with high antioxidant activity of 60.16±0.78 mM Trolox/g d.w. The highest PT content was observed in the ultrasound-assisted ethanol extraction, thus the combination of parameters with 50% ethanol and 0.1 N HCl, after 50 min of treatment at 25 °C, extracted a PT content of 87.41±7.80 mg EAG/g d.w.

In the case of conventional solvent extraction and ultrasound-assisted extraction, it can be observed that in most cases, the temperature of 50 °C resulted in the extraction of a higher content of compounds by increasing the permeability and solubility of the cell walls and decreasing the viscosity of the solvent, compared to the temperature of 25 °C. In other cases, however, a slight degradation of the compounds occurred. For example, the results obtained for conventional extraction with 70% ethanol and citric acid show a decrease in AT content of 2.9% after 15 min of extraction. It is observed that in some cases, the longer time extracted a higher concentration of bioactive compounds, and in others, it led to the degradation of the compounds due to the parameters with which it was combined. For example, in the case of conventional extraction with 70% ethanol acidified with 0.1 N HCl, an increase in TA of 6.3% is observed between 60 and 120 min of extraction, and then after 180 min a slight decrease of 7% occurs. This decrease in AT content is also observed in the ultrasound-assisted extraction, where the content of AT extracted with 96% ethanol and 0.1 N HCl increases from 20 to 35 min by 11.8%, then at 50 min, it decreases by 6.8%.

4.11. Optimization of AT extraction conditions and antioxidant activity of red onion skins by conventional solvent extraction

The complete CCD matrix used for the optimization of the main variables evaluated, as well as the corresponding values, are shown in table 4.18.

Table 4.18. Experimental values of the main independent variables analyzed in the CCD matrix.

Run	Factor 1 A: Citric acid %	Factor 2 B: Ethanol %	Factor 3 C: Temperature °C	Factor 4 D: Time min	Response 1 AA mM TE/g d.w	Response 2 AT mg C3G/g d.w
1	1	40	37	100	26.92	1.02
2	1	40	16.47	100	27.71	1.05
3	2	60	50	20	27.24	1.34
4	1	73.63	37	100	37.2	1.43
5	1	40	37	100	26.84	1.03
6	0.1	20	50	20	28.7	0.6
7	2	60	25	20	25.01	1.32
8	0.1	60	25	180	32.53	1.18
9	1	40	37	100	26.74	1.02
10	1	40	58.52	100	26.73	1.09
11	2.64	40	37	100	25.32	1.11
12	0.1	20	25	20	30.39	0.62
13	2	20	25	180	29.51	0.52
14	1	6.36	37	100	29.41	0.45
15	0.1	60	50	180	31.07	1.21
16	1	40	37	10	24.29	0.52
17	1	40	37	100	26.73	1.04
18	0.05	40	37	100	26.63	1.05
19	1	40	37	234.54	31.14	1.22
20	1	40	37	100	27.64	1.01
21	2	20	50	180	27.24	0.51

* values are expressed as the mean value of three determinations

Influence of extraction parameters on AA

The optimization study aimed to determine the effect of an optimal model of variables on the antioxidant activity of red onion skin extract. The concentration of antioxidant activity varied from 24.29 to 37.20 mM Trolox/g d.w. The regression model resulting from the removal of insignificant model terms was expressed using equation 4.7 which illustrates the relationship between antioxidant activity (R1) and the variables used in coded units.

$$R1\ (AA) = +26.92 + 2.41B - 0.3454C + 2.50D + 1.37AB + 2.41AD + 0.5912BC + 1.51BD - 0.5337CD - 0.4301A^2 + 2.26B^2 \quad (4.7)$$

The correlation between independent and dependent variables was predicted using contour plots (Figure 4.4a-d), which were used to show the synergistic effects of the independent variables on the antioxidant activity of the obtained extract. Also, the response surface plots (three-dimensional response area) describe the correlative impact of the chosen parameters on the antioxidant activity of the extract. Figures 4.4a-b show that ethanol concentration and time influenced the antioxidant activity, it increased with decreasing citric acid concentration. The maximum antioxidant activity could be reached at an ethanol concentration of almost 60% and an extraction time of almost 180 min. Moreover, as the graphs show, lower extraction times and higher percentages of citric acid resulted in decreased DPPH free radical scavenging potential. In addition, lowering the temperature and ethanol concentration decreased the antioxidant activity of the extract from red onion skins (Figure 4.4c). From Figure 4.4d, AA increases when increasing ethanol concentration at a constant extraction temperature.

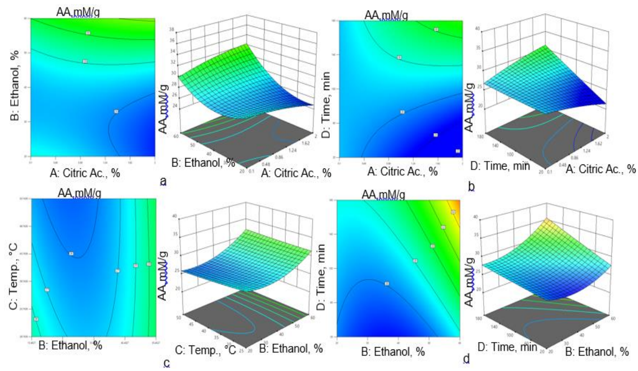

Figure 4.4. Contour plots and response surface plots of AA as a function of ethanol and citric acid concentration (a); extraction time and citric acid concentration (b); temperature and ethanol concentration (c); extraction time and ethanol concentration (d)

Influence of extraction parameters on AT content

The AT content varied from 0.45 to 1.43 mg C3G/g d.w. based on the experimental model. The regression model resulting from the removal of insignificant model terms was expressed using equation 4.8.

$$R2\ (AT) = +1.03 + 0.3114B + 0.3152D + 0.3727AB - 0.0386AD + 0.0247A^2 - 0.0311B^2 + 0.0148C^2 - 0.1208D^2 \quad (4.8)$$

Contour plots and response surface plots (Figure 4.5) were also plotted to show the relationship between independent and dependent variables and to determine the optimal values of AT extraction from red onion skins.

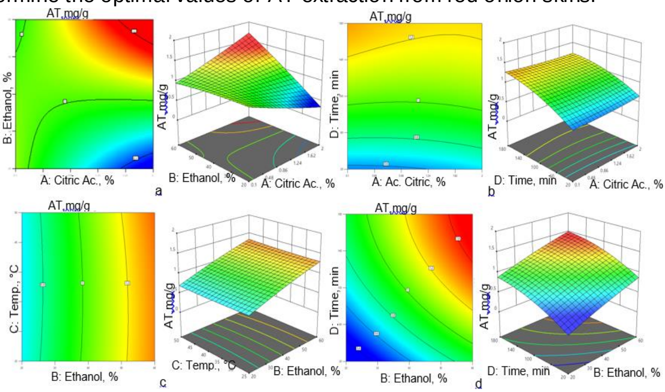

Figure 4.5. Contour plots and response surface plots of AT as a function of ethanol and citric acid concentration (a); extraction time and citric acid concentration (b); temperature and ethanol concentration (c); extraction time and ethanol concentration (d)

When examining the effects of ethanol and citric acid concentration, it was observed that AT increased with increasing ethanol concentration up to 50% and from 0.86% for citric acid concentration (Figure 4.5a). According to the response surface plots, AT concentration was not influenced by temperature variation and extraction time, but was influenced by ethanol concentration (Figure 4.5b-c). AT yield improved steadily as extraction time and ethanol concentration increased simultaneously, according to the analysis of the impact of the two variables (Figure 4.5d). The CCD matrix findings showed that ethanol concentration and extraction time positively influenced anthocyanin extraction.

4.12. Model validation

A desirability score of 1 (0.929) indicated that all selected conditions were in the correct order (Table 4.20). A citric acid concentration of 0.87%, an ethanol concentration of 60%, a temperature of 25 °C, and an extraction time of 180 min were the optimal conditions for maximum antioxidant activity and maximum AT recovery.

Table 4.20. Validation of the mathematical model.

Dependent Variable	Predicted Value	95% Confidence Intervals	Experimental Value
AA (mM Trolox/g d.w.)	35.45	24.29–37.20	37.20
AT (mg C3G/g d.w.)	1.43	0.45–1.43	1.43

The maximum antioxidant activity and maximum AT concentration predicted by the model was 35.45 mM Trolox/g d.w. and 1.43 mg C3G/g d.w., respectively, while the experimental data showed responses close to those predicted by the model, i.e. 37.20 mM Trolox/g d.w. and 1.43 mg C3G/g d.w.

4.13. Partial conclusions

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

- Four different extraction methods were comparatively tested from the perspective of analyzing the content in AT, FT, PT, and antioxidant activity and selecting extracts with a superior phytochemical profile for particular uses. The extraction methods applied were conventional solvent extraction, and three modern extraction methods (ultrasound-assisted, microwave-assisted, and enzyme-assisted). In all these methods the concentration of ethanol, acids, extraction time, and temperature were varied.
- The extract with the highest AT content of 1.75±0.04 mg C3G/g d.w. was obtained for conventional extraction with 70% ethanol and 0.1N HCl after 120 min of extraction at 25 °C.
- The highest FT content of 142.09±10.87 mg EQ/g d.w. was recorded for the extraction with the addition of enzyme preparations, i.e. with the enzyme preparation with xylanase activity, after 120 min of treatment. Also, the extraction with 50% ethanol and 0.1 N HCl assisted by ultrasound extracted the highest PT content of 87.41±7.80 mg EAG/g d.w. after 50 min of extraction at 25 °C.
- Extraction with the enzyme preparation with cellulase activity resulted in the highest antioxidant activity of 60.16±0.78 mMol Trolox/g d.w. after only one hour of extraction of compounds from red onion skins.
- At the same time, it was observed that each combination of parameters extracted biologically active compounds from red onion skins in a different way. Thus, when using pure ethanol and glacial acetic acid, the lowest values for AT, FT, and PT contents were obtained for all extraction methods tested, regardless of the time and temperature chosen, except for the antioxidant activity.
- From the results obtained, it can be seen that the addition of water to ethanol up to a concentration of 50% leads to the extraction of a higher

amount of bioactive compounds than in the case of pure ethanol. Thus, the highest AT content was obtained for 70% ethanol in combination with hydrochloric acid in the case of conventional solvent extraction.

- Conventional solvent extraction was found to be effective in terms of concentration of biologically active compounds and used in subsequent analyses for optimization and validation of AT extraction parameters and antioxidant activity.
- The optimal extraction conditions for maximum recovery of AT and antioxidant activity from red onion skin extract were: 60% ethanol, 0.87% citric acid, 180 min extraction time, and 25 °C temperature.
- Maximum antioxidant activity and maximum concentration of AT predicted by the model were 35.45 mM Trolox/g d.w. and 1.43 mg C3G/g d.w., respectively, while the experimental values were 37.20 mM Trolox/g d.w. and 1.43 mg C3G/g d.w.
- The results revealed that ethanol concentration and extraction time significantly influenced antioxidant activity. The regression equation showed that temperature had a minor negative effect on antioxidant activity, while ethanol concentration and extraction time exerted a positive effect.
- The results highlighted the significant effect of ethanol concentration on AT extraction, while extraction temperature had a minor effect on AT extraction yield. The regression equation obtained showed that ethanol concentration and extraction time positively influenced AT extraction.
- The obtained results showed that conventional solvent extraction under optimal conditions can be considered an effective method of extracting bioactive compounds to obtain valuable extracts from natural and inexpensive sources such as red onion skins. Therefore, the obtained results allowed the selection of optimal extraction conditions to obtain and use compositionally valuable extracts from red onion skins in further experiments.

CHAPTER 5. Advanced phytochemical characterization of optimized extract obtained from red onion skins

5.1. Introduction

The food industry processing onions generate high quantities of onion by-products annually, mainly skins, which require proper management. In this regard, inedible dried skins can be a potential source of bioactive compounds, mainly anthocyanins and flavonols with many benefits for human health (Ren et al., 2020). The health-promoting effects of anthocyanins are related to antioxidant, antiproliferative, anti-inflammatory, and cardioprotective activities, helping to regulate lipid metabolism and improve insulin resistance (Yamaguchi et al., 2015).

5.2. Objectives of the study

The experimental study had the following specific objectives:

- Advanced characterization of the phytochemical profile of optimized extract from red onion skins using high-performance liquid chromatography techniques;
- To evaluate the impact of thermal treatment on the biologically active compounds (AT, FT, PT content and antioxidant activity) in the extract obtained from red onion skins in the temperature range 75-155°C with a holding time between 0-60 min;
- Evaluation of thermal degradation kinetics of biologically active compounds in red onion skin extract to substantiate the thermal degradation behaviour and mechanisms of the mentioned polyphenolic compounds;
- Determination of the thermodynamic parameters corresponding to the degradation of each phytochemical compound studied in order to establish the physical, chemical, and biochemical phenomena of the degradation of bioactive compounds and to decide whether the kinetic model identified in this study is thermodynamically feasible;
- Molecular modeling investigations on the behavior of anthocyanins in red onion skins to the thermal treatment tested;
- Examination of the antidiabetic, anti-obesity, and anti-inflammatory potential of red onion skin extract by investigating the *in vitro* inhibitory activities of α-glucosidase, α-amylase, pancreatic lipase and lipoxygenase, enzymes associated with metabolic syndrome and pro-inflammatory processes;
- Performing molecular docking assays to test the ability of anthocyanins from red onion skins to recognize and interfere with the catalytic site of enzymes associated with metabolic syndrome and pro-inflammatory processes.

5.14. RESULTS AND DISCUSSIONS

5.15. Chromatographic profile of polyphenolic compounds in red onion skin extract

The typical HPLC chromatogram of polyphenolic compounds in red onion skin extract is shown in Figure 5.1. Different detection wavelengths such as 520 nm, 320 nm, and 280 nm were used to identify the compounds. The identification of phenolic compounds was based on retention time and comparison with available standards (chlorogenic acid, ferulic acid, catechin, quercetin, kaempferol) as well as data from the literature. Chromatograms at 320 nm and 280 nm showed the presence of many polyphenolic compounds, of which only six could be tentatively identified, such as peak 1-catechin, peak 2-chlorogenic acid, peak 3-vanillic acid, peak 6-ferulic acid, peak 12-quercetin, and peak 13-kaempferol. Among these, the main compound identified was quercetin. The high levels of quercetin in the outer layers of onion bulbs can be explained by sun exposure leading to increased synthesis of flavonoids, which protect onions from UV light (Albishi et al., 2013). Sagar et al., (2020) obtained similar results in a study on fifteen Indian onion varieties. In this case, the flavonoids identified were quercetin, quercetin 3-β-D-glucoside, luteolin and kaempferol.

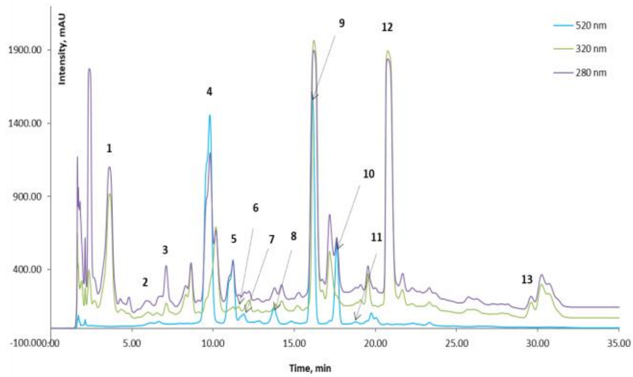

Figure 5.1. HPLC chromatograms of phenolic and polyphenolic compounds quantified in the extracts of red onion skins: peak 1-catechin, peak 2-chlorogenic acid, peak 3-vanillic acid, peak 4-cyanidin 3-laminaribioside, peak 5-cyanidin 3-(3”-malonylglucoside), peak 6-ferulic acid, peak 7-peonidin 3-glucoside, peak 8-cyanidin 3-(6”-malonylglucoside), peak 9-cyanidin 3-(6”-malonyl-laminaribioside), peak 10-peonidin 3-malonylglucoside, peak 11-cyanidin 3-dimalonylaminaribioside, peak 12-quercetin, and peak 13-kaempferol

Figure 5. 1 also illustrates that seven anthocyanins were separated at 520 nm: Peak 4-cyanidin 3-laminaribioside, Peak 5-cyanidin 3-(3″-malonylglucoside), Peak 7-peonidin-3-glucoside, Peak 8-cyanidin-3-(6)"-malonylglucoside), Peak 9-cyanidin 3-(6"-malonyl-laminaribioside), Peak 10-peonidin-3-malonylglucoside and Peak 11-cyanidin-3-dimalonyl-laminaribioside. Our results are in agreement with other studies such as Rice-Evans et al, (1996), Donner et al, (1997), and Sharif et al, (2010).

5.16. Influence of thermal treatment on the content of bioactive compounds in red onion skin extract

5.16.1. Influence of thermal treatment on the content of AT

The effect of thermal treatment on the anthocyanins in the red onion skin extract is shown in Figure 5.3-a. Following thermal treatment, a rapid decrease in the number of anthocyanins is observed. The results show that after 15 min of thermal treatment, the degradation process of the AT content starts slowly and intensifies rapidly, with a rapid decrease in the AT content. Thus, after treatment at 75 °C for 30 min, a decrease in anthocyanin content of 7.6% was recorded, while after thermal treatment at 155 °C for 30 min the degradation reaches 86.5%, (Figure 5.3-a).

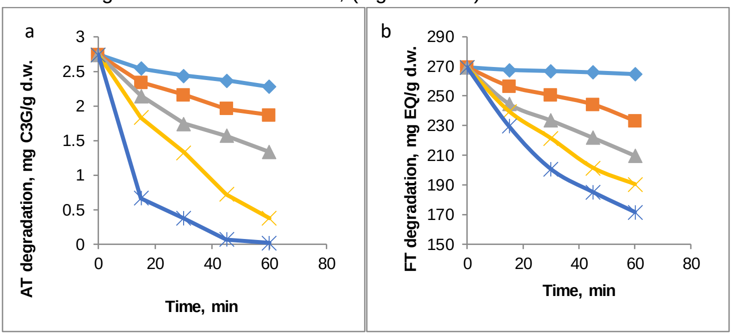

Figure 5.3.-a,-b Influence of thermal treatment on AT and FT stability of red onion skin extract at different temperatures (◆ 75°C, ■95°C, ▲115°C, × 135°C, ✳155°C)

5.16.2. Influence of thermal treatment on the content of FT

The changes produced by thermal treatment in the case of FT in the extract obtained from red onion skins are shown in Figure 5.3-b. Following the thermal treatment of the extract obtained from red onion skins, a decrease in flavonoid content is observed. Thermal treatment with a holding time of 60 min resulted in a reduction of FT concentration by ~13.42% at 95 °C, ~22.16% at 115 °C, ~29.26% at 135 °C and ~36.26% at 155 °C, respectively.

5.16.3. Influence of thermal treatment on the content of PT

The effect of thermal treatment on the PT content of the extract obtained from red onion skins is illustrated in Figure 5.4-c. Following the thermal treatment, the PT content decreased, this decrease of polyphenols in the extract obtained from red onion skins corresponded with the increase in temperature and heating time. Thus, the thermal treatment had a significant effect on the PT content, resulting in a reduction of approximately 21.95% after 60 min of holding at 95 °C and 29% at 115 °C, respectively. At higher temperatures, the loss in PT was significant, ranging from 37.55% at 135 °C to 53.14% at 155 °C after 60 min of holding.

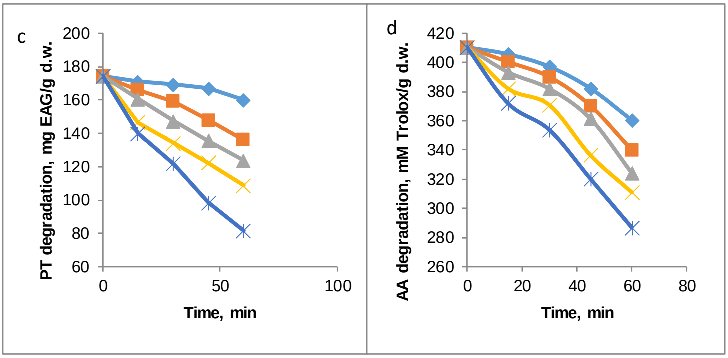

Figure 5.4.-c,-d. Influence of thermal treatment on PT and AA stability of red onion skin extract at different temperatures (◆ 75°C, ■95°C, ▲115°C, × 135°C, ✳155°C)

5.16.4. Influence of thermal treatment on antioxidant activity (AA)

The effect of thermal treatment on AA of the extract obtained from red onion skins is shown in Figure 5.4-d. As can be seen, thermal treatment influences the antioxidant activity of the extract obtained from red onion skins. Similar to the content in biologically active compounds, the thermal-treated extract in the temperature range of 75-115 °C, after 60 min of thermal treatment, showed a decrease from 12.16% to 20.98% in antioxidant activity. It was observed that the degradation process intensified with increasing duration of thermal treatment, reaching 30.09% after 60 min of thermal treatment at 155 °C. Over the temperature range, AA showed a slight reduction, suggesting that other compounds with different thermostability might be involved in this phenomenon. Therefore, even with this reduction and considering the intensely applied time-temperature combination, it can be appreciated that the antioxidant potential of the extract was maintained over the entire temperature range studied.

5.17. Thermal degradation kinetics of biologically active compounds in red onion skin extract

Linear regression equations confirmed that the degradation of phytochemical compounds (AT, FT, PT, and antioxidant activity-AA) in the extract obtained from onion skins follows first-order reaction kinetics and was described in terms of the degradation rate constant (1/min) and activation energy (Ea). Table 5.1 shows the kinetic parameters for bioactive compounds in the extract obtained from red onion skins, namely the degradation rate constant (k), correlation coefficient (R^2), decimal reduction time (D), activation energy (Ea), z_T value and half-life ($t_{1/2}$).

Table 5.1. Kinetic parameters of phytochemical compounds following thermal treatment of red onion skin extract

Compound	Temperature °C	*k ·10^{-2} (min^{-1})*	*R^2*	*$t_{1/2}$ (min)*	*D (min)*
AT	75	0.69±0.08		100.32±1.91	333.33±6.85
			0.96		166.67±4.68
	95	1.38±0.21	0.96	50.16±1.42	90.91±3.45
	115	2.53±0.83	0.98	27.36±0.93	31.25±2.06
	135	7.37±1.33	0.98	9.41±0.72	12.35±1.08
	155	18.65±2.36	0.98	3.72±0.48	
		E*a (kJ·mol^{-1}) =** 50.77±1.71 (R^2*** = 0.97)			
		z$_T$* (°C) =** 55.56±2.91 (R^2*** = 0.98)			
FT	75	0.069±0.15		1003.25±9.84	3333.33±39.21
			0.98		454.55±10.03
	95	0.51±0.31	0.98	136.81±2.32	250.01±8.67
	115	0.92±0.48	0.98	75.24±1.82	172.41±6.12
	135	1.34±0.97	0.99	51.89±1.47	135.13±4.87
	155	1.71±1.01	0.97	40.67±1.53	
		E*a (kJ·mol^{-1}) =** 46.81±1.84 (R^2*** = 0.86)			
		z$_T$* (°C) =** 62.50±4.28 (R^2*** = 0.82)			
PT	75	0.29±0.26	0.92	231.52±3.02	769.23±12.67
	95	0.94±0.49	0.98	73.41±1.72	243.90±7.34
	115	1.31±0.68	0.99	52.80±1.61	175.44±5.47
	135	1.72±0.78	0.98	40.13±1.18	133.33±4.63
	155	2.87±0.92	0.99	24.08±1.05	80.01±3.23
		E*a (kJ·mol^{-1}) =** 32.15±1.32 (R^2*** = 0.94)			
		z$_T$* (°C) =** 90.09±4.72 (R^2*** = 0.92)			
AA	75	0.48±0.27	0.92	143.32±2.42	476.19±10.31
	95	0.69±0.42	0.93	100.33±2.51	333.33±9.57
	115	0.85±0.64	0.93	81.34±1.82	270.27±8.67
	135	1.04±0.75	0.98	66.88±1.74	222.22±7.48
	155	1.33±0.84	0.99	51.89±1.46	172.41±6.12
		E*a (kJ·mol^{-1}) =** 15.13±2.05 (R^2*** = 0.99)			
		z$_T$* (°C) =** 188.68±5.11 (R^2*** = 0.98)			

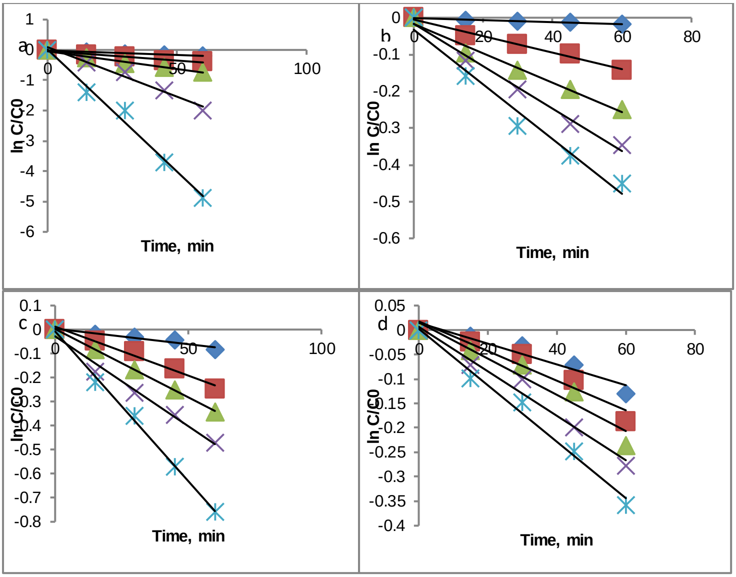

Figure 5.7. Thermal degradation kinetics of bioactive compounds (AT-a; FT-b; PT-c) and AA (d) in red onion skin extract at different temperatures (◆ 75°C, ■95°C, ▲115°C, × 135°C, ✳155°C)

Linear regression analysis confirmed that the thermal degradation of biologically active compounds (AT, FT, PT) and AA in the extract obtained from red onion skins followed a first-order kinetic pattern (Figure 5.7). The changes induced by the thermal treatment on the content of AT, FT, PT, and AA were described in terms of the thermal degradation rate constant (min^{-1}) and activation energy (Ea). For example, the decimal reduction time for AT at 75 °C showed values of 333.33±6.85 min, while a decrease of about 96.3% was observed by increasing the temperature to 155 °C (12.35±1.08 min).

5.18. Temperature behaviour of anthocyanin molecules estimated by molecular modelling techniques

In general, the thermal degradation of anthocyanins starts with the hydrolysis of sugar fragments. A careful analysis of anthocyanin molecules thermaled by the *in silico* approach at temperatures similar to experimental ones showed that the planarity of the aromatic rings in the anthocyanidin structure changed with the intensity of the thermal treatment. Equalization of the anthocyanin models at increasing temperatures from 75 to 155 °C resulted in a more advanced inclination of the aromatic phenyl ring toward benzopyrol. Furthermore, a slight increase in the C-O-C angle was observed providing the link between the sugar molecule and the anthocyanidin with temperature; C-O-C increased from 125.13° (at 75 °C) to 133.05° (at 155 °C) for cyanidin 3-O-laminaribioside and from 125.66° (at 75 °C) to 128.61° (at 155 °C) for cyanidin 3-O-(6″-malonyl-laminaribioside).

5.19. Determination of thermodynamic parameters of biologically active compounds

Table 5.2 shows the enthalpy (ΔH), Gibbs free energy of degradation (ΔG), and entropy (ΔS) at 75, 95, 115, 135 and 155 °C calculated by equations (5.10) - (5.12).

Table 5.2. Thermodynamic parameters (enthalpy (ΔH), Gibbs free energy of degradation (ΔG), and entropy (ΔS)) of the extract obtained from red onion skins

Compound	Temperature (°K)	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (J/(mol·K))
AT	348	47.88±0.62	111.91±1.37	-184.02±2.18
	368	47.71±0.28	116.40±1.32	-186.65±2.15
	388	47.54±0.30	120.94±3.49	-189.16±1.43
	408	47.38±0.34	123.72±2.14	-187.12±1.33
	428	47.21±0.27	126.65±2.18	-185.61±1.56
FT	348	43.92±0.45	118.58±1.14	-214.54±3.16
	368	43.75±0.57	119.47±1.17	-205.75±2.92
	388	43.59±0.65	124.20±1.16	-207.77±3.01
	408	43.42±0.78	129.52±1.13	-211.02±3.11
	428	43.25±0.25	135.17±2.18	-214.75±3.12
PT	348	29.25±0.45	114.34±1.20	-244.49±3.05
	368	29.09±0.43	117.56±1.26	-240.43±3.06
	388	28.92±0.38	123.06±1.31	-242.63±2.58
	408	28.75±0.36	128.64±1.16	-244.83±2.21
	428	28.59±0.33	133.31±2.46	-244.66±3.02
AA	348	12.24±0.16	112.95±3.53	-289.39±1.41
	368	12.07±0.23	118.52±1.41	-289.25±0.92
	388	11.91±0.15	124.45±2.83	-290.07±0.71
	408	11.74±0.28	130.37±2.57	-290.77±0.87
	428	11.57±0.40	136.03±2.12	-290.79±1.06

In addition, the 8.5% extent of bonds involved in defining this angle supports the hypothesis of potential de-glycosylation of anthocyanins at elevated temperatures. De-glycosylation of anthocyanins results in the formation of anthocyanidins, which are further degraded into chalcones. Decomposition of chalcones results in the formation of phenolic acids and carboxaldehyde (Stoica et al., 2021).

5.20. *In vitro* studies on the activity of enzymes involved in metabolic syndrome and pro-inflammatory processes

The inhibitory activity of polyphenolic extract obtained from red onion skins was evaluated in relation to metabolic syndrome-associated enzymes including α-glucosidase, α-amylase, and pancreatic lipase but also the pro-inflammatory enzyme LOX. The *in vitro* inhibitory activity of red onion skin extract at three different concentrations (0.5, 1, 5 µg/mL) on the four enzymes tested (α-glucosidase, α-amylase, lipase, and LOX) is shown in table 5.3 and table 5.4.

Table 5.3. Percentage enzyme inhibition of red onion skin extract and standard inhibitors on α-amylase, α-glucosidase, lipase and LOX enzymes at different concentrations

Sample	Sample conc. µg/mL	α-Amilase inhibition, %	α-Glucozidase inhibition, %	Lipase inhibition, %	LOX inhibition, %
Extract	5	90.31±0.18^{aA}	96.02±0.46^{aA}	87.45±0.57^{aA}	33.39±0.27^{aA}
	1	89.75±0.36^{aA}	95.69±0.67^{aA}	84.79±1.74^{abA}	32.11±1.45^{aA}
	0.5	89.63±0.43^{aA}	95.56±0.68^{aA}	83.46±1.14^{bA}	31.48±1.67^{aA}
Acarbose	5	9.93±0.19^{bB}	3.22±0.18^{bB}	-	-
	1	7.77±0.49^{cB}	2.15±0.12^{cB}	-	-
	0.5	5.17±0.39^{dB}	1.13±0.08^{dB}	-	-
Orlistat	5	-	-	94.86±1.38^{cB}	-
	1	-	-	93.05±0.52^{cdB}	-
	0.5	-	-	91.84±0.90^{dB}	-
Quercetin	5	-	-	-	65.97±1.20^{bB}
	1	-	-	-	64.93±0.31^{bcB}
	0.5	-	-	-	63.88±0.30^{cB}

Values in a column that do not share the same lowercase letter for the three concentrations are significantly different ($p<0.05$). Values in a column that do not share the same capital letter of extract and standard inhibitor for the same concentration are significantly different ($p<0.05$). Measurements are expressed as mean±standard deviation of three replicates.

Table 5.4. Enzyme inhibition results (IC50 values; µg/mL) of red onion skin extract on α-amylase, α-glucosidase, lipase and LOX

Sample	IC50 (µg/mL)			
	α-Amilase	α-Glucozidase	Lipase	LOX
Extract	1.02±0.30^{a}	0.57±0.16^{a}	4.57±0.86^{a}	2.40±0.71^{a}
Acarbose	4.49±0.44^{b}	2.09±0.14^{b}	-	-
Orlistat	-	-	3.18±0.33^{a}	-
Quercetin	-	-	-	1.95±0.20^{a}

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

5.21. *In silico* testing of anthocyanin (ligand) binding to enzymes

Molecular docking assays were further used to test whether any of the two major anthocyanins identified in red onion skin extract by HPLC bind directly to the active site of enzymes involved in metabolic syndrome and pro-inflammatory processes, so as to interfere with substrate recognition or transformation. Further analysis of the three top-scoring complexes showed that cyanidin 3-O-laminaribioside and/or cyanidin 3-O-(6″-malonyl-laminaribioside) could contribute to the inhibition of α-amylase, α-glucosidase, lipase and LOX activity (Figure 5.8), thus explaining the experimental findings shown in table 5.4.

The activity of α-amylase appears to be potentially affected by the presence of cyanidin 3-O-(6″-malonyl-laminaribioside) which has a good affinity towards the active site of the enzyme, establishing contacts with the catalytic amino acids Asp197, Asp300, and Glu233 (Axer et al., 2021). The best-predicted fit for the α-glucosidase—cyanidin 3-O-laminaribioside complex involves the direct contact of the ligand with the catalytic acid Asp616 (Roig-Zamboni et al., 2017).

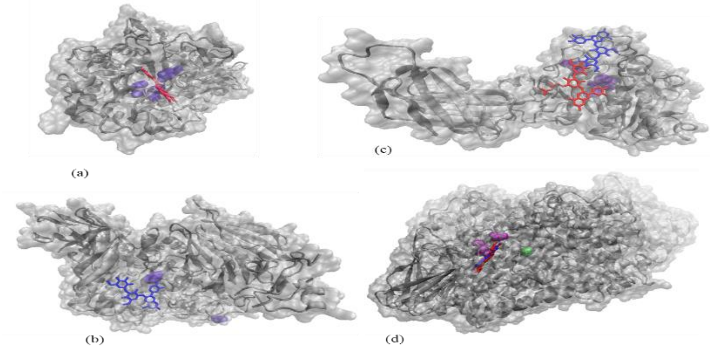

Figure 5.8. The results of the molecular docking tests showing the complexes formed by α-amylase (a) α-glucosidase (b), lipase (c), and LOX (d) shown in silver, with cyanidin 3-O-laminaribioside and/or cyanidin 3-O-(6″-malonyl-laminaribioside) represented in Licorice style in blue and red, respectively. The catalytic amino acids establishing contacts with the ligands are represented in purple in Van der Waals style. Images were prepared using VMD software (Humphrey și colab., 1996, Stoica și colab., 2021).

In the case of lipase, both ligands (anthocyanins) investigated appear to be able to bind to the surface of the enzyme near the hydrogen triad formed by Ser152, Asp176, and His263 (Hadvary et al, 1991). As for LOX, both ligands investigated bind in the vicinity of Phe177 and Tyr181 located at one end of the active site cavity (Gilbert et al., 2011), thus potentially responsible for limiting substrate access to the channel leading to the catalytic iron.

5.22. Partial conclusions

The experimental results allowed the following partial conclusions to be drawn:

- The extract obtained by conventional solvent extraction was analyzed for the individual chromatographic profile of polyphenolic compounds. Chromatographic analysis of the extract from red onion skins revealed the presence of thirteen phenolic and polyphenolic compounds, but the concentration of flavonoids was the majority, especially quercetin. Following the identification and chromatographic separation of anthocyanins in the red onion skin extract, the two major compounds were cyanidin 3-(6"-malonyl-laminaribioside), and cyanidin 3-laminaribioside. However, the chromatographic profile of flavonoids in the red onion skin extract identified the presence of quercetin and quercetin 3,4-diglycoside as major compounds.

- The results obtained for anthocyanin stability at high temperatures showed that after 15 min of thermal treatment, the degradation process of AT content starts slowly and intensifies rapidly, with a rapid decrease of AT content from 7.6% at 75 °C to 86.5% degradation at 155 °C after a thermal treatment duration of 30 min.
- The half-life required to degrade 50% of the AT content at temperatures of 75 °C, 95 °C, 115 °C, 135 °C and 155 °C were 100.32 min, 50.16 min, 27.36 min, 9.41 min, and 3.72 min respectively.
- From a kinetic point of view, following thermal treatment, anthocyanins, flavonoids, polyphenols and antioxidant activity from the extract obtained from red onion skins degrade according to a first-order kinetic model.
- After prolonged heating at the studied temperatures up to 60 min different final levels of biologically active compounds and antioxidant activity were obtained thus FT content decreased by 36.26%, PT content by 53.14%, and antioxidant activity by 30.09% at 155 °C, suggesting that the final degree of degradation is temperature dependent.
- The estimated activation energy (Ea) value for the extract obtained from red onion skins was 50.77±1.71 kJ/mol and indicated that anthocyanins exhibit the highest temperature dependence. In the case of the other bioactive compounds studied, the calculated Ea values were: 46.81±1.84 kJ/mol for flavonoids, 32.15±1.32 kJ/mol for polyphenols, and 15.13±2.05 kJ/mol for antioxidant activity.
- In this study, the estimated kinetic parameters indicate a higher temperature sensitivity of AT in the extract obtained from red onion skins compared to FT, PT, and AA. The difference in Ea values may be due to changes occurring in the samples during heating.
- The values of the degradation rate constantly increased with temperature, indicating that more degradation occurs at higher processing temperatures, intensifying the degradation process, a phenomenon revealed by the activation energy (Ea) values.
- It can also be seen that the thermal degradation of biologically active compounds is characterized by different kinetic parameters, which describe a different thermostability depending on the matrix, the type of compounds present in the extract, and the particularities of the

environment (pH, presence or absence of oxygen and specific thermal processing conditions).

- The data presented suggest that thermal treatment reduced the content of bioactive components in the extract obtained from red onion skins, as well as the antioxidant activity of the extract studied. Modeling of the results of thermal degradation kinetics showed that changes in bioactive components and antioxidant activity can be realized with the first-order kinetic model, which allows for predicting the content of biologically active compounds in the studied temperature range.
- The determination of thermodynamic parameters confirmed the previous assumptions of endothermic, irreversible, and non-spontaneous reaction for the first-order kinetic model followed by bioactive compounds.
- This information on the influence of temperature on bioactive compounds will help to optimize the conditions of industrial technological processes to minimize the loss or degradation of important biologically active compounds, such as various polyphenols and antioxidant activity in value-added foods.
- The study aimed to evaluate the *in vitro* antidiabetic, anti-obesity, and anti-inflammatory potential of red onion skin extract by testing its activity as an inhibitor of α-glucosidase, α-amylase, pancreatic lipase and LOX, enzymes associated with metabolic syndrome and pro-inflammatory processes.
- The results confirmed that this extract acts as a potent inhibitor of α-glucosidase and α-amylase, and suggest that the extract obtained from onion skins has the potential to contribute effectively as a dietary supplement for both postprandial blood glucose control and diabetes-related cellular oxidative stress.
- The phytochemical constituents of onion skins extract can inhibit pancreatic enzymes (lipase) and therefore could be considered effective preparations in the prevention and control of hyperlipidemia-related diseases.
- In addition, polyphenolic extract from red onion skins exerts moderate anti-inflammatory potential by inhibiting the pro-inflammatory enzyme, LOX.
- The results demonstrate the bioactive potential and functional value of red onion skin extracts from red onion processing that can be exploited as a valuable alternative resource for the development of value-added foods.

CHAPTER 6. Obtaining bioactive compound-based ingredients from red onion skins for use in the food industry

6.1. Introduction

To satisfy consumers who demand natural ingredients, major food and beverage companies are committed to eliminating artificial substances, including synthetic colors, from their products. This marketing strategy is in line with the so-called "clean label" trend (Cortez și colab., 2017).

6.2. Objectives of the study

The objectives of this chapter were the selection of complex biopolymer microencapsulation matrices for biologically active compounds extracted from red onion skins, from the perspective of developing functional ingredients for the food industry, and the characterization of the resulting powders.

The following specific objectives were defined:

- Microencapsulation of bioactive compounds (anthocyanins) in whey protein isolate (IPZ) and soy protein isolate (IPS) with polysaccharides such as pectin (P), carboxymethyl cellulose (CMC), gum arabic (GA), maltodextrin (MD), by testing three microencapsulation techniques, namely complex coacervation, encapsulation as liposomes, cold gelation and characterization of the resulting ingredients;
- Selection of promising experimental variants in terms of anthocyanin encapsulation efficiency and bioactive compound content and their overall phytochemical characterization (AT, FT, PT, and antioxidant activity);
- Characterisation of microcapsules in terms of structural and morphological appearance;
- Determination of *in vitro* digestibility and storage stability of the chosen experimental variants.

6.3. Materials and methods

Various methods and encapsulation matrices were used to microencapsulate anthocyanin compounds from red onion skin extract. Fifteen microencapsulation variants were performed, in which anthocyanin extract dissolved in ultrapure water, whey protein isolate (IPZ) and soy protein isolate (IPS) in different concentrations, soy plant protein (lecithin), carboxymethyl cellulose (CMC), pectin (P), gum arabic (GA), maltodextrin (MD) were used. The methods of obtaining encapsulated powders are complex coacervation, encapsulation as liposomes, and the combined technique of cold gelation and freeze-drying.

6.13. RESULTS AND DISCUSSIONS

6.14. Comparative analysis of encapsulation efficiency and phytochemical characterisation of microencapsulated experimental variants

Fifteen variants of powders were tested, table 6.1 shows the values recorded for anthocyanin encapsulation efficiency (EÎ) and phytochemical profiles of the experimental variants obtained in terms of AT, FT, PT content, and antioxidant activity.

Table 6.1. Encapsulation efficiency (EÎ) and phytochemical profile of experimental powder variants

Experim. variants	EÎ, %	AT, mg C3G/g d.w.	PT, mg EAG/g d.w.	FT, mg EQ/g d.w.	AA, mM Trolox/g d.w.
V1	91.17±0.75	0.51±0.01	90.37±0.38	51.45±0.72	54.12±0.21
V2	92.09±0.87	0.53±0.02	92.69±1.55	50.82±1.21	55.87±0.16
V3	96.39±1.02	0.67±0.02	69.60±0.62	50.31±0.38	46.61±0.26
V4	95.53±0.97	0.55±0.03	72.65±0.28	46.37±0.31	48.42±0.15
V5	83.75±0.42	0.43±0.05	35.52±1.02	49.25±0.53	62.37±0.24
V6	86.03±0.67	0.27±0.03	31.94±1.17	51.90±0.47	52.47±0.18
V7	82.53±0.42	0.31±0.03	46.09±1.01	56.12±1.08	51.41±0.12
V8	85.46±0.62	0.39±0.02	31.36±0.96	50.12±0.26	47.74±0.23
V9	81.15±0.62	0.19±0.01	41.76±1.31	49.34±0.17	51.16±0.17
V10	97.36±2.61	1.83±0.05	123.96±1.08	133.50±1.47	53.21±0.32
V11	85.92±1.14	1.42±0.04	116.15±1.12	129.49±0.74	50.78±0.06
V12	87.18±0.92	1.45±0.02	89.67±0.33	111.14±0.46	50.29±0.12
V13	84.3±1.02	1.40±0.02	84.91±0.74	107.20±0.93	47.92±0.21
V14	93.19±1.12	1.41±0.02	113.72±0.20	123.75±0.24	48.19±0.16
V15	82.46±0.92	1.23±0.04	104.60±0.20	116.79±0.15	46.04±0.17

A different overall EÎ and phytochemical profile can be observed for the experimental powder variants obtained. The highest EÎ was recorded for V10 powder obtained by gelation technique being 97.36±2.61% and the lowest for V9 of 81.15±0.62 obtained with IPS and lecithin by encapsulation as liposomes. Therefore, it can be appreciated that the gelation encapsulation technique using the combination of 2% IPS with 4% CMC and 4% P resulted in excellent encapsulation efficiency.

Considering the results obtained for EÎ, the phytochemical profile **variants 10, 12 and 14 were selected for further experiments**.

6.15. Phytochemical characterization of selected microencapsulated experimental variants

Microencapsulation is a complex technique that has been successfully applied to protect sensitive active compounds from environmental factors. Three experimental powder variants were analyzed and the phytochemical profile and antioxidant activity of the selected powder variants are presented in Table 6.2.

Table 6.2. Phytochemical and antioxidant profile of selected microencapsulated variants

Phytochemical characteristics	V10	V12	V14
AT, mg C3G/g d.w.	1.83±0.05[a]	1.45±0.02[b]	1.41±0.02[c]
FT, mg EQ/g d.w.	133.50±1.47[a]	111.14±0.46[c]	123.75±0.24[b]
PT, mg EAG/g d.w.	123.96±1.08[a]	89.67±0.33[c]	113.72±0.19[b]
AA, mM Trolox/g d.w.	53.21±0.32[a]	50.29±0.12[b]	48.19±0.16[c]
EÎ %	97.36±2.61[a]	87.18±0.92[c]	93.19±1.12[b]

For each category of bioactive compound and powder variant tested, mean values in the same row that do not share a letter are statistically different at $p<0.05$ based on the Tukey method.

The powder variant with 2% IPS, 4% CMC, and 4% P (V10) resulted in a higher content of TA encapsulated in the powder with a high EÎ of 97.36±2.61%. Thus, V10 (1.83±0.05 mg C3G/g d.w.) showed significantly higher AT content and EÎ (%) compared to the other variants analyzed ($p<0.05$). Significantly higher PT (123.96±1.08 mg EAG/g d.w.) and FT (133.50±1.47 mg EQ/g d.w.) contents were also obtained for the V10 variant with IPS, CMC, and P ($p<0.05$). Phytochemical compounds were found in higher concentrations in V10, which resulted in higher antioxidant activity of 53.21±0.32 mM Trolox/g d.w.

6.17. *In vitro* digestibility of microcapsules

The gastrointestinal release profile of microencapsulated AT from the powders analyzed was studied in simulated gastric and intestinal juices for 4 h and is represented in figure 6.1. In SGS, a maximum release of up to 15.42±0.19% after 120 min of digestion was observed in the case of V12 powder having good stability of AT content in the gastric system. Among the samples investigated, V10 powder showed the highest protective effect in SGS with a slight decrease of a maximum of 9.55±0.11% after 120 min of digestion followed by V14 powder with 13.50±0.15% after 120 min of digestion. In SIS, the results show that a maximum amount of anthocyanins is released during the 120 min of digestion in all the powders variants analyzed, with a maximum value recorded for V14 powder of 66.21±0.13%, followed by V12 with 66.05±0.22%, and V10 with 55.99±0.17%, respectively. Therefore, it can be appreciated that microparticles microencapsulated with IPS and different polysaccharides (CMC, P, MD, and GA) are resistant to the gastrointestinal system. Thus, a greater protective effect of anthocyanins can be observed by the tertiary matrix containing IPS, CMC, and GA biopolymers (V14).

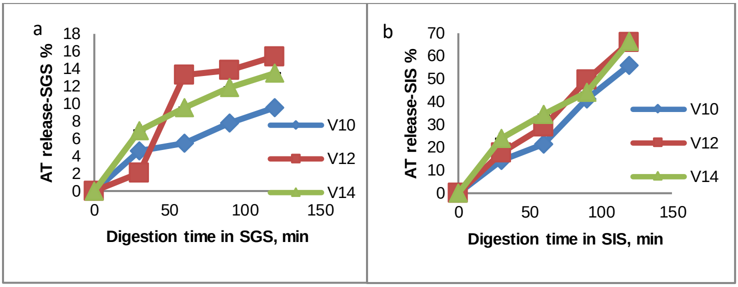

Figure 6.1. *In vitro* digestibility of V10, V12 and V14 powder variants in SGS (a) and SIS (b)

6.18. Stability of phytochemical compounds during storage

The test powders were stored at 4 ºC for 28 days and characterized in terms of phytochemical content and antioxidant activity (Table 6.4).

Table 6.4. Initial phytochemical content and stability of powders after 28 days of storage

Phytochemical profile	Storage time(days)	V10	V12	V14
AT (mg C3G/g d.w.)	0	1.83±0.05^{bA}	1.45±0.02^{bA}	1.41±0.02^{aA}
	28	2.06±0.02^{aB}	1.61±0.03^{aB}	1.49±0.01^{aA}
FT (mg EQ/g d.w.)	0	133.50±1.47^{bA}	111.14±0.46^{aA}	123.75±0.24^{dA}
	28	138.07±1.02^{aB}	113.97±0.62^{aB}	130.11±0.54^{bB}
PT (mg EAG/g d.w.)	0	123.96±1.08^{aB}	89.67±0.33^{aB}	113.72±0.19^{aA}
	28	128.84±0.27^{aA}	92.83±0.65^{bA}	118.03±0.35^{cA}
AA(mM Trolox/g d.w.)	0	53.21±0.32^{bA}	50.29±0.12^{aA}	48.19±0.16^{dA}
	28	58.32±0.13^{bB}	55.14±0.16^{aB}	52.60±0.30^{dB}

For each category of the bioactive compound tested and each powder variant tested, values that are on the same row that do not share the same lowercase letters are statistically different at p<0.05. For each category of the bioactive compound tested and each storage time (0, 28 days), values on the same column do not share the same capital letters are statistically different at p<0.05.

Thus, there is a significant increase ($p<0.05$) in AT content by about 11% in V10 powder, 10% in V12, and 5% in V14 powder. A trend of increase over time in the concentration of the studied compounds was also recorded for the FT and PT content of the selected powders after 28 days of storage. This release of bioactive compounds also resulted in an increase of antioxidant activity by about 8% in V14 powder and 9% in V10 and V12 powders.

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

Scanning electron microscopy was used to observe the morphology and surface structure of the microcapsule powders for the three experimental variants (V10, V12, and V14).

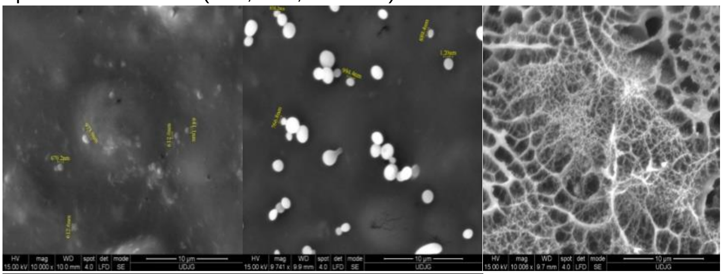

Figure 6.2. SEM images of V10, V12 and V14 powder variants

In the SEM images, it can be seen that the selected powder variants have complex, spherical-shaped structures with sizes ranging from 1 µm to 1.2 µm in the V10 powder and larger and more agglomerated in the V12 variant, ranging from 1 µm to 1.5 µm. In the V14 powder, the globules are merged, in the form of a network, while the surface showed some protuberances.

6.20. Partial conclusions

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

- For microencapsulation, complex coacervation, encapsulation as liposomes, and cold gelation were used as techniques, using different polysaccharides (CMC, MD, P, and GA) and proteins (IPS, IPZ) as encapsulation materials; the most efficient method of microencapsulation of anthocyanins from red onion skin extract was the complex combination of cold gelation and freeze-drying obtaining powders with high EÎ (82.46±0.92% - 97.36±2.61%).
- In general, regardless of the encapsulation technique and material, fine, dark reddish powders with microparticles of various sizes resulted. Among the powder variants tested, 3 variants showed high values for phytochemical profile, antioxidant activity, and EÎ. Therefore, variants 10, 12, and 14 were chosen for further experiments.
- During storage, the powders tested showed variations in the content of phytochemical compounds thus, the study on the storage stability of bioactive compounds showed an increasing trend over time in the content of AT, PT, FT, and antioxidant activity for the variants studied. V10 powder encapsulated with IPS:P:CMC showed higher values of

phytochemical profile as well as better stability in terms of bioactive compounds content and antioxidant activity.

- *In vitro* digestibility study indicated that anthocyanins from red onion skins encapsulated in soy protein with polysaccharides showed good stability in the gastric system, while anthocyanins retained in microcapsules were gradually released into the intestinal system, which may lead to better absorption. Thus, *in vitro* studies highlighted that the best protection was provided when using a blend of biopolymers with encapsulating materials such as IPS, GA, and CMC (V14), allowing better bioavailability and gradual release of anthocyanins into the intestinal system.
- From the SEM images, it can be concluded that the selected powder variants have complex structures with spherical shapes, ranging from 1 µm to 1.2 µm in the V10 powder, and larger and more agglomerated in the V12 variant, ranging from 1 µm to 1.5 µm. In the V14 powder, the globules are merged, in the form of a network, while the surface showed some protuberances.
- These results demonstrate that microencapsulation can lead to stable, functional colored powders that can be used as value-added natural ingredients due to their beneficial functional properties on human health, in particular the demonstrated antioxidant activity.

CHAPTER 7. Development of value-added food products by incorporating the powders

7.1. Introduction

The development of functional foods involves the incorporation of specific compounds that are beneficial to health by reducing the risk of diseases such as cardiovascular disease, diabetes, cancer, etc. Recently, there has been an increasing demand for safe and nutritionally complex foods, which has led to the diversification of functional foods rich in bioactive compounds. Attention has focused on polyphenolic compounds of plant origin because of their nutritional and functional benefits, among the most studied being antioxidant activity and anti-inflammatory effect (Helkar și Sahoo, 2016).

7.2. Objectives of the study

The main objectives of this study were:

- to develop technological recipes for obtaining value-added products that exploit the functional potential of microencapsulated red onion skin extract, namely a technology for obtaining a tahini salad dressing with added powder (V10), a technology for obtaining a yogurt salad dressing with added powder (V12) and a technology for obtaining value-added appetizer biscuits (V14);

- comparative analysis of the phytochemical, physicochemical, textural and rheological characteristics and analysis of the sensory characteristics of the products obtained.

7.3. Materials and methods

7.3.1. Technology of tahini salad dressing with the addition of powder (V10)

The commercial products (sesame paste (40 g), extra virgin olive oil (12 g), apple cider vinegar (12 g), water (40 g), salt (0.80 g), pepper (0.20 g)) were purchased from a supermarket in Galati. In addition to the ingredients listed, red onion skin powder was added in different concentrations (S1-1% (1.05 g), S2-2% (2.10 g), and S3-3% (3.15 g)).

The salad dressing was obtained by mixing the ingredients listed above, with red onion skin powder added as an ingredient in different percentages and added to the amount of dressing. It was homogenized so that the composition was uniform in color and texture. After preparation, the dressing samples were packed in glass containers and stored at 4 °C for further analysis. A control sample (M) of dressing was also made, in which no powder was added to the dressing sample. The stability of phytochemical compounds was also determined after 14 days of storage.

7.3.2. Technology of yogurt salad dressing with the addition of powder (V12)

The following ingredients and raw materials were used to make the yogurt dressing: 2% Greek yogurt (80 g), extra virgin olive oil (11 g), red wine vinegar (10 g), granulated garlic (1.50 g), ground black pepper (0.20 g), salt (0.60 g) and red onion skin powder (S1-1% (1.05 g) and S3-3% (3.09 g)).

The process is simple and involves mixing the above ingredients, red onion skin powder was added as an ingredient in different percentages. Homogenization of the composition followed, thus ensuring uniform incorporation in terms of color and texture of the salad dressing. After preparation, the samples were packed and stored at 4 °C for experimental analysis. The stability of the phytochemical compounds was also determined after 14 days of storage. For comparison, a control sample (M) was also made in which no powder was added.

7.3.3. Technology of the value-added aperitif biscuit (crackers) with the addition of powder (V14)

The aperitif biscuit recipe contains the following ingredients: 43 g brown rice flour (21.54%), 23 g buckwthermal flour (11.52%), 20 g butter 65% (10.02%), 5 g olive oil (2.50%), 10 g yolk (5.01%), 35 g water (17, 53%) 60 g cheese (30.6%), 1.40 g cumin (0.70%), 0.20 g black pepper (0.1%) and 2 g salt (1%) and added powder red onion skins (B1-1% (1.99 g) and B2-3% (5.98 g)). Thus, butter, salt, and yolk were weighed into the bowl of a mixer, followed by mixing for 2 min at 120 rpm. The water and powder from the red onion skins (in various proportions) were then added and blended for 5 min at 120 rpm. The cheese is added in the next stage and blended for 10 min at ~250 rpm to a firm paste. In the end, the two types of flour were added, followed by 1 min of mixing at level 1 to form the dough. The resulting dough was chilled at 4 °C for 20 min, shaped, and then baked at 160 °C for 15 min. The biscuits were tempered, packed in paper bags, and stored in the dark at room temperature (20-25°C and relative air humidity (65-70%) until further analysis. For comparison, a control sample was also made, which followed the same technology, but in which no powder from red onion skins was added (M). The stability of the phytochemical compounds was also determined after 28 days of storage.

7.12. RESULTS AND DISCUSSIONS

7.12.1 Characterization and evaluation of the stability of phytochemical compounds during storage of value-added tahini dressing

Table 7.1 shows the results obtained for the phytochemical profile of tahini salad dressing with added red onion skin powder as well as the storage stability during 14 days of storage.

Table 7.1. Initial phytochemical profile of value-added tahini salad dressings and storage stability over 14 days of storage

Sauce samples	Time, days	AT, µgC3G/g d.w.	FT, mg EQ/g d.w.	PT, mg EAG/ d.w.	AA, mM Trolox/g d.w.
Control	0	-	1.59 ± 0.04^{a}	0.99 ± 0.03^{a}	2.61 ± 0.19^{a}
	14	-	1.61 ± 0.02^{a}	1.01 ± 0.02^{a}	2.98 ± 0.05^{b}
D1 (1 %)	0	12.59 ± 0.16^{a}	1.88 ± 0.01^{b}	1.56 ± 0.01^{b}	7.20 ± 0.08^{c}
	14	12.33 ± 0.96^{a}	2.23 ± 0.01^{c}	1.62 ± 0.01^{c}	7.68 ± 0.11^{d}
D2 (2 %)	0	22.46 ± 0.20^{b}	2.62 ± 0.02^{d}	2.01 ± 0.02^{d}	10.52 ± 0.12^{e}
	14	21.24 ± 0.51^{c}	2.71 ± 0.03^{e}	2.11 ± 0.01^{e}	10.72 ± 0.02^{f}
D3 (3 %)	0	32.92 ± 0.11^{d}	3.14 ± 0.02^{f}	2.62 ± 0.03^{f}	12.37 ± 0.28^{g}
	14	31.30 ± 1.07^{e}	3.51 ± 0.03^{g}	2.71 ± 0.01^{g}	12.90 ± 0.05^{h}

Values with different letters in the same column are significantly different ($p<0.05$).

From table 7.1 it can be seen that the incorporation of powder into salad dressings, led to an increase in all bioactive compounds tested, especially with increasing powder concentrations. Also, AT content increases with increasing concentration of microencapsulated ingredient with red onion skin extract and the highest value (32.92±0.11 µg/g d.w.) was recorded for the dressing with 3% microencapsulated ingredient. After storage for 14 days at 4 ºC, a slight decrease in AT content was observed in D1 dressing, while in D2 and D3 salad dressings the AT content decreased significantly ($p<0.05$). On the other hand, FT and PT contents showed an increasing trend after 14 days of storage. From Table 7.1, it can be observed an increase in the antioxidant activity values for the three tahini salad dressings tested (D1, D2, D3) during the whole storage period, probably due to the release from the microcapsules of compounds other than anthocyanins, such as phenolics, flavonoids, etc.

7.12.2. Physico-chemical characterisation of value-added tahini dressing

Moisture, lipid, protein, sugars, and ash contents for tahini salad dressing fortified with 1 and 3% red onion skin powder are shown in table 7.2. From table 7.2 it can be seen that the addition of the ingredient had a significant contribution to the fiber content, increasing it by 23%, 42%, and 47% respectively compared to the control sample.

Table 7.2. Physico-chemical characteristics of tahini salad dressings prepared with different amounts of powder (0% - Control, 1% - D1, 2% - D2 și 3% - D3)

Physical-chemical characteristics	Sauce samples			
	M	D1	D2	D3
Humidity (g/100 g)	49.51 ± 0.11^{a}	48.45± 2.09^{a}	46.79± 1.85^{a}	45.99± 1.37^{a}
Lipids (g/100 g)	41.19 ± 1.32^{a}	40.64 ± 1.07^{a}	40.21 ± 0.12^{a}	39.81 ± 1.03^{a}
Proteins (g/100 g)	4.11± 0.02^{d}	4.82± 0.06^{c}	5.66± 0.03^{b}	6.34± 0.08^{a}
Sugars (g/100 g)	1.05± 0.01^{a}	1.04± 0.01^{ab}	1.02± 0.01^{bc}	1.01± 0.01^{c}
Fibers (g/100 g)	2.82 ± 0.01^{d}	3.64± 0.14^{c}	4.83± 0.32^{b}	5.33± 0.15^{a}
Ash (g/100 g)	1.32± 0.08^{b}	1.41± 0.03^{ab}	1.49± 0.01^{a}	1.52± 0.05^{a}
Energy value Kcal	404.22± 10.39^{a}	401.98±10.23^{a}	401.34± 9.31^{a}	400.36± 9.08^{a}
kJ	1689.64±28.79^{a}	1680.27±27.48^{a}	1677.61± 27.08^{a}	1673.52± 26.58^{a}

Values with different letters in the same row are significantly different ($p<0.05$).

There is also a significant increase in protein content ($p<0.05$) in the added-ingredient salad dressing variants due to the presence of IPS in microcapsules. Regarding the fat content, the results show that the added powder salad dressing showed a lower fat content. However, the energy value of the salad dressing obtained with added powder in different concentrations is close to that of the control salad dressing.

7.12.4. Texture analysis of value-added tahini dressing

Table 7.4 shows the textural parameter values for the tahini salad dressing samples. Texture analysis showed that the addition of microencapsulated red onion skin extract powder improved the textural properties of tahini salad dressing in proportion to the concentration.

Table 7.4. Textural parameters of tahini salad dressings prepared with different amounts of powder (0% - Control, 1% - D1, 2% - D2 and 3% - D3)

Textural properties	Sauce samples			
	Control	D1	D2	D3
Firmness, N	0.32±0.04^{b}	0.40±0.03^{b}	0.41±0.02^{b}	0.84±0.06^{a}
Adhesiveness, mJ	0.87±0.12^{b}	1.17±0.30^{b}	1.19±0.12^{b}	3.57±0.13^{a}
Cohesiveness	0.70±0.06^{a}	0.71±0.01^{a}	0.72±0.01^{a}	0.80±0.01^{a}
Springiness, mm	7.73±0.65^{a}	8.11±0.01^{a}	8.17±0.08^{a}	8.49±0.20^{a}

Mean values in a row with different letters are significantly different ($p<0.05$).

7.12.5. Rheological behaviour of value-added tahini dressing

The influence of microcapsule powder addition on the rheological properties of tahini dressing samples was evaluated by measuring the dynamic viscoelastic and flow behavior. The addition of red onion skin powder most likely enabled new types of intermolecular interactions between complex matrix constituents, such as the interaction between polysaccharides and proteins or lipids. However, given the high dependence of the complex viscosity values on the frequency scanned during the oscillatory test, observed for all samples investigated, it could be assumed that the addition of microcapsule powder to the dressing did not modify the overall interaction forces ensuring the aggregation of droplets into the network (Diftis și colab., 2005).

Table 7.5. Rheological properties of tahini salad dressings prepared with different amounts of powder (0% - Control, 1% - D1, 2% - D2 and 3% - D3)

Rheological properties		Samples			
		Control	D1	D2	D3
Critical strain, %		2.03±0.002^{d}	2.54±0.008^{c}	3.19±0.016^{b}	5.17±0.087^{a}
Viscosity (Pa·s) at shear rate of	$1 s^{-1}$	4.44±0.23^{dA}	8.70±0.02^{cA}	10.28±0.09^{bA}	27.93±1.90^{aA}
	$10 s^{-1}$	1.18±0.05^{dB}	1.97±0.03^{cB}	2.63±0.01^{bB}	8.21±0.004^{aB}
	$100 s^{-1}$	0.288±0.012^{dC}	0.59±0.003^{cC}	0.96±0.02^{bC}	2.66±0.013^{aC}

In a row, the values of means with different lower case letters are significantly different ($p<0.05$). In a column, the values of means with different upper case letters are significantly different ($p<0.05$).

Regardless of shear rate, the addition of red onion skin powder to tahini dressing resulted in a significant ($p<0.05$) increase in viscosity (table 7.5), suggesting the synergistic effect of the powder in limiting the flow behavior of the complex matrix.

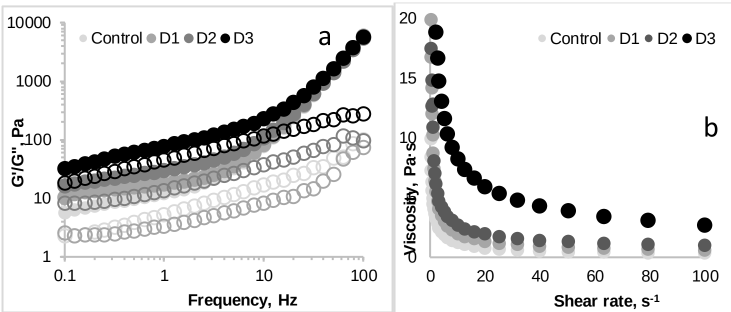

Figure 7.1. Rheological behavior of the dressing samples with different amounts of microcapsule powder (0% - Control, 1% - D1, 2% - D2 and 3% - D3) under (a) frequency sweep (G′- storage modulus - represented with full symbols and G″- loss modulus represented with empty symbols) and (b) forced flow conditions.

The reduction in viscosities caused by the gradual destruction of the semi-solid structure and the alignment of the system macromolecules with the shear direction is more pronounced in the early part of the shear rate range tested (up to 10 s^{-1}), becoming constant at higher shear rate values (figure 7.1).

7.12.6. Sensory analysis of value-added tahini dressing

Tahini salad dressing is a semi-solid sesame seed dressing, without food additives, with a sesame and apple cider flavor - ready to use for salads. Figure 7.2 shows the results obtained for the sensory evaluation of the salad dressing analyzed. The sensory analysis revealed that the dressing with 2% added powder (D2) received higher scores for most of the attributes analyzed (external appearance, odor, consistency, taste, flavor, aftertaste, and overall acceptability) compared to the other salad dressing.

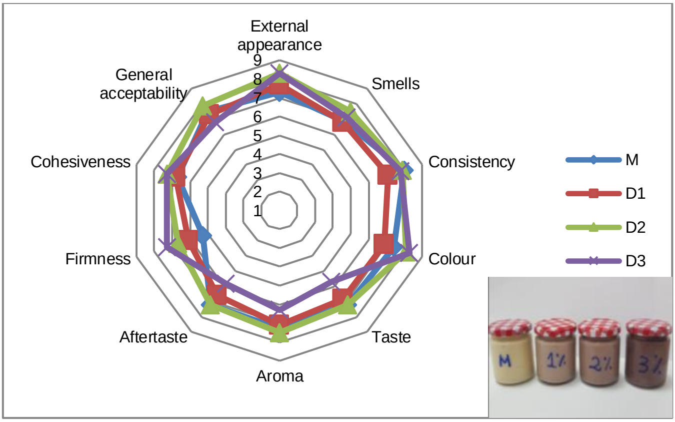

Figure 7.2. Comparative diagram of sensory attributes specific to tahini dressing types: M- tahini dressing without added red onion skin powder, D1, D2 and D3 - tahini dressing with added 1; 2 and 3% red onion skin powder.

Analyzing the results of the sensory evaluation of the value-added dressing samples, it is noted that the D2 dressing variant was the most appreciated by the tasting team, having balanced, pleasant taste, smell, and aroma, with a semi-solid, smooth consistency, the powder improving the color properties and overall acceptability of the salad dressing.

7.12.7. Characterization and evaluation of the stability of phytochemical compounds during storage of value-added yogurt dressing

In order to highlight the added value of the yogurt dressing samples, phytochemical characterization was carried out and the results are presented in table 7.6.

Table 7.6. Initial phytochemical profile of value-added salad yogurt dressing and stability over 14 days of storage

Sauce samples	Time, days	AT, µgC3G/g d.w.	FT, mg EQ/g d.w.	PT, mg EAG/ d.w.	AA, mM Trolox/g d.w.
Control	0	-	1.97 ± 0.05^{a}	1.64 ± 0.06^{a}	2.28 ± 0.19^{a}
	14	-	2.11 ± 0.03^{a}	1.76 ± 0.02^{a}	2.43 ± 0.15^{b}
P1 (1 %)	0	30.02 ± 2.47^{a}	5.44 ± 0.06^{b}	3.40 ± 0.03^{b}	15.64 ± 0.14^{c}
	14	29.30 ± 1.08^{a}	5.52 ± 0.06^{c}	3.62 ± 0.03^{c}	16.44 ± 0.25^{d}
P2 (3 %)	0	53.25 ± 2.28^{b}	8.30 ± 0.18^{d}	5.67 ± 0.02^{d}	23.05 ± 0.14^{e}
	14	52.23 ± 1.07^{c}	8.40 ± 0.05^{e}	5.78 ± 0.01^{e}	24.01 ± 0.10^{f}

Values with different letters in the same column are significantly different ($p<0.05$).

From Table 7.6 it can be seen that the addition of powder in the salad dressings obtained resulted in an increase in the content of biologically active compounds compared to the control sauce. The FT content varied according to the percentage of added powder so P1 increased by 2.76 times compared to the control sample and P2 by 4.21 times compared to the control sample. Similar behavior is observed for the AT and PT content. The antioxidant activity of yogurt salad dressing fortified with microencapsulated ingredients was significantly higher than that of the control sauce.

Throughout the storage period, a slight decrease of about 2% in AT content after 14 days of storage at 4 ºC can be observed in P1 and P2 fortified salad dressings. On the other hand, the phenolic contents (FT, PT) and antioxidant activity of the powder-enriched salad dressings were significantly higher ($p<0.05$) than those of the control sauce throughout the storage period, probably due to the release of polyphenolic compounds, etc. from the microcapsules.

7.12.8. Physico-chemical characterisation of value-added yogurt dressing

The data in table 7.7 indicate that salad dressing with added powder from red onion skin are characterized by a lipid content similar to that of the control sauce. The sugar content increased by 45% in P1 dressing and 48% in P2 dressing, respectively, compared to the control sauce. In terms of protein content, there was a significant increase ($p<0.05$) in relation to the percentage of added powder. The increase in protein content compared to the control sample showed that red onion skin powder was a very good value-added food ingredient due to its high nutritional value. The energy value of salad dressing with added red onion skin powder is higher compared to the control yogurt dressing.

Table 7.7. Physico-chemical characteristics of value-added yogurt dressing prepared with different amounts of powder (0% - Control, 1% - P1 și 3% - P2)

Physical-chemical characteristics		Sauce samples		
		Control	P1	P2
Humidity (g/100 g)		75.86 ± 1.56^{a}	72.10 ± 3.11^{a}	71.92 ± 5.21^{a}
Lipids (g/100 g)		12.61 ± 0.91^{a}	12.11 ± 0.09^{a}	12.01 ± 1.03^{a}
Proteins (g/100 g)		6.52 ± 0.76^{b}	7.82 ± 0.03^{a}	7.74 ± 0.01^{a}
Sugars (g/100 g)		3.04 ± 0.05^{c}	5.56 ± 0.01^{b}	5.81 ± 0.02^{a}
Ash (g/100 g)		2.10 ± 0.03^{c}	2.41 ± 0.02^{b}	2.52 ± 0.06^{a}
Energy value	Kcal	156.47 ± 4.77^{b}	167.48 ± 1.01^{a}	167.25 ± 5.45^{a}
	kJ	654.04 ± 6.79^{b}	700.07 ± 4.18^{a}	699.10 ± 9.68^{a}

Values in a row with different letters (a, b, c) are significantly different ($p<0.05$).

7.12.10. Texture analysis for value-added yogurt dressing

Instrumental analysis of the texture of yogurt dressing based on the addition of microencapsulated ingredient from red onion skins aimed to determine the firmness, adhesion, cohesiveness, and elasticity of the samples in response to their deformation by a double compression, the results of which are presented in table 7.9.

Table 7.9. Textural parameters of value-added yogurt dressing samples prepared with different amounts of powder (0% - Control, 1% - P1 și 3% - P2)

Textural properties	Sauce samples		
	Control	P1	P2
Firmness, N	0.26±0.03[c]	0.42±0.03[b]	0.88±0.02[a]
Adhesiveness, mJ	0.42±0.06[c]	1.67±0.07[b]	3.01±0.10[a]
Cohesiveness	0.86±0.01[a]	0.75±0.02[b]	0.55±0.01[c]
Springiness, mm	8.72±0.11[a]	8.85±0.01[a]	8.13±0.02[b]

Values with different letters in the same row are significantly different ($p<0.05$).

Instrumental texture analysis revealed that the addition of powder obtained by microencapsulation of red onion skin extract at a rate of more than 1% improves the textural characteristics of yogurt salad dressings.

7.12.11. Rheological behaviour of value-added yogurt dressing

The rheological properties of yogurt-based salad dressing with different concentrations of added powder were analyzed after 24 h of storage at 4 °C (Figure 7.4).

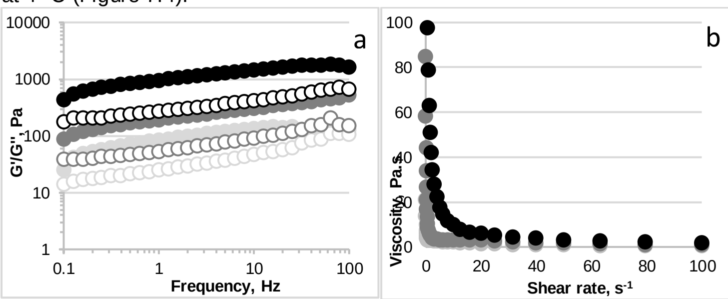

Figure 7.4. Rheological behaviour of yogurt-based dressings prepared with different amounts of microcapsule powder (0% - Control, 1% - P1, 3% - P2). (a) Results of frequency sweep scanning test (storage modulus (G′) represented with filled symbols and loss modulus (G″) with empty symbols. (b) Forced flow test results.

Table 7.10. Rheological properties of yogurt-based dressings prepared with different amounts of microcapsule powder (0% - Control, 1% - P1, 3% - P2)

	Rheological properties		**Samples**		
			Control	**P1**	**P2**
Frequency scanning test	G', Pa		71.54±4.29^{c}	197.6±2.55^{b}	1097.2±6.79^{a}
	G'', Pa		21.4±0.88^{c}	54.85±1.34^{b}	303.55±19.45^{a}
	Tan(δ)		0.30±0.01^{a}	0.278±0.003^{b}	0.278±0.003^{b}
Flow test	Viscosity (Pa·s) at shear rate of	$1s^{-1}$	3.29±0.31^{cA}	7.58±0.42^{bA}	77.22±2.45^{aA}
		$10s^{-1}$	2.11±0.10^{cB}	3.32±0.16^{bB}	10.53±0.78^{aB}
		$100s^{-1}$	0.52±0.02^{cC}	1.045±0.001^{bC}	2.12±0.01^{aC}

In a row, the values of means with different lower case letters are significantly different ($p<0.05$). In a column, the values of means with different upper case letters are significantly different ($p<0.05$).

Independent of the concentration of powder introduced into the yogurt-based matrix, a decrease in apparent viscosity is observed over the entire shear rate range. The value-added yogurt salad dressing showed higher values of rheological parameters compared to the control sample. Analyzing the results presented in table 7.10 it can be seen that the apparent viscosity increased significantly with the concentration of the powder ($p<0.05$).

7.12.12. Sensory analysis of value-added yogurt dressing

Yogurt salad dressing is a creamy, food additive-free yogurt dressing with a garlic flavor - ready to use for salads. The mean scores of the sensory attributes obtained from the sensory analysis are shown in figure 7.7.

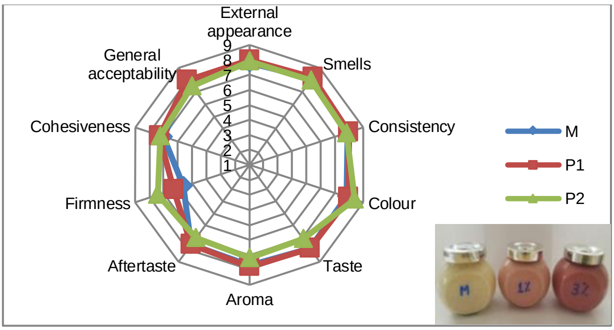

Figure 7.7. Comparative diagram of specific sensory attributes of dressing types: M (control) - yogurt salad dressing without added red onion skin powder, P1 and P2 - yogurt salad dressing with added 1 and 3% red onion skin powder.

The sensory analysis revealed that the yogurt dressing obtained with the addition of 1% red onion skin powder (P1) received higher scores for most of the attributes analyzed (external appearance, odor, consistency, taste, aroma, aftertaste, cohesiveness, and overall acceptability) compared to the other dressings analyzed. Analyzing the results of the sensory evaluation of the value-added yogurt dressing, it is noted that the P1 dressing with the addition of 1% powder was the most appreciated by the panelists having a smooth and creamy consistency with a balanced, pleasant color corresponding to red onions.

7.12.13. Characterization and evaluation of the stability of phytochemical compounds during storage of value-added aperitif biscuit (crackers)

To test red onion skin powder (V14) as a multifunctional food ingredient, it was added to a recipe of crackers in different percentages of 1% (B1) and 3% (B2).

Table 7.11 shows the phytochemical content of the cracker samples and stability over 28 days of storage. The addition of powder to the biscuits resulted in increases in the levels of AT, FT, and PT. *In vitro*, the antioxidant activity of biscuits with added powder was positively influenced by bioactive compounds extracted from red onion skin extract. The packaged biscuits were exposed to storage conditions such as room temperature of approximately 25 °C for 4 weeks. After 28 days of storage, a significant decrease ($p<0.05$) in AT content was observed in the obtained variants, 24% and 17% for samples B1 and B2. Moreover, a significant decrease was observed throughout the storage period in the PT content for the two obtained biscuit variants, while the FT content of the biscuit variants remained constant. From table 7.11, a significant decrease of 8% and 6% ($p<0.05$), respectively, in the antioxidant activity of B1 and B2 biscuits, can be observed throughout the storage period tested.

Table 7.11. Initial phytochemical profile of value-added crackers and storage stability over 28 days

Biscuit samples	Time, days	AT, μgC3G/g d.w.	FT, mg EQ/g d.w.	PT, mg EAG/ d.w.	AA, mM Trolox/g d.w.
Control	0	-	$1.13±0.02^a$	$1.47±0.04^a$	$4.18 ±0.11^a$
	28	-	$1.16±0.01^a$	$1.50±0.01^a$	$4.38 ±0.07^a$
B1 (1 %)	0	$27.57±0.24^a$	$2.52 ±0.03^b$	$2.44 ±0.03^b$	$9.88 ±0.06^b$
	28	$22.24 ±0.13^b$	$2.49 ±0.02^b$	$2.38 ±0.01^c$	$9.18 ±0.08^c$
B2 (3 %)	0	$39.18 ±0.65^c$	$4.57 ±0.19^c$	$3.62 ±0.01^d$	$11.57 ±0.06^d$
	28	$33.51 ±0.33^d$	$4.53 ±0.01^c$	$3.56 ±0.01^e$	$10.96 ±0.03^e$

Values with different letters in the same column are significantly different ($p<0.05$).

7.12.14. Physico-chemical characterisation of value-added crackers

The value-added aperitif biscuits were analyzed from a physicochemical point of view and the results are shown in Table 7.12.

Table 7.12. Physico-chemical characteristics of value-added biscuits prepared with different amounts of powder (0% - Control, 1% - B1 și 3% - B2)

Physical-chemical characteristics	Biscuit samples		
	Control	B1	B2
Humidity (g/100 g)	7.31 ± 0.16^a	7.22 ± 0.14^a	7.36 ± 0.04^a
Lipids (g/100 g)	22.43 ± 0.03^a	22.38 ± 0.01^a	22.39 ± 0.04^a
Proteins (g/100 g)	10.43 ± 0.18^a	10.59 ± 0.11^a	10.62 ± 0.06^a
Sugars (g/100 g)	58.54 ± 0.01^a	58.51 ± 0.02^a	58.31 ± 0.02^b
Fibres (g/100 g)	4.34 ± 0.32^a	4.54 ± 0.09^a	4.68 ± 0.13^a
Ash (g/100 g)	1.29 ± 0.03^a	1.30 ± 0.01^a	1.33 ± 0.01^a
Energy value Kcal	491.37 ± 1.05^a	491.44 ± 0.62^a	490.84 ± 0.69^a
kJ	2053.94 ± 4.39^a	2054.22 ± 2.59^a	2051.72 ± 2.89^a

Values in a row with different letters (a, b) are significantly different ($p<0.05$).

The results presented in table 7.12 indicate that the crackers with added powder of red onion skin are characterized by a similar protein and lipid content as the control sample. In contrast, the carbohydrate content of the B2 value-added crackers is significantly lower than that of the control appetizer biscuits ($p<0.05$). The fiber content increased by 4.40% and 7.30% respectively compared to the control sample. In terms of energy value, the energy values of all fortified biscuit variants are very close.

7.12.16. Sensory analysis of value-added crackers

The sensory attributes recorded for the samples of the aperitif biscuits enriched with different concentrations of red onion skin powder are shown in figure 7.9. The biscuit samples obtained with the addition of powder were preferred by all panelists, mainly because of the reddish-brown color given by the anthocyanins in the red onion skin. The biscuit samples with added powder from red onion skins were assessed as having a balanced taste, smell, and color. Moreover, the tender, unctuous consistency and the firmness upon breaking were appreciated.

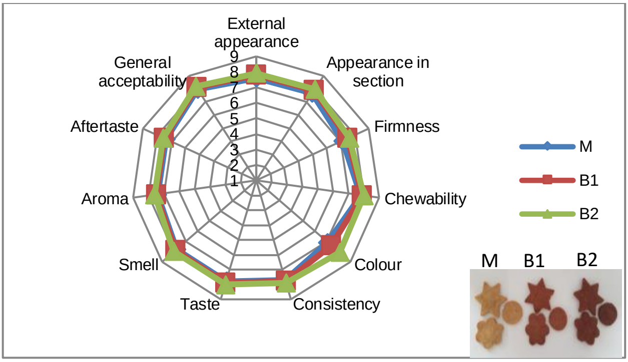

Figure 7.9. Comparative diagram of the specific sensory attributes of the types of biscuits: M - biscuits without added red onion skin powder, B1 and B2 - biscuits with added 1 and 3% red onion skin powder.

Enriching the biscuits with powder resulted in improved biscuit color with an attractive reddish-brown color due to the higher amounts of anthocyanins present.

7.13. Partial conclusions

The scientific objectives of the study have been achieved as follows:

- In this chapter, the possibility of valorization of experimental variants of powders (V10, V12, V14) with remarkable phytochemical profiles, obtained by microencapsulation of extracts from red onion skins, was tested, with the main objective of developing innovative technologies to obtain food products with added value, expressed in terms of the content of biologically active compounds and antioxidant activity.
- Taking into account the specific properties (color, content of biologically active compounds) of the three experimental variants of powders, three value-added products were developed: two salad dressings and crackers. For these products, the experimental powder variant V10 was added at 1, 2, and 3% in a tahini-based dressing, the powder variant V12 at 1 and 3% in a yogurt dressing, and the powder variant V14 at 1 and 3% in crackers.
- In the tahini dressing samples, it was observed that with the addition of powder, the antioxidant activity increases significantly, by almost 2.76 times in sample D1 with 1% addition, 4.03 times in sample D2 with 2% addition, and about 4.74 times in sample D3 with 3% encapsulated ingredient compared to the control sauce sample. In the case of the experimental yogurt dressing variants to which the V12 powder variant was added, higher AT content values of 30.02±2.47 µg C3G/g d.w. were

obtained in the experimental P1 variant of dressing with 1% and 53.25±2.28 µg C3G/g d.w. in the P2 variant of dressing with 2% microencapsulated ingredient.

- Appetizer biscuits showed higher values of antioxidant activity, 4.18±0.11 mM Trolox/g d.w. in the control sample, 9.88±0.06 mM Trolox/g d.w. for variant B1 that used V14 powder in a percentage of 1% and 11.57±0.06 mM Trolox/g d.w. for the B2 variant that used V14 powder in a percentage of 3%, with an increase in antioxidant values of 58% in the case of the first variant and 64% in the B2 biscuit variant.
- The salad dressings were tested for rheological properties, highlighting the fact that the G' values were superior to G", over the entire frequency range tested, suggesting that all the dressing samples kept their specific structure, presenting a solid-like behavior as well as thinning behavior under shear conditions.
- Instrumental texture analysis for salad dressings based on powder from red onion skins aimed at determining the firmness, cohesiveness, adhesion and elasticity of the samples in response to their deformation (TPA method). The analysis of the textural parameters shows that the addition of the microencapsulated ingredient, at a percentage of more than 1%, improved the textural properties of the salad dressings based on red onion skin powder.
- The evaluation of the products through the sensory analysis demonstrated that the products with the addition of powder were more appreciated as sensorial, and the powder from the red onion skins added improved the appearance of the products, both from a visual, color and textural point of view. The incorporation of powders into the analyzed products did not lead to negative effects on the sensory properties.
- Salad dressings are semi-solid, acidic, oil-in-water emulsions with a creamy texture. The use of red onion skin powder as a colorant in the obtained salad dressing maintains the nutritional characteristics and improves the sensory acceptance and stability of the salad dressing.
- This study demonstrated that the addition of natural ingredients obtained from red onion skins represents a promising means of obtaining products with added value, given the antioxidant activity, as well as increasing the nutritional values of the products obtained.

CHAPTER 8. FINAL CONCLUSIONS

Recent studies have shown that agri-food by-products should be considered renewable and cheap resources of bioactive compounds with added value in the production of food additives, functional foods, supplements, and nutraceuticals. As red onion skins are particularly rich in phytochemicals, the recovery and valorization of these compounds from onion by-products is an important issue both in terms of reducing environmental impact, food waste disposal, and the use of high added-value substances with beneficial effects on health. Therefore, the proper management of red onion skins will influence the shift towards a circular economy model and, a new concept perceived as an efficient long-term option for processing and increasing the value of these by-products.

In this context, the aim was to extract the main bioactive compounds from this matrix in order to further incorporate them into value-added natural ingredients that could serve different purposes. Therefore the studies in the PhD thesis, entitled ,,*Development of ingredients based on biologically active compounds from red onion skins for use in the food industry*" aimed at the valorization and quantification of biologically active compounds from red onion skins in order to develop functional ingredients to support consumers. All the objectives initially assumed have been achieved, in the partial conclusions at the end of each chapter as well as in the general conclusions summarised in this chapter.

- ✓ By comparison, four extraction techniques were tested for their content of biologically active compounds and antioxidant activity. The extract obtained by conventional extraction with 70% ethanol and 0.1N HCl at 25°C and 120 min of extraction was considered superior in terms of concentration in biologically active compounds.
- ✓ Following the analysis of the four factors: citric acid concentration, ethanol concentration, extraction time and temperature, and the use of the Central Composite Design matrix, it was found that ethanol concentration and extraction time positively influenced AT extraction while temperature had a minor negative effect on antioxidant activity.
- ✓ Optimal conditions for maximum extraction of anthocyanins and compounds conferring antioxidant activity were established as ethanol concentration of 60%, citric acid concentration of 0.87%, and extraction time of 180 min at 25°C.
- ✓ Chromatographic analysis of anthocyanins in the optimized extract of red onion skins revealed two major compounds represented by cyanidin 3-(6"-malonyl-laminaribioside) and cyanidin 3-laminaribioside.
- ✓ In order to quantitatively describe the impact of thermal treatment on the bioactive compound content and antioxidant activity of red

onion skin extract, thermal degradation kinetic studies were performed in the temperature range of 75-155 °C. These highlighted that anthocyanins exhibit high thermal stability in the temperature range of 75-135 °C. Thermal degradation kinetics of phytochemical content and antioxidant activity in red onion skin extract followed the first-order kinetic model.

- ✓ Thermal degradation parameter values for antioxidant activity were significantly lower than those estimated for thermal degradation of anthocyanins, indicating different thermostability of bioactive compounds in the extract. The determination of thermodynamic parameters confirmed previous assumptions of irreversible and non-spontaneous reactions for the first-order kinetic model. The thermal treatment results provide important information on the degradation kinetics of phytochemical compounds, which may be useful for optimizing thermal treatment conditions in the food industry (pasteurization or sterilization process) to obtain anthocyanin pigment-based products from red onion skins to minimize the loss of color and bioactive components.
- ✓ The effect of polyphenolic components in red onion skin extract against enzymes relevant to hyperglycemia, dyslipidemia, and oxidative stress associated with metabolic syndrome and pro-inflammatory processes was studied. Red onion skin extract exhibited higher inhibitory activity compared to reference compounds on α-amylase and α-glucosidase enzymes with antidiabetic potential. The extract inhibited pancreatic lipase and LOX *in vitro* and therefore may exert moderate anti-inflammatory and preventive potential against hyperlipidemia-related disease.
- ✓ In this study, a series of microencapsulation variants of red onion skin extract was developed using techniques such as complex coacervation, liposome encapsulation, and cold gelation using different polysaccharides (CMC, MD, P, and GA) and proteins (IPS, IPZ) as encapsulation materials. Efficient encapsulation of anthocyanins from red onion skin extract was achieved by the complex combination of cold gelation and lyophilization, obtaining powders with high encapsulation efficiency (82.46±0.92%-97.36±2.61%).
- ✓ Powders with superior characteristics in terms of phytochemical profile and encapsulation efficiency were selected and showed satisfactory content of phytochemical compounds and outstanding biological activity, especially antioxidants. The fine powders showed different morphological structures and different micrometer sizes, depending on the encapsulation matrices used.

- ✓ The fine, dark red powders were characterized by good storage stability, with *in vitro* digestibility studies suggesting a protective effect on anthocyanin release and a controlled, gradual release of anthocyanins into the intestinal environment.
- ✓ The *in vitro* digestibility results are supported by the results of SEM analysis of the microcapsules (V14) thus due to the spherical surface of the microparticles showing some protuberances and fissures there is an easier release of anthocyanin compounds into the intestinal environment.
- ✓ In order to evaluate the potential for industrial application of natural ingredients from red onion skins obtained by microencapsulation, different technological variants were developed to obtain three value-added products, their functionality was evaluated by the content of phytochemical compounds and antioxidant activity.
- ✓ A significant increase in the content of bioactive compounds was observed with the addition of microencapsulated ingredients, while rheological properties showed that the obtained dressing samples exhibited solid-like behavior regardless of the percentage added. Texture analysis of the obtained salad dressing led to the conclusion that the addition of powder in salad dressing improves the textural properties of the final product.
- ✓ The results of the instrumental analyses are supported by the results of some sensory attributes evaluated by the panelists, thus the red shade of the products obtained with the addition of powder was particularly appreciated by the panelists, as it emerged from the determination of the CIELAB color parameters, the intensity of the red color being attributed to the parameter a*. The firmness of the instrumentally determined products increases with increasing concentration of added powder, which was also perceived and appreciated by the panelists in the sensory evaluation of the firmness of the salad dressing. Analyzing the results of the rheological properties it can be observed that the viscosity increased with the concentration of powder added to the products suggesting the synergistic efficiency of the powder in limiting the flow behavior of complex matrices (salad dressing).
- ✓ Incorporating powders into tested products such as salad dressing, and biscuits is a novel idea that may offer the possibility to expand the use of ingredients obtained from red onion skins while increasing the nutritional value of the food. This also facilitates the development of innovative and patentable food products.

- ✓ All the products obtained have added value noted by high antioxidant activity, improved color, nutritional value, and textural and rheological properties that change consumer perception.
- ✓ The optimal concentration of red onion skin powder added to products was up to 3%, as concentrations higher than 3% were not sensory and technologically preferable. Analysis of the results confirmed that the use of polyphenolic compounds in powders obtained with a microencapsulated extract from red onion skins with a coloring and antioxidant role improves the sensory and nutritional quality of the food products obtained, due to the fact that the powders have good solubility, intense color and high stability.
- ✓ The results obtained allowed the conclusion that extracts from red onion skins have value in terms of phytochemical content, while microencapsulation offers the possibility to develop functional ingredients that can substitute synthetic additives and contribute to the development of value-added food products.

CHAPTER 9. Original contributions and prospects for further studies

The PhD thesis entitled "*Development of ingredients based on biologically active compounds from red onion skins for use in the food industry*" is original research, which allows it to go through several stages of fundamental and applied research aimed at establishing strategies for recovery and reintegration into the food system of bioactive compounds, which offer multiple positive health benefits.

The original contributions of the PhD thesis derive from the following aspects:

- ➢ Testing and comparing different extraction techniques from the perspective of establishing the optimal conditions for maximizing the extraction of bioactive compounds (anthocyanins) and antioxidant activity from red onion skins;
- ➢ Advanced characterization of extracts obtained from red onion skins by different spectrophotometric and chromatographic techniques for detailed phytochemical profiling;
- ➢ Study of kinetic degradation parameters of biologically active compounds in order to optimize industrial processing conditions and maintain biochemical properties in the final processed products, all from the point of view of molecule-process-function-product relationships;
- ➢ Evidence of the inhibitory effect of polyphenols in red onion skin extract on the enzymes α-amylase, α-glucosidase, lipase, and lipoxygenase, thus the extract has a potential antidiabetic, anti-

inflammatory, and preventive effect against hyperlipidemia-related disease;

- Development of microencapsulated variants (gelation and freeze-drying) in the form of stable powders with the role of natural, innovative ingredients, the main advantage being to stabilize the biological and technological functions of hydrophilic compounds (anthocyanins) in extracts from red onion skins;
- The valorization and reintegration of biologically active compounds from red onion skins resulting from the industrial processing of red onion into food products with added value, especially antioxidants, contribute practically to the application of the circular economy concept in Romania.

As regards the prospects for further research studies on red onion skins, the following are envisaged:

- Determination of the antibacterial and antifungal activity of extracts from red onion skins;
- Testing the probiotic, cytotoxic, and antiproliferative potential of microencapsulated powders based on red onion skin extract;
- Evaluation of the binding mechanisms between anthocyanins and protein-polysaccharide matrices by molecular docking experiments, molecular modeling, and fluorescence spectroscopy;
- Development of other value-added food products (bakery, pastry and confectionery products, fermented dairy products);
- Obtaining intellectual property rights for all food products obtained.

Therefore, **the molecule-process-function-product** relationship is the original element of the PhD thesis and this approach can be applied to other plant by-products (tomato, cabbage, cauliflower, peas, black carrot, purple sweet potato, pumpkin) for the incorporation of biologically active compounds into food products, with a positive impact on the potential to improve the quality of life through the development of diverse food products.

CHAPTER 10. DISSEMINATION OF RESULTS

Dissemination of the results of the research carried out throughout the doctoral studies resulted in the following scientific articles published or communicated at national and international conferences, and awards, as follows:

I. Articles published in ISI-listed journals

1. Stoica, F.; Aprodu, I.; Enachi, E.; Stanciuc, N.; Condurache, N.N.; Duță, D.E.; Bahrim, G.E.; Râpeanu, G. Bioactive's Characterization, Biological Activities, and In Silico Studies of Red Onion (Allium cepa L.) Skin Extracts. *Plants* 2021, 10, 2330. https://doi.org/10.3390/plants10112330 Q1, Impact Factor = 4,658
2. Stoica, F.; Condurache, N.N.; Horincar, G.; Constantin, O.E.; Turturica, M.; Stanciuc, N.; Aprodu, I.; Croitoru, C.; Râpeanu, G. Value-Added Crackers Enriched with Red Onion Skin Anthocyanins Entrapped in Different Combinations of Wall Materials. *Antioxidants* 2022,11(6), 1048; https://doi.org/10.3390/antiox11061048 Q1, Impact Factor = 7.675
3. Stoica, F.; Aprodu, I.; Enachi, E.; Andronoiu D.G.; Stanciuc, N.; Condurache, N.N.; Croitoru, C.; Bahrim, G.E.; Râpeanu G. Value-added salad dressing enriched with red onion skin anthocyanins entrapped in different biopolymers. *Food Chemistry: X* 2022, 15, 100374; https://doi.org/10.1016/j.fochx.2022.100374 Q1, Impact Factor = 6,443

II. Articles published in ISI indexed journals

1. Stoica, F.; Râpeanu, G. Nistor, O.V.; Enachi, E.; Stanciuc, N.; Mureșan, C.; Bahrim, G.E.; Recovery of bioactive compounds from red onion skins using conventional solvent extraction and microwave assisted extraction. The Annals of the University Dunarea de Jos of Galati, Fascicle VI–*Food Technology* *2020,* *44(2)*, 104-126. https://doi.org/10.35219/foodtechnology.2020.2.07
2. Stoica, F.; Constantin, O.E.; Stănciuc, N.; Aprodu, I.; Bahrim,G.E.; Râpeanu, G. Optimization of the Parameters Influencing the Antioxidant Activity and Concentration of Anthocyanins Extracted from Red Onion Skins Using a Central Composite Design. *Inventions 2022, 7*, 89. https://doi.org/10.3390/inventions7040089

III. Patent applications

1. Stoica, F.; Andronoiu D.G.; Râpeanu G. Aprodu, I.; Bahrim, G.E.; Stanciuc, N.; Croitoru, C. Universitatea "Dunărea de Jos" Galați, Yogurt dressing with added microencapsulated anthocyanin extract powder from red onion (Allium cepa L.) skins - product with added value and

manufacturing technology - nr. de înregistrare OSIM A/00131/03.05.2022

IV. Participation in national and international conferences

1. Stoica, F., Râpeanu, G., Stănciuc, N., Bahrim, G.E. 2020 Red onion by-products as a functional ingredients in food formulations, poster, *8th edition of Scientific Conference of Doctoral Schools SCDS-UDJG, "Dunarea de Jos" University of Galați*
2. Stoica, F., Râpeanu, G., Stănciuc, N., Bahrim, G.E. 2020 Phytochemical characterization of red onion skin extracts, prezentare, *8th edition of Scientific Conference of Doctoral Schools SCDS-UDJG, "Dunarea de Jos" University of Galați*
3. Stoica, F., Râpeanu, G., Stănciuc, N., Bahrim, G.E. 2020 Extraction and characterization of bioactive compounds found in red onion (*Allium cepa L*.) skins, poster, *9th edition of the Intrenational Conference Agriculture for Life, Life for Agriculture, University of Agronomic Sciences and Veterinary Medicine, București*
4. Stoica, F., Râpeanu, G., Stănciuc, N., Nistor, O.V., Bahrim, G. E. 2020 Efficient extraction of bioactive compounds from red onion skins (*Allium Cepa L*.), poster, *19th International Conference "Life Sciences for Sustainable Development" University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca*
5. Stoica, F., Râpeanu, G., Stănciuc, N., Aprodu, I., Bahrim G.E. 2020 Enzyme assisted extraction of phytochemicals from red onion skins as an approach to novel extraction technology poster, *Scientific Symposium "Young people and multidisciplinary research in applied life sciences", 7th edition, Section: Food Engineering, Banat`s University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" Timisoara*
6. Stoica, F., Bahrim, G.E., Râpeanu, G., Aprodu, I., Stănciuc, N. 2021 Red onion skins as a rich source of anthocyanins: thermal stability and biological activity poster, *EuroAliment, "Dunărea de Jos" University of Galați*
7. Stoica, F., Aprodu, I., Râpeanu, G., Bahrim, G.E. Stănciuc, N. 2021 Microencapsulation of red onion skins anthocyanins by freeze drying method, poster, *Multidisciplinary Conference on Sustainable, Development, Section: Food Chemistry, Engineering & Technology, Faculty of Food Engineering Timişoara*
8. Stoica, F., Aprodu, I., Râpeanu, G., Bahrim, G.E., Stănciuc, N. 2021 Flavonoides profile of red onion (*Allium cepa L*.) skins extract, poster, *9th edition of Scientific Conference of Doctoral Schools SCDS-UDJG, "Dunarea de Jos" University of Galați*

9. Stoica, F., Aprodu, I., Râpeanu, G., Bahrim, G.E., Stănciuc, N. 2021 Thermal degradation kinetics of anthocyanins extracted from red onion skins (Allium cepa L.), prezentare, *9th edition of Scientific Conference of Doctoral Schools SCDS-UDJG, "Dunarea de Jos" University of Galați*

10. Stoica, F., Râpeanu, G., Stănciuc, N., Aprodu, I., Bahrim G.E. 2021 Stability evaluation of red onion skin anthocyanins liposomes, poster, *20th International Conference „Life sciences for sustainable development'', University of Agricultural Sciences and Veterinary Medicine, Cluj- Napoca*

11. Stoica, F., Stănciuc, N., Aprodu, I., Bahrim, G.E., Râpeanu, G. 2021 Phytochemical characterization of red onion skin anthocyanins liposomes, poster, International scientific symposium "*Young researchers and scientific research in life sciences" Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" Timişoara*

12. Stoica, F., Stănciuc, N., Aprodu, I., Bahrim, G.E., Râpeanu G. 2021 Encapsulation of anthocyanins rich extract from red onion skin with different carrier agents poster, *PhD students' days Faculty of Food Engineering, Tourism, and Environmental Protection The First Edition, Arad*

13. Stoica, F., Râpeanu,G., Stănciuc, N., Aprodu, I., Bahrim, G.E. 2022 Functional ingredients based on anthocyanins from red onion skins extract and soy protein isolates and different biopolymers for use in the food industry poster*, 10th edition of Scientific Conference of Doctoral Schools, "Dunarea de Jos" University Galați*

14. Stoica, F., Râpeanu,G., Stănciuc, N., Aprodu, I., Bahrim, G.E. 2022 Functional potential of natural microencapsulated ingredients based on anthocyanins from red onion skins, soy protein isolates and different biopolymers prezentare, *10th edition of Scientific Conference of Doctoral Schools, "Dunarea de Jos" University Galați*

15. Stoica, F., Andronoiu D.G., Râpeanu,G., Stănciuc, N., Aprodu, I., Bahrim, G.E., Croitoru C. 2022 Yogurt dressing with added microencapsulated anthocyanin extract powder from red onion (Allium cepa L.) skins - product with added value and manufacturing technology*,* Patent applications NR. OSIM A/00131/03.05.2022 poster, *Scientific Research, Innovation And Invention Exhibition Pro Invent, XX Edition, Technical University of Cluj-Napoca*

IV. <u>Awards</u>

1. Honorable Mention, Prezentare, Thermal degradation kinetics of anthocyanins extracted from red onion skins (*Allium cepa L.*), Stoica, F., Aprodu, I., Râpeanu, G., Bahrim, G.E., Stănciuc, N., 9th edition of

Scientific Conference of Doctoral Schools SCDS-UDJG, “Dunarea de Jos” University of Galați, 10-11 of June 2021

2. Honorable Mention, Prezentare, Functional potential of natural microencapsulated ingredients based on anthocyanins from red onion skins, soy protein isolates and different biopolymers, Stoica, F., Râpeanu,G., Stănciuc, N., Aprodu, I., Bahrim, G.E., 10th edition of Scientific Conference of Doctoral Schools, “Dunarea de Jos” University of Galați 9-10 of June 2022

3. Prof. Constantin Moraru Award - for the research results presented at the Scientific Conference of the SCDS-UDJG 2022 Doctoral Schools, organized by the Danube University of Galati.
4. Second prize, Poster, Enzyme assisted extraction of phytochemicals from red onion skins as an approach to novel extraction technology, Stoica, F., Râpeanu, G., Stănciuc, N., Aprodu, I., Bahrim, G.E. Scientific Symposium "Young people and multidisciplinary research in applied life sciences", 7th edition, Section: Food Engineering, Banat`s University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" 27 november, 2020 Timisoara
5. 3rd prize, Poster, Phytochemical characterization of red onion skin anthocyanins liposomes Stoica, F., Stănciuc, N., Aprodu, I., Bahrim, G.E., Râpeanu G. International scientific symposium “Young researchers and scientific research in life sciences” Banat’s University of Agricultural Sciences and Veterinary Medicine “King Michael I of Romania” 25 November 2021 Timişoara
6. Pro Invent Medal and diploma of excellence, Yogurt dressing with added microencapsulated anthocyanin extract powder from red onion (Allium cepa L.) skins - product with added value and manufacturing technology, Stoica, F., Andronoiu D.G., Râpeanu,G., Stănciuc, N., Aprodu, I., Bahrim, G.E., Croitoru C. Scientific Research, Innovation And Invention Exhibition Pro Invent, XX Edition, Technical University of Cluj-Napoca, 26-28 octombrie 2022

V. Projects

1. Project "*Innovative and emerging solutions for the design of natural co-microcomposites to improve food functionality* ", nr. RF 3637/30.09.2021.
2. Project "*Innovative strategies to valorize agro-food by-products into products with economic value promoting the principles of circular economy* ", nr. 14888/11.05.2022.