„Dunărea de Jos" University of Galaţi

Doctoral school of Fundamental Sciences and Engineering

PhD thesis

The kinetic behavior during fruit anthocyanins processing in model and real food systems
(PhD thesis summary)

PhD student,

Mihaela TURTURICĂ

Scientific coordinator,

Prof.univ.dr.eng Gabriela Elena BAHRIM

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Content

Introduction

Currently, the chemical composition and the antioxidant properties of fruits determined an increased interest in regards to experimental reasearch and healthy eating principles that leads to a better life quality. Consumers are becoming more and more interested in eating foods rich in bioactive compounds, due to the fact that not all products have the same chemical composition or biological properties (Legua et al., 2016). It is widely accepted that a diet rich in fruits and vegetables will have major health implications by reducing the risk of coronary heart disease, cancer, stroke, etc. These human health benefits are attributed to the phytochemical compounds, among which polyphenolic compounds, mainly anthocyanins (Bors and Michel, 2003; Chaovanalikit and Wrolstad, 2004). The use of biologically active compounds from fruits with health benefits is continuously expanding, the main purpose being to introduce these compounds in fortified foods such as jams, juices, wines, etc., or in the form of food supplements (Koss-Mikołajczyk et al., 2015). Fruit juices offer the same nutritional and functional benefits as fresh fruits but drinking the juice is much simpler and handier than eating the fruit, being especially preferred by children and the elderly (Falguera and Ibarz, 2014).

Numerous factors influence the composition and the quality of processed plant products, of which temperature, pH, pressure, the most important of them being the extraction conditions that can exert a major influence on the biochemical properties, total polyphenols and flavonoids contents (Dos Santos Lima et al., 2015).

There are numerous studies that highlight the implications of anthocyanins in the food industry, in particular regarding the conditions of fruit preservation and more than this several researchers consider that there are still many untapped possibilities to improve the manufacture and processing steps in order to expand the range of functional products. Furthermore, the kinetic behavior and the determination of the thermal degradation conditions of anthocyanins have an impact on both the fundamental and applicative research and also on the optimization of the processing steps in order to preserve the functional properties of fruit products.

The PhD thesis entitled "**The kinetic behavior during fruit anthocyanins processing in model and real food systems**" aimed the study of the biochemical and functional behavior of vegetal pigments, mainly anthocyanins, in order to optimize the parameters under minimal processing conditions. Hence, through modern analytical techniques (fluorescence spectroscopy, high performance liquid chromatography), kinetic modeling and statistical analysis of experimental data, the structural and functional changes of anthocyanins from two varieties of local red fruits, plums (*Prunus domestica*) and cherries (*Prunus avium*) were analyzed both in model and real systems (natural matrices). The stability concerning different treatments (temperature and pH) was also assessed and the processing treatments have been optimized to maintain unaltered the functional properties under processing conditions, while ensuring the biochemical stability of food products.

The research carried out during the PhD studies focused on the following scientific objectives:

• Separation, identification and quantification of anthocyanins from plum skins (*Prunus domestica*) and cherrie skins (*Prunus avium*) as well as the evaluation of their behavior during processing in order to determine the optimal conditions to obtain and preserve products rich in polyphenolic compounds.

• Structural changes analysis of polyphenolic compounds from *Prunus domestica* skins and *Prunus avium* skins by altering the pH and temperature, in order to establish the correlations between processing, structural conformation and biologically active function maintenance.

• The study of the physico-chemical conditions in which the anthocyanins from plum skins (*Prunus domestica*) and cherries skins (*Prunus avium*) suffer structural modifications by employing kinetic modeling and statistical analysis.

The PhD thesis is structured into two parts, as follows:

I. THE DOCUMENTARY STUDY consists of three chapters, structured into 22 subchapters, and presents the most recent data from the literature regarding the biochemical and technological characteristics of anthocyanins and their impact in the food industry and on the quality of human life. Moreover, several modern techniques associated with food industry regarding the extraction, identification, quantification, purification, biochemical behavior and processing stability of anthocyanins are also presented.

II. THE EXPERIMENTAL STUDY presents the results of the original investigations carried out during the doctoral program and it is structured into three chapters, as follows:

Chapter 4, entitled ”**Characterization of the phenolic composition of plums and cherries derived matrices**”, displays the extraction, separation, identification, quantification and also the biochemical characterization of anthocyanins from local plum skins (*Prunus domestica*) and cherry skins (*Prunus avium*), using spectrophotometric methods and high performance liquid chromatography techniques (HPLC).

Chapter 5, entitled ”**pH influence on anthocyanins from plum and cherry-derived matrices**”, shows the results of the investigations on the behavior and stability of anthocyanins under different pH conditions and also in the presence of several chemical compounds that are used as food ingredients such as citric acid, glucose and fructose.

Chapter 6, entitled ”**Chemical and kinetic behavior of bioactive compounds from plums and cherries derived matrices after thermal processing**”, presents the results regarding the chemical and biochemical behavior of anthocyanins under similar temperature conditions to those used in the fruit processing industry. The degradation mechanisms were described by using kinetic models, mainly the first order and/or the fractional conversion kinetic models.

Each chapter of the experimental study is structured in the following subchapters: *Introduction*, which presents the importance of the research and the scientific objectives; *Materials and methods*, describes the materials, the used reagents and the methods for determining, processing and interpreting the experimental data; *Results and discussions*, outlines the obtained results, as well as comparing them to other scientific studies from literature; *Partial Conclusions* and *References*.

Chapter 7, **General Conclusions**, presents the main conclusions of the investigations, which focused on the study of anthocyanins from plum and cherry derived matrices, by monitoring the biochemical behavior under different pH and temperature conditions, identifying the processing conditions in which the biological properties alteration of these bioactive compounds is minimal.

The PhD thesis consists of 174 pages, including 52 figures and 13 tables. The documentary study represents 30% and the experimental part 70%.

Finally, the original contributions of the PhD thesis are presented, with an impact on the knowledge development in the biological active compounds field and the perspectives for future research, as well as the dissemination of the results obtained in the research field. Thus, the obtained results were disseminated through the elaboration of four scientific articles, published

or to be published: 3 articles in ISI journals (*Journal of Food Engineering*, *Food and Bioprocess Technology*, *Chemical Papers*) and one article indexed in the international databases (*Annals of the University Dunarea de Jos of Galati Fascicle VI - Food Technology*) as well as ten communications at national and international scientific events that are representative for the biotechnology field.

The research activities of the PhD thesis were carried out using the modern research infrastructure of the Integrated Research Center, Expertise and Technology Transfer (BioAliment-Tehnia) (www.bioaliment.ugal.ro), within the Faculty of Food Science and Engineering, "Dunarea de Jos" University of Galati.

During the PhD studies, the PhD student was involved in the research team of the following projects, with convergent themes to the PhD thesis, as follows:

- IDEI, PN-II-ID-PCE-2012-4-0509/2013-2016 (www.biostab.ugal.ro), entitled "*Thermal and/or non-thermal technology as a tool to increase the health functionality of bioactive compounds in fruit based food*", project director Prof.dr.eng. Gabriela Rapeanu.

- PN-II-RU-TE-2014-4-0115/2015-2017 (www.funfood.ugal.ro), entitled "*Functional composites based on whey protein and vegetable extracts for food applications*", project director Prof.dr.eng. Nicoleta Stanciuc.

The thesis was conducted under the scientific coordination of Prof.dr.eng. Gabriela-Elena BAHRIM, as a PhD supervisor, and a scientific committee composed of: Prof.dr.eng. Gabriela RAPEANU, Prof.dr.eng. Nicoleta STANCIUC and Prof.dr.eng. Iuliana APRODU.

4. Characterization of the phenolic composition of plums and cherries derived matrices

This chapter presents the studies regarding the phenolic composition and the antioxidant activity in plum derived matrices (*Prunus domestica Vanette* variety) and cherry derived matrices (*Prunus avium Uriaşă de Bistriţa* variety) by using UV-Vis spectrophotometry and chromatographic techniques.

The obtained results present a fundamental and applicative importance because they provide a great deal of information on the composition in bioactive compounds, with a particular impact on the food industry.

4.1. Introduction

The food industry produces large amounts of organic waste that depend both on the raw material subjected to processing and on the processing conditions (González-García et al., 2014). It is considered that the industries that generate a significant amount of agro-food waste are the ones that process fruits and vegetables. A part of the waste products are used in animal feed while the rest are exploited in different ways. One of the alternatives is the recovery of bioactive compounds that usually are present in a high concentration in order for them to be later used in the food, cosmetics or pharmaceutical industry (Deng et al., 2012).

The present study aimed the assessment of the biologically active compounds content, compounds such as polyphenols, flavonoids and total monomeric anthocyanin from Romanian varieties plums (*Prunus domestica, Vanette* variety) and cherry (*Prunus avium, Uriaşă de Bistriţa* variety), from juice or after the extraction from fresh fruit skins. Furthermore, the antioxidant activity of the extracts and also of the juices was also evaluated. These studies are important and justified due to the fact that the bioactive compounds from these red fruits are very little studied.

4.2. Materials and methods

Plums and cherries were purchased from the local market and were kept in a frozen state until processing. The following reagents such as 2,2-Diphenyl-1-picrilhidrazil (DPPH), acid 6-Hydroxy-2,5,7,8-tetrametilcroman-2-carboxylic acid (Trolox), sodium acetate, potassium chloride, sodium hydroxide, aluminum chloride, nitrite of sodium, ethanol, methanol, and formic acid (HPLC grade) were purchase from Sigma Aldrich Steinheim, Germany whereas the standards corresponding to cyanindin and peonidin were purchased from Extrasynthèse (Z. I Lyon Nord, France). The Zymorouge enzyme was purchased from Sodinal (Bucharest, Romania) and was used without any further purification steps.

The study followed:

- the extracts preparation
- the natural and simulated juices preparation
- the biologically active compounds analysis:
  - total polyphenolic content (TPF)
  - total flavonoidic content (TF)
  - total monomeric anthocyanins (TMA)
  - the antiradical activity (DPPH RSA)
  - the chromatographic analysis of anthocyanins from the plant matrix

4.3. Results and discussion

4.3.1. Analysis of phenolic composition and antiradical activity of skins plum extract

From the compositional point of view, the fruits differ depending on the development conditions, the soil on which they were grown, the geographical and climatic conditions, the degree of maturity at the time of harvest and on the genetic differences.

The extract from the lyophilized plum skins powder presented a total polyphenolic content (TPF) of 0.017 ± 0.001 mg gallic acid/g dry weight (dw). The flavonoids are well-known antioxidant compounds, so that the plants that are rich in these compounds have antioxidant activity. The total flavonoidic content (TF) from the plum skins extract was 2.794 ± 0.176 mg catehinic equivalent (CE)/g dw. The content of the TMA in the plum skins composition also varies depending on the climatic conditions, validity period, genetic factors, etc. The content of the TMA of the plum skins extract (*Prunus domestica* var. *Vanette*) was 2.112 ± 0.168 mg cyanidin 3-glucoside/g dw.

The chromatographic analysis of the plum skins extract analysed in this study revealed the presence of four peaks at 520 nm (figure 4.4), which correspond to five anthocyanins: cyanidin 3-xyloside/cyanidin 3-glucoside (peak 1 – 0.0303 mg/mL), cyanidin 3-rutinoside (peak 2 – 0.182 mg/mL), peonidin 3-glucoside (peak 3 – 0.0008 mg/mL) and peonidin 3-rutinoside (peak 4 – 0,069 mg/mL).

The reducing ability of a compound generally depends on the presence of compounds which have a reducing or an antioxidant potential so that by breaking the chain of free radicals they donate a hydrogen atom. The percentage of inhibition was measured to determine the antiradical activity of the plum skins extract. The heat-treated samples showed a DPPH RSA value of 71.42±2.31%.

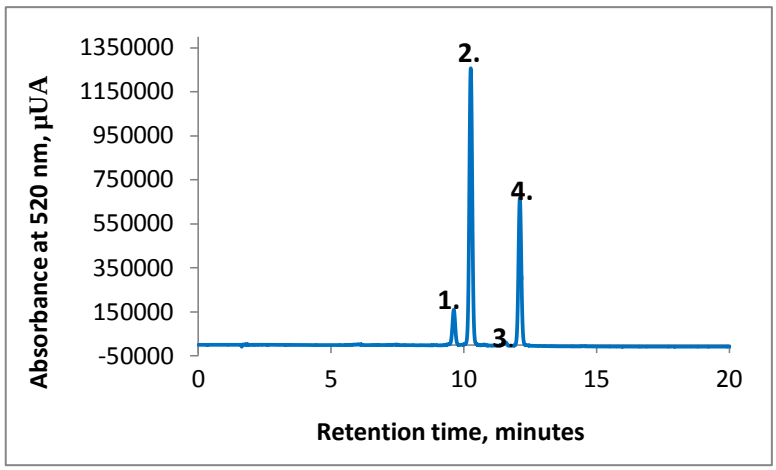


Figure 4.4. The chromatographic profile of anthocyanins from the plum skins extract (λ=520 nm). The peaks correspond to: cyanidin 3-xiloside and cyanidin 3-glucoside(1); cyanidin 3-rutinoside (2); peonidin 3-glucoside (3) and peonidin 3-rutinoside(4).

4.3.2. Analysis of phenolic composition and antiradical activity of plum juices

The content of total polyphenols in the juices from plums, coded PJW, PJCA, PJG, PJM and NPJ presented the following values such as: 1.54 ± 0.09, 0.69 ± 0.025, 0.70 ± 0.012, 0.78 ± 0.009 mg gallic acid (GA)/mL juice and 0.14 ± 0.002 mg gallic acid (GA)/mL, respectively.

The content of total flavonoids in the thermally untreated juices PJW, PJCA, PJG, PJM and NPJ varied as follows: 1.35 ± 0.07, 0.49 ± 0.015, 0.48 ± 0.011, 0.53 ± 0.005 mg CE/mL and 0.22 ± 0.006 mg catehinic equivalent/mL, respectively.

The antioxidant activity of all the five tested juices (coded PJW, PJCA, PJG, PJM and NPJ) also varied, being of 66.07 ± 1.22, 25.00 ± 0.65, 30.31 ± 0.84, 26.34 ± 0.31 and 52.57 ± 2.5 %, respectively.

The total content of anthocyanins in the juices, coded as PJW, PJCA, PJG, PJM and NPJ, had the subsequent values 0.144 ± 0.11, 0.052 ± 0.01, 0.053 ± 0.007, 0.051 ± 0.007 mg cyanidin 3-glucoside/mL and 0.038 ± 0.01 mg cyanidin 3-glucoside/mL, respectively.

The chromatographic analysis of the plum juice with various additions revealed the presence of four peaks (figure 4.5), corresponding to five anthocyanins: cyanidin 3-xiloside/cyanidin 3-glucoside (peak 1), cyanidin 3-rutinoside (peak 2), peonidin 3-glucoside (peak 3) and peonidin 3-rutinoside (peak 4). Thus, in the plum juice with the addition of water and with the addition of sugars has revealed the presence of three anthocyanins: cyanidin 3-glucoside, cyanidin 3-rutinoside and peonidin 3-rutinoside, whereas peonidin 3-glucoside was found as traces and could not be quantified. The major anthocyanin in all the simulated juices was cyanidin 3-rutinoside, which displayed the following contents: 0.435 ± 0.012 mg/mL (in the case of the juice with the addition of the water - PJW), 0.559 ± 0.052 mg/mL (in the case of the juice with the addition of citric acid - PJCA), 0.403 ± 0.014 mg/mL (in the case of the juice with the addition of sugars - PJG), 0.421 ± 0.016 mg/mL (in the case of the juice with the addition of all the compounds mentioned above - PJM).

In the case of natural plum juice, the presence of eight anthocyanins was revealed: cyanidin 3-xiloside/cyanidin 3-glucoside (peak 1), cyanidin 3-rutinoside (peak 2), peonidin 3-glucoside (peak 3) and peonidin 3-rutinoside (peak 4), whereas peaks 5 – 8 could not be identified. In the case of natural juice, the major anthocyanin was represented by cyanidin 3-rutinoside with a content of 0.526 ± 0.013 mg/mL (figure 4.5).

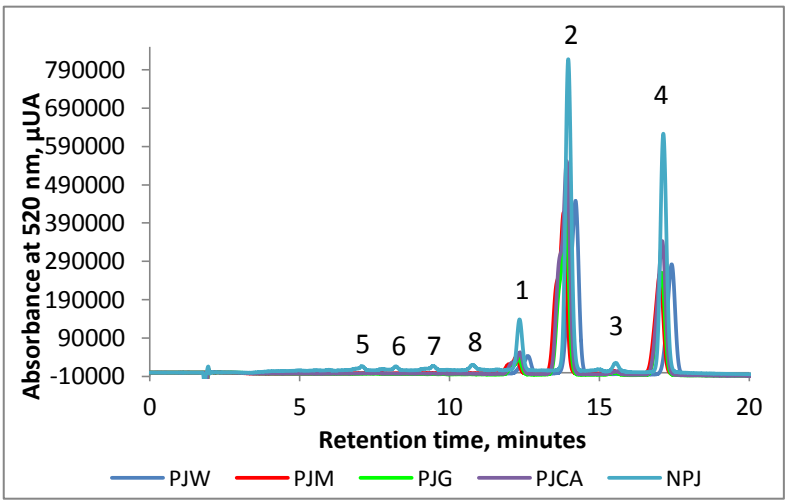


Figure 4.5. Chromatographic profile of anthocyanins from *Prunus domestica* var. *Vanette* (λ = 520 nm). The peaks represent: cyanidin 3-glucoside (1); cyanidin 3-rutinoside (2); peonidin 3-glucoside (3); peonidin 3-rutinoside (4).

According to the results obtained in this study, the addition of sugars, citric acid or that of a mixture resulted in smaller values of the TMA, TPF, TF and DPPH RSA in the juices by

approximately 50% compared to the juice with the addition of water. After this value the contents became stable.

Wrolstad et al., (1990) have shown that sucrose addition has a protective effect on anthocyanins and slows down both the browning process and the polymerization process of frozen strawberries. The explanations for the protective effect of sucrose were the inhibition of the degradative enzymes (*β*-glucosidases, polyphenoloxidases, peroxidases), the polymerization reaction between the anthocyanin and the phenol group, or that it provides a partial barrier to oxygen. The exact mechanism behind the stabilization of anthocyanins is difficult to determine, but it is known that in addition to glucose and organic acid molecules, the phenolic acids can also help stabilize the compounds (Shaheer et al., 2014).

4.3.3. Analysis of phenolic composition and antiradical activity of cherry skins extract

Numerous studies have focused on the distribution of anthocyanins in fruits, so that it has been investigated that most of them are found in the fruit skin, although in some cases they are also present in the fruit pulp (cherry *Bing* variety) (Chaovanalikit and Wrolstad, 2004). For this reason, it has been decided that the total polyphenolic content (TPF), total flavonoidic content (TF), total monomeric anthocyanin content (TMA) and the antioxidant capacity will be evaluated from the surface layer of the cherry fruits (the skins). Furthermore, in this case lyophilized cherry skin powder was used for the extraction.

The total polyphenolic content (TPF), total flavonoidic content (TF) and total monomeric anthocyanin content (TMA) as well as the antioxidant activity of the cherry skins extract (*Uriaşă de Bistriţa*) were 0.011 ± 0.001 mg GA/g dw, 2.38 ± 0.193 mg CE/g dw, 2.44 ± 0.182 mg C3G/g dw and 86.86%, respectively.

According to the chromatographic analysis (figure 4.6.), two anthocyanins were identified in the Romanian cherry skins extract (*Uriaşă de Bistriţa* variety) in the presence of peonidin 3-glucoside and pelargonidin 3-rutinoside.

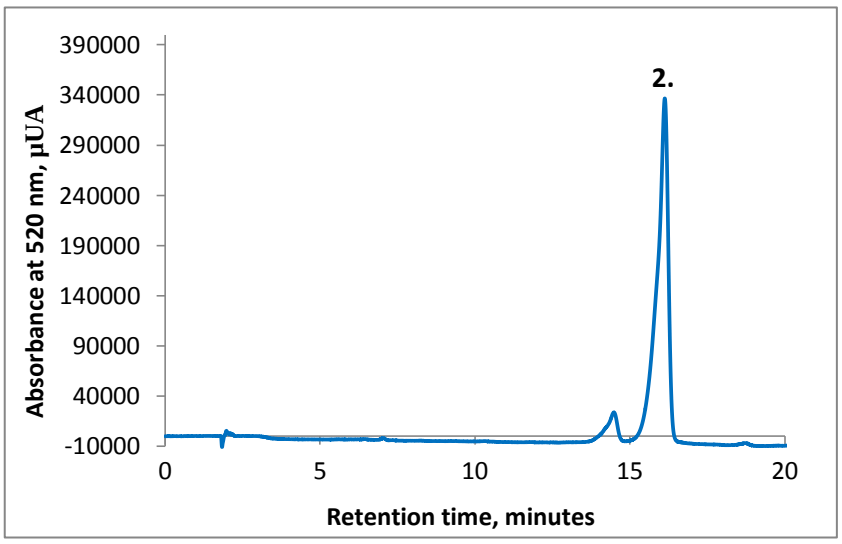


Figure 4.6. Chromatographic profile of anthocyanins from cherry skins extract (λ = 520 nm). The peaks correspond to the subsequent compounds (1) peonidin 3-glucoside; (2) pelargonidin 3-rutinoside

It can also be observed that the compositional variation of Romanian cherries is probably due to the cultivation conditions, the type of soil used, the geographical position, the environmental conditions, and also the degree of maturity at the time of harvest.

4.3.4. Analysis of phenolic composition and antiradical activity of natural cherry juice

The values for the total polyphenolic content (TPF), total flavonoidic content (TF), total monomeric anthocyanins content (TMA) and the antioxidant activity of the natural cherry juice (*Prunus avium* var. *Uriaşă de Bistriţa*) were: 0.94 ± 0.021 mg gallic acid/g dw, 0.63 ± 0.08 mg catechin equivalents/g dw, 0.176 ± 0.0325 mg C3G/g dw, and 87% respectively.

Following the HPLC analysis of the studied natural cherry juice (*Uriaşă de Bistriţa* variety), five anthocyanins were identified: cyanidin 3-rutinoside, cyanidin 3-glucoside, peonidin 3-rutinoside, peonidin 3-glucoside and pelargonidin 3-rutinoside. The major anthocyanin found was cyanidin 3-rutinoside (0.124 ± 0.008 mg/mL), closely followed by cyanidin 3-glucoside (0.116 ± 0.003 mg/mL), pelargonidin 3-rutinoside (0.039 ± 0.002 mg/mL), peonidin 3-rutinoside (0.009 ± 0.0009 mg/mL) and peonidin 3-glucoside (0.006 ± 0.0006 mg/mL) (figure 4.7).

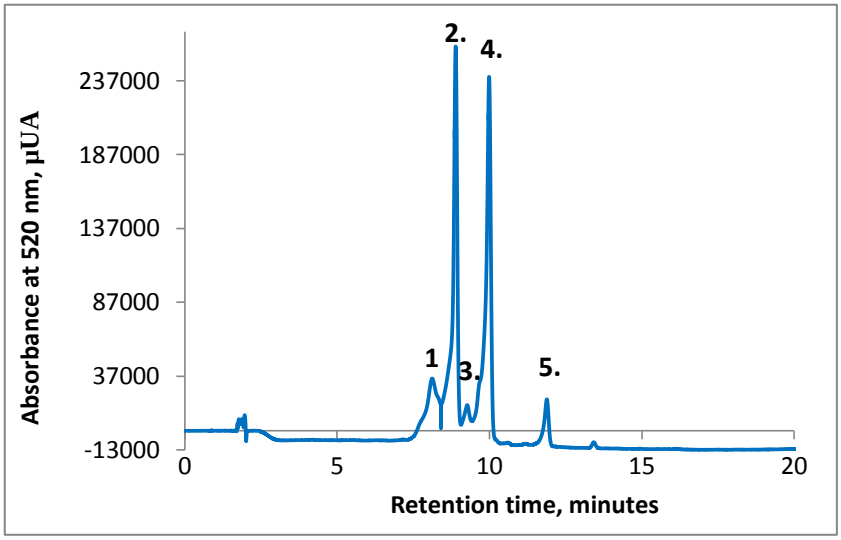


Figure 4.7. Chromatographic profile of anthocyanins from natural cherry juice (λ = 520 nm). Peaks correspond to the following compounds: (1) cyanidin 3-rutinoside; (2) cyanidin 3-glucoside; (3) peonidin 3-rutinoside; (4) peonidin 3-glucoside; (5) pelargonidin 3-rutinoside

4.4. Partial conclusions

1. Red fruits represent extremely valuable natural resources with a high content of bioactive compounds, antioxidant activity and many health benefits.
2. In our country, local red fruits as plums (*Prunus domestica* Vanette variety) and cherries (*Prunus avium Uriaşă de Bistriţa* variety) are poorly studied in regards to their content of bioactive compounds, especially phenolic compounds and their antioxidant activity.
3. The studies carried out focused on the compositional analysis of plums and cherries in concentrated skins fruit extracts, in natural fruit juice as well as in simulated matrices so that it was demonstrated that the bioactive potential is variable depending on the processing techniques.

4. The freeze-dried plum skins powder extract has a total polyphenolic content (TPF) of 0.017 ± 0.001 mg gallic acid/g dw, a total flavonoidic content (TF) of 2.794 ± 0.176 mg of catechin equivalents (CE)/g dw, a TMA content of 2.132 ± 0.168 mg cyanidin 3-glucoside/g dw and a DPPH RSA value of 71.42 ± 2.31%.
5. The chromatographic analysis of the plum extract revealed the presence of four peaks that correspond to five anthocyanins: cyanidin 3-xyloside/cyanidin 3-glucoside (0.0303 mg/mL), cyanidin 3-rutinoside (0.182 mg/mL), peonidine 3-glucoside (0.0008 mg/mL) and peonidin 3-rutinoside (0.069 mg /mL).
6. The total polyphenolic content was found to be much higher for the simulated plum juice with added water (PJW - 11.29 ± 3.13 mg GA/mL) while the highest content of flavonoids was assessed for the plum skins extract (*Prunus domestica Vanette* variety), 2.794 ± 0.176 mg CE/mL.
7. The major anthocyanin found in the simulated juices was cyanidin 3-rutinoside that presented different values such as: 0.435 ± 0.012 mg/mL (in the case of the juice that contained water), 0.559 ± 0.052 mg/mL (in the case of the juice with citric acid), 0.403 ± 0.014 mg/mL (in the case of the juice with glucose), 0.421 ± 0.016 mg/mL (in the case of the juice that contained all the above-mentioned compounds).
8. In the case of the simulated plum juices, the highest anthocyanin content was recorded for the PJCA juice (0.559 ± 0.052 mg C3G/mL), possibly due to the copigmentation effect.
9. In the case of the natural juice, the major anthocyanin is cyanidin 3-rutinoside with a content of 0.526 ± 0.013 mg/mL.
10. The lyophilized skin cherry extract had a total polyphenolic content of 0.011 ± 0.001 mg GA/g dw, a total flavonoidic content of 2.38 ± 0.193 mg CE/g dw, a total monomeric anthocyanin content of 2.436 ± 0.182 mg C3G/g dw and the antioxidant activity of 86.86 ± 0.003 %.
11. The high-performance liquid chromatography techniques allowed the identification of two anthocyanins in the cherry skins extract, while five anthocyanins were found in the natural cherry juice, cyanidin 3-rutinoside having the highest concentration (0.124 ± 0.009 mg/mL).
12. Preserving the biochemical and physiological properties as well as maintaining the bioactive properties during raw material processing or after the extraction process is a very important approach to achieve a good stability of the phenolic compounds under different physicochemical conditions.
13. The obtained results can be a good benchmark to understand the biologically active compounds behavior in model and food systems.

5. pH influence on anthocyanins from plum and cherry-derived matrices

This chapter presents the studies regarding the conformational properties that assess the kinetic behavior of the anthocyanins derived from plums (*Prunus domestica Vanette* variety) and cherries (*Prunus avium Uriaşă de Bistriţa* variety) by using fluorescence spectroscopy techniques that describe the conformational and structural changes induced by different pH values.

The methods used to describe the pH behavior of anthocyanins were the phase diagram and the emission spectra.

The obtained results present a fundamental and applicative importance because they provide a great deal of information on the behavior of anthocyans during processing, with a particular impact on the food industry.

5.1. Introduction

Anthocyanidins are a unique subgroup of flavonoids, which give the plant distinctive colors. From a chemical point of view, anthocyanidins are flavin cations and are most commonly found as chlorinated salts. Depending on the pH, anthocyanidins have different colors, from red (under very acidic conditions) to blue-violet (intermediate/neutral pH conditions) and yellow-green (alkaline conditions) (Anderson and Jordheim, 2006).

This study aimed to evaluate the effect of the pH variation (pH 1.0 - 8.0) on anthocyanins from Romanian varieties of plum (*Prunus domestica*) and cherries (*Prunus avium*) skins in different environments with different complexities. The effect of pH on the fruit extracts and on the juices obtained from these fruits was assessed. In order to have a comparison, the studies were carried out also on anthocyanins standards, which are found in the composition of Romanian plums and cherries. In this respect, fluorescence spectroscopy techniques, such as emission spectra and phase diagram, have been used to accurately describe the structural changes of the polyphenolic compounds in order to establish the process-structure-function relationships. Although fluorescence spectroscopy is less used, this technique is proposed as an advanced analysis alternative to determine the pH-induced changes of the polyphenolic compounds from plant matrices.

5.2. Materials and methods

Plums and cherries have been purchased from the local market and have been stored in a frozen state until processing. The used reagents are similar to those presented in subchapter **4.2.1.** In addition, a number of standards, namely cyanidin 3-xyloside, cyanidin 3-rutinoside, peonidin 3-glucoside and peonidin 3-rutinoside, were used for the identification and quantification of anthocyanins and were purchased from Extrasynthese (France). Sodium acetate, hydrochloric acid, sodium hydroxide, citric acid, glucose, fructose were purchased from Sigma (Sigma-Aldrich Co., St. Louis, MO). Standards were used without any further purification steps. All used reagents were analytically pure.

5.3. Results and discussion

5.3.1. The influence of pH variation on the behavior of anthocyanins in standard solution

For the study relevance, the behavior of four standard compounds, mainly cyanidin 3-xyloside, cyanidin 3-rutinoside, peonidin 3-glucoside and peonidin 3-rutinoside was studied initially.

Figure 5.6. shows the variations of the fluorescence intensity and λ_{max} of standard anthocyanin compounds at different pH values. Following the excitation at 270 nm, the fluorescence intensity varied for cyanidin 3-xyloside between 107.72 - 390 AU, for cyanidin 3-rutinoside between 177.93 - 382.21 UA, in the case of peonidin 3-glucoside between 57.29 - 314.36 UA and between 50.53 and 357.66 UA for peonidin 3-rutinoside. When λ_{ex} was at 300 nm, the fluorescence intensity of peonidin 3-glucoside standard varied between 53.41 - 96.59 UA and for peonidin 3-rutinoside between 52.87 - 108.9 UA. Following the excitation at 340 nm, the fluorescence intensity varied for cyanidin 3-xyloside between 61.02 - 97.09 UA, for cyanidin 3-rutinoside between 74.26 - 108.32 UA, for peonidin 3-glucoside between 35.99 - 101, 98 UA and between 36.77 and 112.35 UA for peonidin 3-rutinoside. The fluorescence intensity registered a significant decrease following the excitation at the wavelength of 410 nm as follows: 25.43-52.44 UA for Kuromanin, 21.71-57.74 UA for Keracyanine, 22.9-54.5 UA for peonidin 3-glucoside and 20.24-51.89 UA for peonidin 3-rutinoside, respectively.

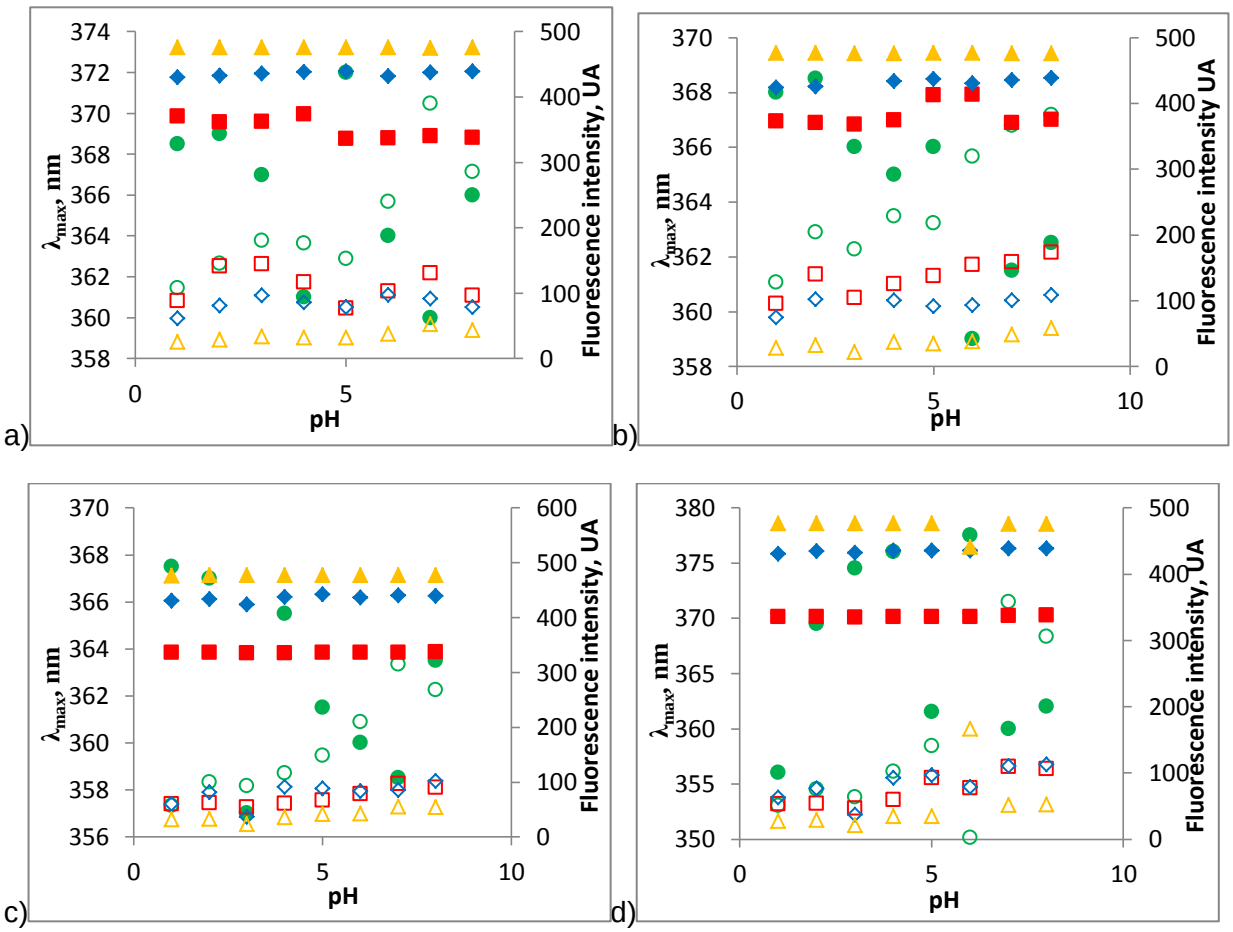


Figure 5.6. The variation at different pH values of the fluorescence intensity (empty symbols) and λ_{max} (full symbols) of the standard anthocyanin compounds, which certifies the structural changes: a) cyanidin 3-xyloside b) cyanidin 3-rutinoside c) peonidin 3-glucoside, d) peonidin 3-

rutinoside; Excitation wavelengths λ=270 nm (emission λ=310-420 nm, circles), λ=300 nm (emission λ=320-420 nm, squares), λ=340 nm (emission λ = 360-660 nm; diamonds), λ= 410 nm (emission λ= 430 - 800 nm, triangles)

Based on the studies performed on the anthocyanin standards, it was revealed that the most suitable excitation wavelength is 270 nm, because at this wavelength the recorded fluorescence intensity for the four studied anthocyanins is maximum.

Figure 5.7. shows a phase diagram that describes the conformational changes of anthocyanins (standards) induced by varying the pH of the reaction environment.

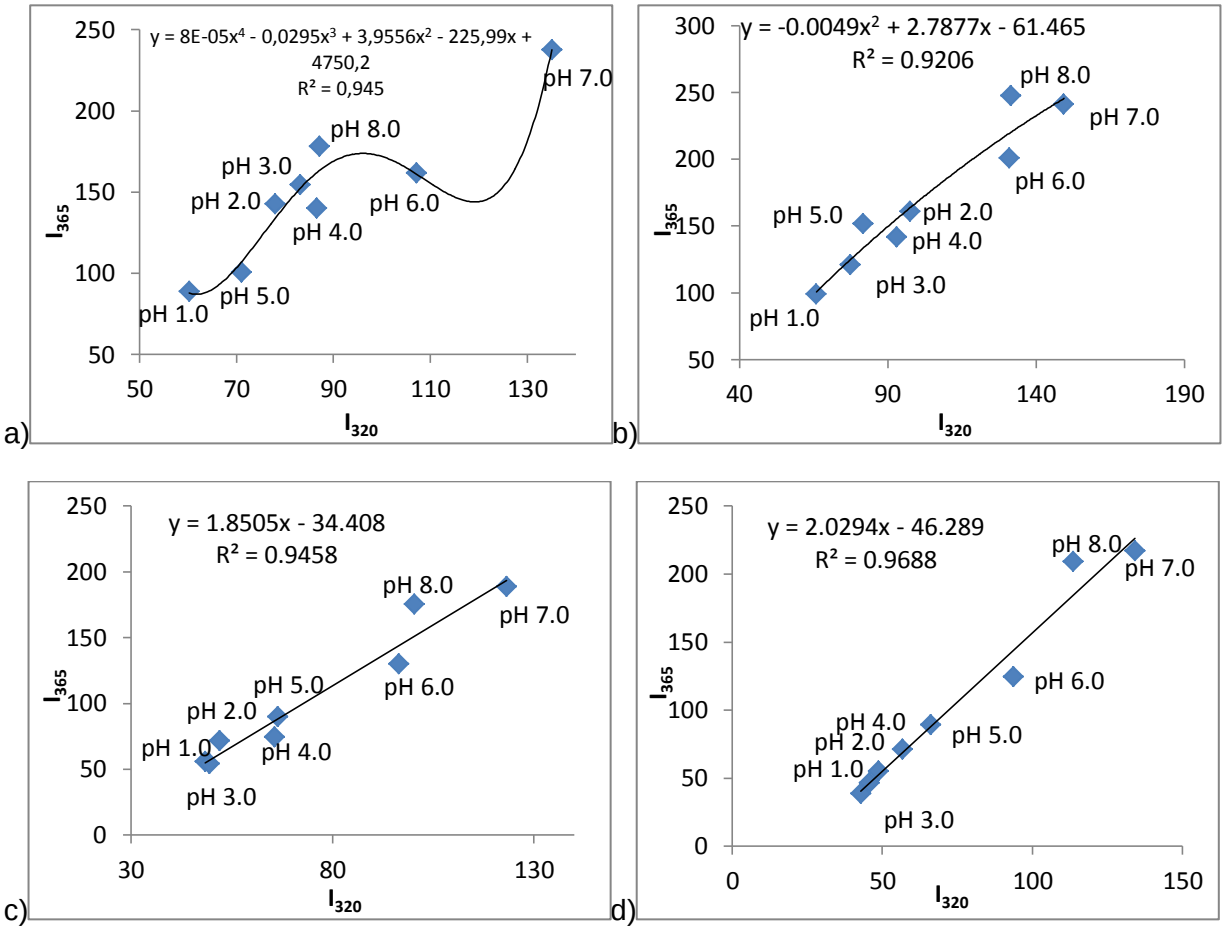


Figure 5.7. Phase diagrams that describe the conformational changes induced by different pH values of the standard compounds: a) cyanidin 3-xyloside b) cyanidin 3-rutinoside c) peonidin 3-glucoside d) peonidin 3-rutinoside

The peonidin 3-glucoside and peonidin 3-rutinoside standards (figure 5.7.c, d) showed a linear variation, the correlation indicating the presence of at least two molecular species. By changing the pH value, there was a decrease in the fluorescence intensity, especially in the acidic range (pH values below 6.0). Regarding the cyanidin 3-rutinoside standard (figure 5.7.b), it exhibited a second-order polynomial variation, whereas the cyanidin 3-xyloside anthocyanin (Figure 5.7.a) showed a variation after a fourth-order polynomial model, which also indicated the presence of more than two molecular species.

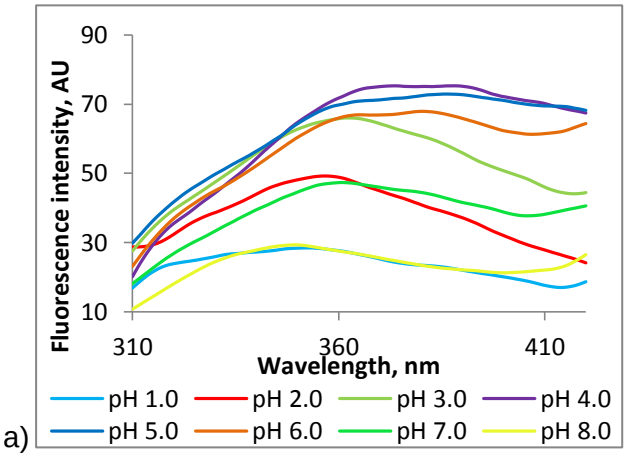
5.3.2. The pH variation on the anthocyanin behavior from plum derived matrices

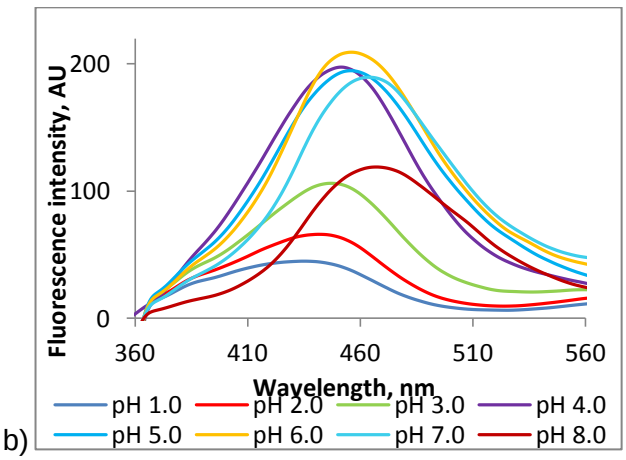
5.3.2.1. Spectrofluorimetric analysis of anthocyanin behavior in skins plum extract

The ethanolic extracts from *Prunus domestica* skins were analyzed and the studies were performed under conditions similar to those of the standards by using the excitation wavelengths of 270 nm, 300 nm, 340 nm and 410 nm, at a pH variation in the range 1.0 to 8.0. The obtained emission spectra are shown in figure 5.8.

When the plum skins extract was subjected to the excitation at λ=270 nm, 340 and 410 nm, specific spectra were obtained. The spectra was positioned within the wavelength range of λ=350 - 388 nm, 450 - 470 nm and 440 - 476 nm, which indicated the presence of cyanidin 3-xyloside/cyanidin 3-glucoside, cyanidin 3-rutinoside, peonidin 3-glucoside and peonidin 3-rutinoside, all these compounds being also identified by the HPLC analysis.

In figure 5.8.a) the spectra obtained in the pH range of 4.0 - 6.0 displayed different shapes due to the fact that this excitation wavelength indicates the presence of anthocyanins in a carbinol pseudobase form (pH 4.0-5.0) as well as the quinoindale base form (pH 6.0-7.0). According to the studies assessed by Rakic et al., (2015), the compound that absorbs in the 270-280 nm wavelength range was identified as cyanidin.





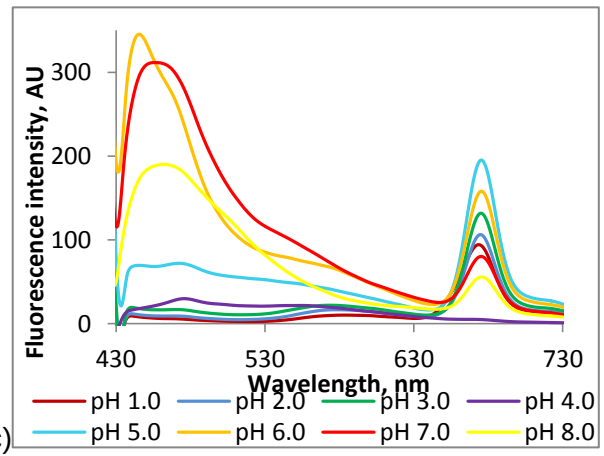


Figure 5.8. Fluorescence spectra of anthocyanins from plum skin extract at different pH values: a) excitation at λ=270 nm, emission at λ=310 nm - 420 nm; b) excitation at λ=340 nm, emission at λ=360 nm - 660; c) excitation at λ=410 nm, emission at λ=430 nm - 800 nm.

When the plum skins extract was subjected to an excitation wavelength of 300 nm, no clear spectra were obtained. Thus, in order to obtain the full spectra, the collected emission was registered between 280 nm and 500 nm.

According to the research conducted by Rakic et al., (2015), the chalcones are highlighted at the excitation wavelength of 340 nm (figure 5.8 b), these compounds exhibiting the maximum intensity within the 420 - 450 nm wavelength range.

According to the data from figure 5.8.c), the presence of a second peak in the wavelength range between 673.5 - 675.5 nm is observed. The maximum intensity was recorded at pH 5.0, which may suggest the existence of two molecular species.

5.3.2.2. Spectrofluorimetric analysis of anthocyanin behavior in natural plum juice

The spectral analysis at different pH values (pH 1.0-8.0) was also performed for the natural plum juice under similar conditions to those described in the anthocyanin standards analysis or for the plum skins extract. The results are presented in figure 5.10.

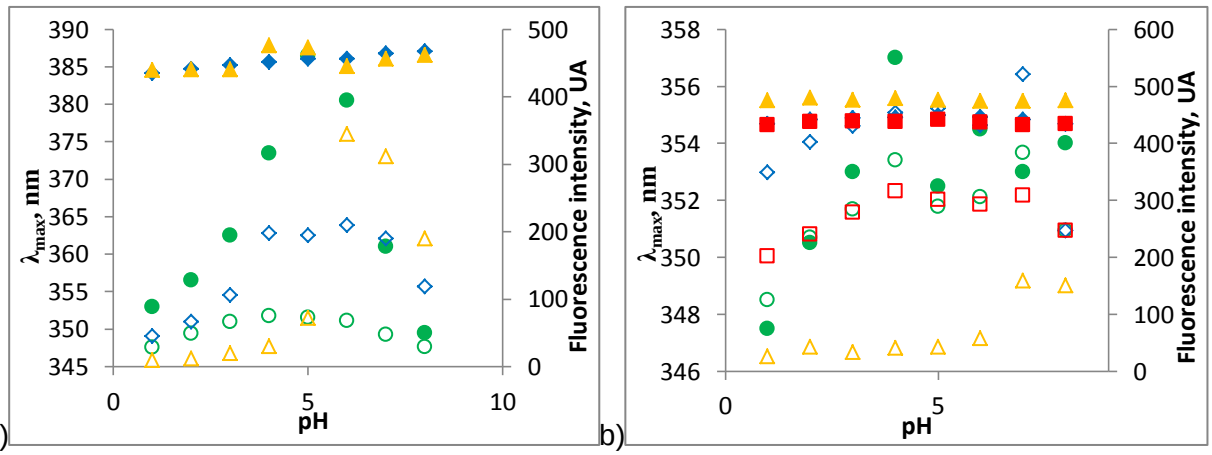


Figure 5.10. The variation at different pH values of the fluorescence intensity of anthocyanins from a) plum skins extract b) natural plum juice (empty symbols) and λ_{max} (full symbols); excitation wavelength: 270 nm (emission 310 - 420 nm, circles), 300 nm (emission 320 - 420 nm, squares), 340 nm (emission 360 - 660 nm, diamonds), 410 nm (emission 430 - 800; triangles)

From figure 5.10.a, it can be seen that after the excitation at 270 nm of the plum skin extract, several structural changes were assessed that led to a fluorescence intensity increase at pH 4.0, followed by a decrease at the other pH values. The λ_{max} values ranged from 353 nm, at pH 1.0 to 388 nm at pH 4.0. Thus, at the other pH values, several changes were observed in the spectrum and also the presence of blue-shifts. The pH value of 8.0 indicates the occurrence of a 38.5 nm blue shift (349.5 nm).

However, the form of the spectra from figure 5.10.a) indicates the presence of two compounds with different spectral properties, for example cyanidin 3-rutinoside and peonidin 3-rutinoside, respectively, that were identified by HPLC analysis from the plum skin extract.

After the plum skin extract excitation at 340 nm, several structural changes occurred, so that the λ_{max} values ranged from 442 nm at pH 1.0 to 470 nm at pH 7.0. Furthermore, at pH 2.0, one can see a change of the spectrum and the presence of a 13 nm (455 nm) red shift.

The pH value of 3.0 also induced a 8 nm red shift (450 nm). The fluorescence spectrum at the excitation wavelength of 340 nm, at pH 4.0, showed a 13 nm (455 nm) red shift. At pH

5.0, a red shift of 18 nm (460 nm) was recorded, and at pH 7.0 the presence of a red shift was indicated at a λ_{max} value of 468 nm (26 nm).

According to the data presented in figure 5.10.b), the fluorescence intensity of the first peak varied with the pH so that the highest fluorescence intensity was recorded at pH 7.0 at the emission wavelength of 353 nm (383.63 UA) while the lowest fluorescence intensity was highlighted at pH 1.0 at the emission wavelength of 347.5 nm (125.23 UA). As for the second peak, it exhibited the highest fluorescence intensity at pH 4.0, at λ_{em} =426 nm (226.8 UA) and the lowest intensity was registered at pH 1.0 at λ_{em} =430 nm (138.61 UA).

In figure 5.10.b), at the excitation wavelength of 340 nm, the presence of two peaks at pH 8.0 was observed, the first one being present at λ_{max}=383 nm (126.18 UA) and the second peak at 474 nm (247.18 UA).

Figure 5.11. presents the phase diagram that describes the conformational changes assessed by representing the dependence between the fluorescence intensity at 320 nm and the intensity at 365 nm, changes induced by different pH values of the natural plum juice.

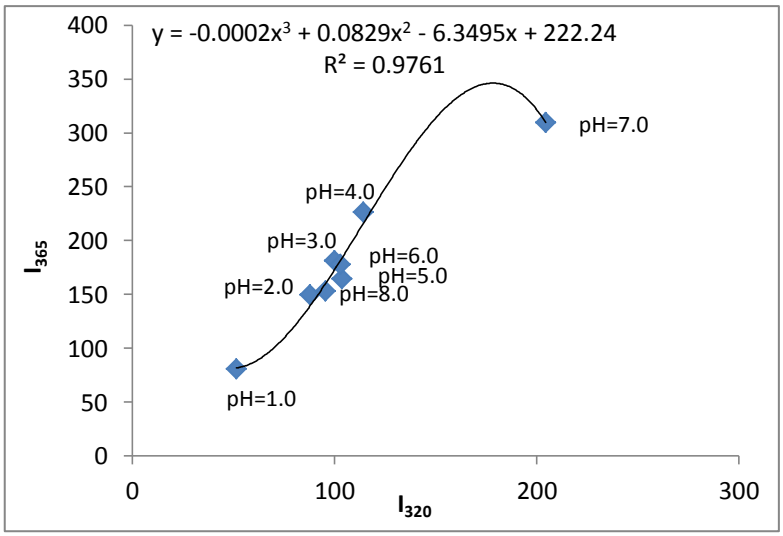


Figure 5.11. Phase diagram that describes the conformational changes of anthocyanins from the natural plum juice induced by different pH values

The spectrofluorimetric variation of the natural plum juice behavior (figure 5.11) respected a polinomial pattern, the correlation indicating the presence of many molecular species in its composition that may react specifically under the analysed conditions. By modifying the pH value of the juice, it exhibited a fluorescence intensity decrease, especially at pH values smaller than 7.0.

5.3.2.3. Spectrofluorimetric analysis of anthocyanin behavior in simulated plum juices

In figure 5.15. the pH structural changes of the anthocyanins found in the simulated juices are shown, changes evidenced by the fluorescence intensity and the excitation wavelength.

Following the excitation at λ = 270 nm, the polyphenolic compounds from the plum juice had a maximum λ_{max} at 372 nm for the PJW juice, at pH 5.0, at 358.5 nm for the PJCA and PJM juices, and at 359 nm for the PJG juice, at pH 6.0. Increasing the pH value in the case of the juice with the water addition, from 1.0 to 3.0, caused a 9 nm red shift of the λ_{max}, followed by a 21 nm red shift, after the decrease of the pH from 8.0 to 5.0 (figure 5.15.a). In the case of the

juice with citric acid and glucids, the decrease of the pH from 5.0 to 3.0 induced a red shift of 6 nm, while the adjustment of the pH at 8.0, a blue shift of 7 nm occured.

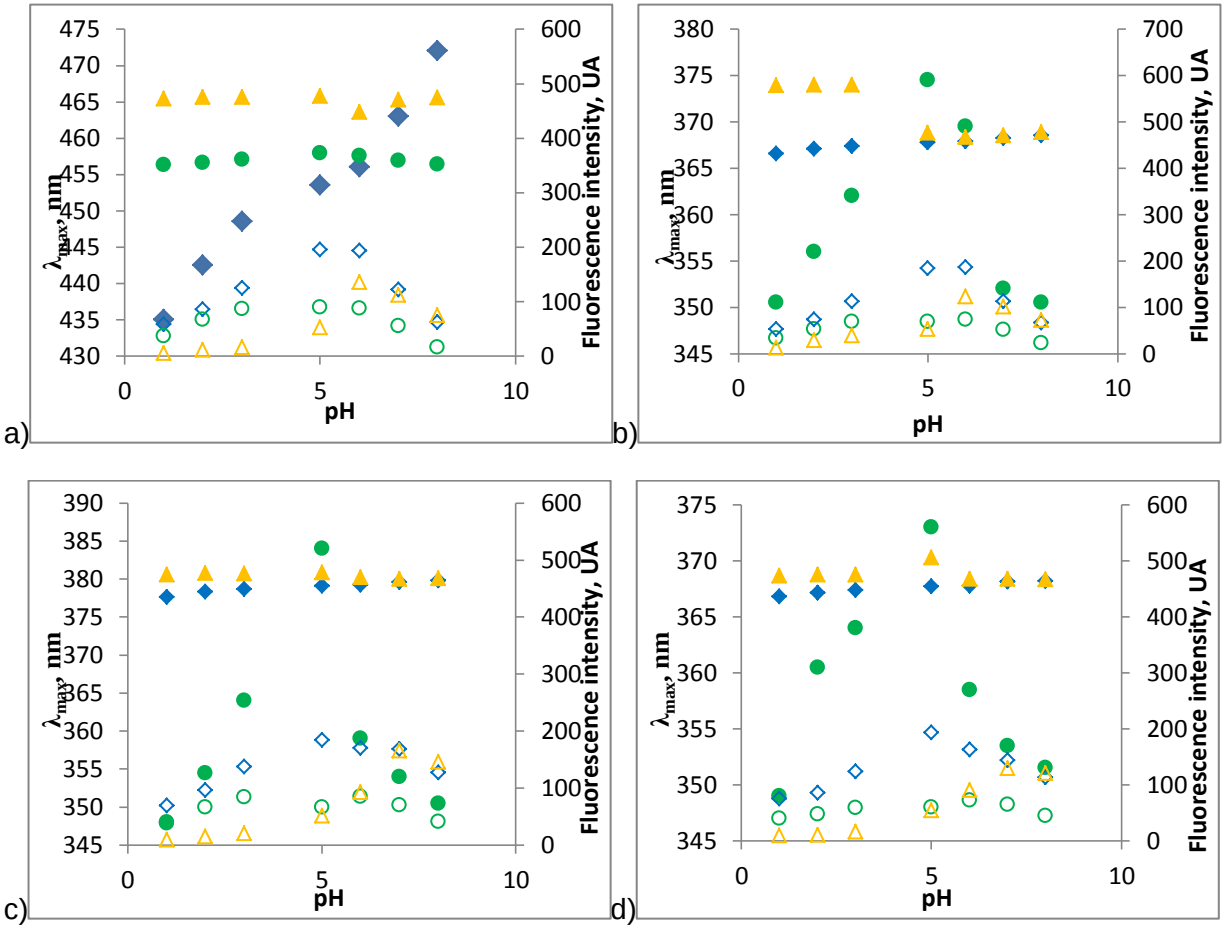


Figure 5.15. The variation at different pH values of the fluorescence intensity of anthocyanins in different juices a) PJW, b) PJCA, c) PJG and d) PJM (empty symbols) and λ_{max} (full symbols); excitation at λ = 270 nm, (emission λ = 310 nm - 420 nm; circles), λ = 340 nm, (emission λ = 360 nm - 660 nm; diamonds), λ = 410 nm, (emission λ = 430 nm - 800 nm; triangles)

Comparing the results obtained after the excitation at 270 nm, it can be observed that by exciting at 340 nm the maximum intensity in the case of the juice with citric acid was recorded at pH 6.0, while for the other simulated juices the maximum intensity was recorded at pH 5.0. The λ_{max} values in the case of the PJW juice ranged from 435 nm (58,236 UA) at pH 1.0 to 472 nm (61,917 UA) at pH 8.0.

Changes were also evidenced in the case of PJCA juice, where a 10 nm red shift (465 nm) and 15 nm (470 nm) were present, with a maximum intensity at pH 6.0 (186.54 UA - 458 nm).

After exciting the juice with the addition of water at λ = 410 nm, several structural changes were revealed, correlated with an increase of the fluorescence intensity in the pH range between 1.0 and 6.0. In the case of the citric acid juice, the presence of the second peak could be observed at pH 1.0 (13.324 UA - 579 nm), 2.0 (28.622 UA - 580 nm) and 3.0 (39.738 UA - 579.5 nm).

The PJG and PJM simulated juices had the same fluorescence behavior, with the highest fluorescence intensity at pH 7.0 for the first peak and at pH 5.0 for the second peak, respectively.

In figure 5.16., the simulated juices phase diagram was obtained by plotting the intensity of the plum-derived matrices at the 320 nm wavelength versus the 365 nm wavelength (Yang Jr. et al., 2006).

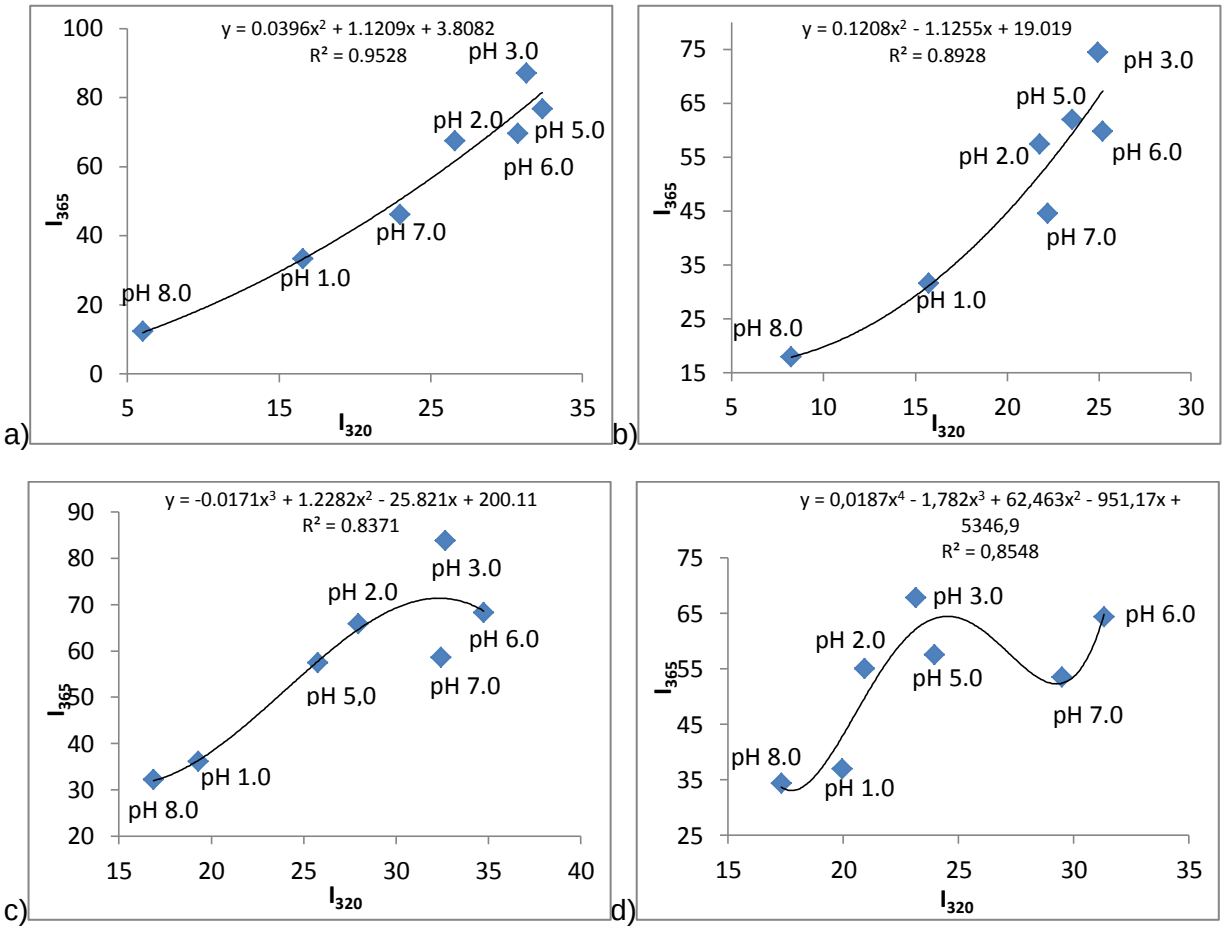


Figure 5.16. Phase diagrams that describes the conformational changes of anthocyanins induced by the pH variation in: a) simulated juice with water addition; b) simulated juice with citric acid; c) simulated juice with glucose addition and d) simulated juice with the addition of citric acid and glucose

A non-linear dependance could be observed, a dependance described by a second order polynomial equation in the case of PJW and PJCA juices, a third order polynomial equation for the PJG juice and a fourth order polynomial ecuation for the SPAm juice. These equations suggested the presence of several distinct molecular species with different chemical behavior that were induced by the change of the pH (figure 5.16).

5.4. The pH variation on the anthocyanins behavior from cherry skins extract and natural cherry juice

In this subchapter, the behavior of the biologically active compounds from the skin cherry extract and the natural cherry juice was monitored in accordance to the pH. The stability of bioactive compounds study in regards to different pH values is of particular importance due to

the fact that this type of study may be used to obtain novel foods or food supplements in the food industry.

Different studies have demonstrated the structural changes of anthocyanins that occur in the cherry skins extract at different pH values. Hence, the extract, at a wavelength of 250 nm (figure 5.17), showed a maximum emission value at λ = 353 nm.

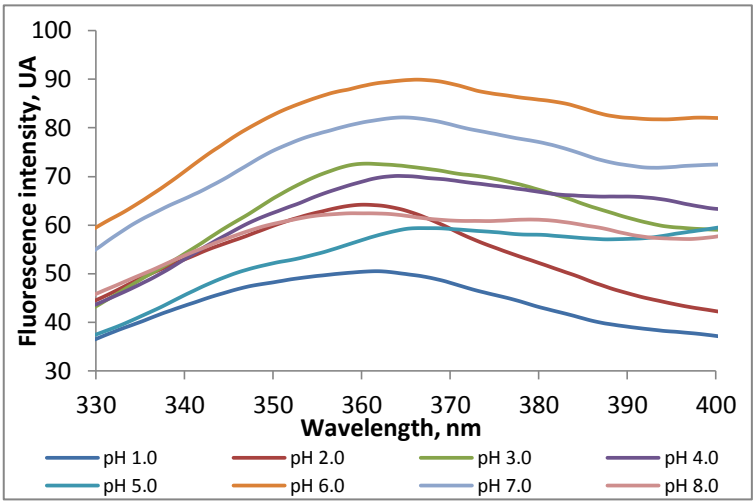
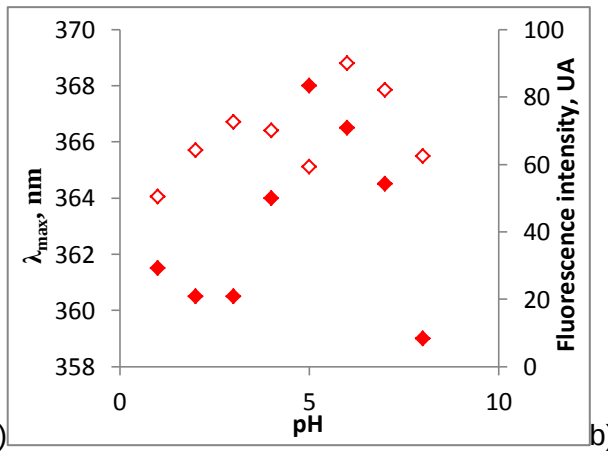


Figure 5.17. Fluorescence spectra of anthocyanins from cherry skins extract at different pH values; excitation at λ = 250 nm, emission at λ = 270-600 nm

The profile of the obtained spectra (figure 5.17) indicated that the pH determines multiple structural changes of anthocyanins. When the cherry skins extract was subjected to an excitation at λ= 250 nm, specific spectra were obtained, being positioned within the wavelength range 350 - 370 nm and thus suggesting the presence of anthocyans that emit differently depending on the pH value.

In figure 5.19., the structural changes of anthocyanins from cherry skins extract and natural cherry juice are reported and analysed by fluorescence intensity and the λ_{max} in the pH range between 1.0 and 8.0.

a)



b)

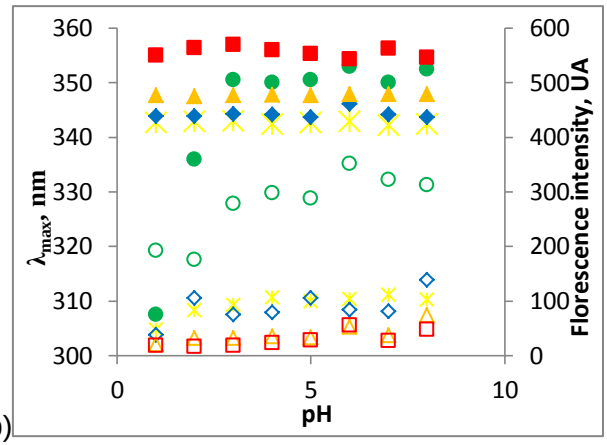


Figure 5.19. The variation at different pH values of the fluorescence intensity of anthocyanins in a) cherry skins extract and b) natural cherry juice, (empty symbols) and λ_{max} (full symbols). The excitation wavelength was 250 nm (emission 270 - 420 nm, red diamond), 270 nm (emission 310 - 420 nm, circles), 300 nm (emission 320 - 420 nm, asterix), 340 nm 660 nm, diamond), 410 nm (emission 430 - 800 nm, triangles), 500 nm (emission 520 - 800 nm, squares)

After the excitation of the cherry skins extract (figure 5.19.a) at the wavelength of 250 nm, several structural changes resulted, which in turn led to an increase of the fluorescence intensity in regards to different pH values. A 18 nm red shift (379 nm) was registered at pH 8.0 whereas at pH 5.0, a 4 nm red shift (λ = 365 nm) was observed.

Depending on the pH, it can be seen from figure 5.19.b) the presence of two different peaks, the first peak having a slight variation. In the case of the first peak, the lowest fluorescence intensity was obtained at pH 1.0, followed by an increase of the fluorescence intensity at pH 2.0. Increasing the fluorescence intensity while increasing the pH was correlated to the copigmentation process, which was better observed for the second peak at pH 2.0, 5.0 and 8.0. For the second peak, the lowest fluorescence intensity was obtained at pH 1.0, at the wavelength of 439 nm. For the peak obtained at pH 5.0, a 3 nm red shift (from 439 nm to 436 nm) was highlighted. At pH 8.0 the presence of three peaks (I - 386 nm, II - 420 nm and III - 436 nm) was observed.

The low fluorescence intensity of anthocyanins after the excitation at 500 nm indicated that these pigments are in a monomeric form in the cherry juice.

Figure 5.20. presents the phase diagram that describes the conformational changes of the natural cherry juice anthocyanins induced by different pH values.

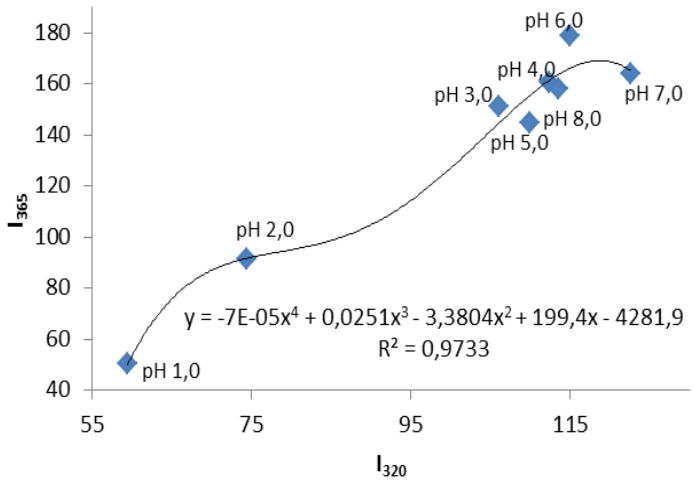


Figure 5.20. The phase diagram that describes the conformational changes of anthocyanins from the natural cherry juice induced by different pH values

The natural cherry juice (figure 5.20) presented a polinomial pattern, the correlation indicating the presence of many molecular species. This natural juice displayed a decrease of the fluorescence intensity by changing the pH value to values less than 3.0.

5.5. Partial conclusions

1. The study aimed to investigate the pH stability of anthocyanins that were extracted from plum and cherry skins, from simulated plum juice as well as from natural fruit juices by using fluorescence spectroscopy.
2. For the relevance of the studies, the behavior of four standard compounds, namely cyanidin 3-xyloside, cyanidin 3-rutinoside, peonidin 3-glucoside and peonidin 3-rutinoside, previously identified in Romanian plum and cherry skins by high performance liquid chromatography techniques. Fluorescence spectra were obtained in correlation

with the pH variation following the sample excitation at several wavelengths such as 270, 300, 340 and 410 nm.

3. Anthocyans have an important role when it comes to color quality of processed fruits and vegetables. Anthocyans differ from other flavonoid compounds due to their ability to form different structures depending on the pH of the environment.
4. After the excitation of anthocyanin standards at 270 nm, the λ_{max} was recorded at 362.5 nm for cyanidin 3-rutinoside, at 360 nm for cyanidin 3-xyloside, at 358.5 nm for peonidin 3- glucoside and at 360 nm for peonidin 3-rutinoside. The results obtained at the excitation wavelength of 300 nm, in the case of cyanidin 3-xyloside (Kuromanin) and cyanidin 3-rutinoside (Keracianin), did not produce well-defined spectra. For the standard solutions of peonidin 3-glucoside and peonidin 3-rutinoside, the λ_{max} varies in the wavelength range 335-337 nm. Following the excitation at 340 nm, two emission bands were recorded for each anthocyanin standard by obtaining two distinct peaks with different fluorescence intensities in the wavelength range 336-340 nm (peak 1) and 423 - 442 nm (peak 2).
5. The presence of anthocyanins as well as the presence of the polyphenolic compounds (ferulic acid, coumaric acid, gallic acid, coumarin, etc.) and flavonoids was highlighted in the plum skin extracts and the plum juices, by applying the fluorescence spectroscopy technique with the excitation at 270 nm. At the excitation wavelength of 300 nm, the presence of polyphenols was highlighted. At the excitation wavelength of 340 nm, the anthocyanins emerged in the chalcone form and at the wavelength of 410 nm, quercetin and the group E vitamins emited.
6. When the plum skin extract was excited at 270 nm, 340 and 410 nm, specific spectra were obtained that were positioned within the wavelength range λ = 350-388 nm, 450-470 nm and 440 - 476 nm, respectively. Thereby, the presence of cyanidin 3-xyloside/cyanidin-3-glucoside, cyanidin-3-rutinoside, peonidin 3-glucoside and peonidin 3-rutinoside was indicated, compounds previously identified through HPLC analysis.
7. Anthocyans from plum and cherry-derived matrices exhibited different sensitivity to the pH variations. The results obtained for the phase diagram and fluorescence spectra suggested a deglycosylation and a cleavage process, accompanied by an increase of the fluorescence intensity in the basic pH range.
8. When the plum juice was excited at the wavelength of 270 nm, specific spectra were obtained, located within the wavelength range λ = 350 - 360 nm, that indicated the presence of anthocyans, former identified through HPLC analysis of the analysed juice. The peak positioned at 440 ± 10 nm is characteristic for a wide variety of polyphenolic compounds, including hydroxycinnamic acids, coumarins, stilbenes, isoflavones, etc.
9. The spectrofluorimetric variation of the natural plum juice behavior (figure 5.11) respected a linear pattern, the correlation indicating the presence of at most two molecular species in its composition, which react specifically under the analysed conditions.
10. Following the excitation at 270 nm, the polyphenolic compounds from the simulated plum juice presented a maximum wavelength emission (λ_{max}) at 372 nm for the juice with added water at pH 5.0, 358.5 nm for the juices with added citric acid and the one with the mixture between these compounds and 359 nm for the juice with sugars, at pH 6.0.

11. Regardless of the studied pH values, the phase diagram for the simulated plum juice did not have a linear evolution, hence indicating the presence of several distinct molecular species with different behavior as a consequence of the reaction environment. The results suggested that juices are complex systems, for which the phase diagrams corresponded to polynomial models of different order (second, third or fourth).
12. If the pH of the cherry skins extract is reduced to 1.0, the presence of a 28 nm blue-shift (307.5 nm - 192.24 UA) can be observed. This modification of the spectrum can be attributed to the presence of chlorophyll, but also to the hemiketal form which is dominant when an excitation takes place in the wavelength range between 260 nm-280 nm.
13. The obtained results can contribute to a better understanding of the chemical behavior of anthocyanins depending on the pH, as to better explain the structure-function relationship and to obtain products derived from plums and cherries with an increased functionality.

6. Chemical and kinetic behavior of biologically active compounds from plums and cherries derived matrices after thermal processing

In this chapter, the studies on the biochemical behavior of polyphenolic compounds from plums (*Prunus domestica* var. *Vanette*) and cherries (*Prunus avium* var. *Uriaşă de Bistriţa*) derived matrices are presented in terms of fluorescence spectroscopy techniques and kinetic degradation studies that describe the conformational and structural changes induced at different temperature values.

The studies aims describe the behavior of polyphenolic compounds in the mentioned matrices at different temperature-heat treatment time combinations in terms of optimizing the process-structure-function relationship by using kinetic thermal degradation models. Moreover, to provide additional information on the thermal behavior of polyphenolic compounds, various fluorescence spectroscopy techniques were used, mainly phase diagram, emission spectra and three-dimensional spectra.

The obtained results present both fundamental and applicative value because they provide a lot of information on the behavior of polyphenolic compounds during processing, in correlation with their functionality with a particular impact on the food industry.

6.1. Introduction

The processing and the formulation of new products can substantially affect the quality and the functional properties of the bioactive compounds in fruits (Nicoli et al., 1999; Paixao et al., 2007; Seruga et al., 2011). Also, the study of these effects is of fundamental and applicative importance in order to optimize processes, to improve the quality and to diversify the range of functional foods.

The determination of the degradation mechanisms led to the establishment of some kinetic parameters that can be used to predict the kinetic behavior of the targeted compounds under the concerned conditions. Thus, the degradation kinetics study can be of real use to determine the influence of processing on food quality, especially the degradation of compounds with a high nutritional and functional value.

6.2. Materials and methods

Plums and cherries were purchased from the local market and were stored at a freezing temperature until processing. A series of reagents such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-Hydroxy-2,5,7,8-tetramethylcroman-2-carboxyl (Trolox), sodium acetate, potassium chloride, sodium hydroxide, aluminum chloride, sodium nitrite, ethanol, methanol and formic acid (HPLC grades) were purchased from Sigma Aldrich Steinheim, Germany. Cyanidin and peonidin standards were purchased from Extrasynthèse (Z.I Lyon Nord, France). The Zymorouge enzyme was purchased from Sodinal (Bucharest) and was used without any further purification. All reagents were analytically pure.

6.3. Results and discussion

6.3.1. Chemical and thermal degradation kinetic of bioactive compounds from plum derived matrices

6.3.1.1. The evaluation of the polyphenolic compounds behavior from a spectrofluorimetric point of view

Among the polyphenols, only a few polyphenolic compounds emit natural fluorescence, including isoflavones that do not have an -OH group in the fifth position and flavonoids that possess a -OH group in the third position, such as catechin and methoxylated flavones (Lamuela-Raventos et al., 2014). The fluorescence spectra of the molecular species from the plum skins extract were analyzed by exciting at different wavelengths: 270 nm, 300 nm, 340 nm and 410 nm (Figure 6.1). The spectrum position and fluorescence intensity varied in accordance to the excitation wavelength.

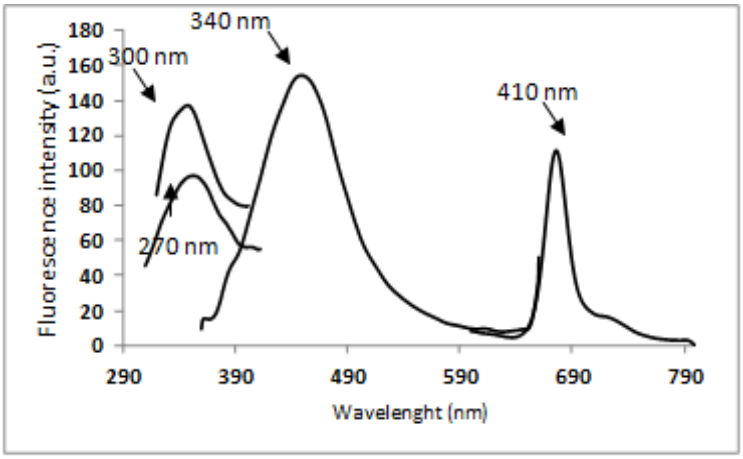
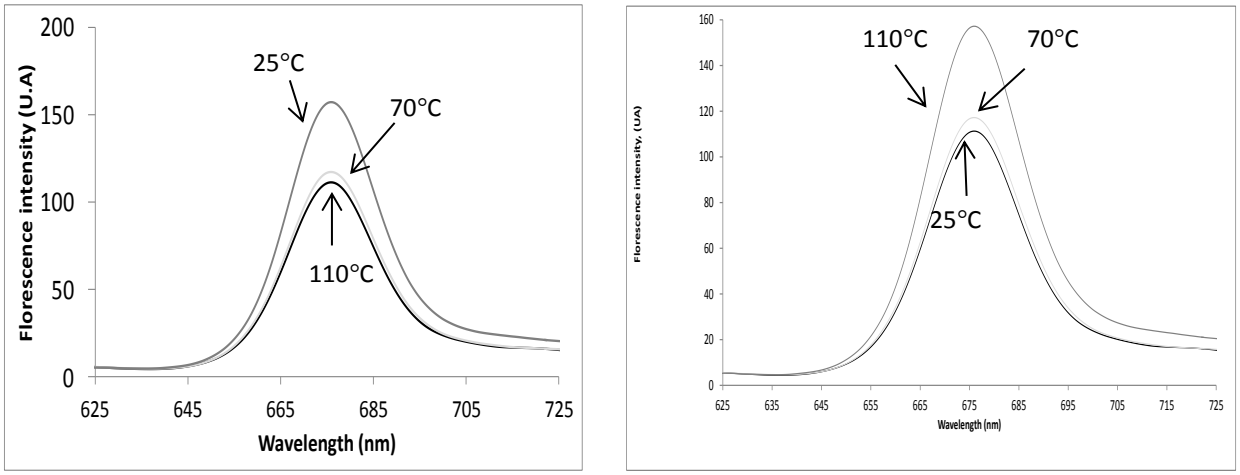


Figure 6.1. The fluorescence spectra of plum skins extract without any heat treatment at different excitation wavelengths (Turturică et al., 2016a)

The data presented in figure 6.1. indicated that the plum skins extract is a multicomponent system that contains different molecular species. The experimental results suggested that at least four fluorescent molecular species are present in the analysed plum extract. These data also allowed the location of the absorption peaks as follows: fraction I is characterized by a wavelength spectrum at λ = 353 ± 1 nm, fraction II at λ = 358 ± 1 nm, fraction III at λ = 448 ± 1 nm and fraction IV at λ = 678 ± 0.5 nm.

The thermal treatment applied to the plum skins extract led to different structural changes with a decrease of the fluorescence intensity (FI) at high temperatures, after the excitation at 300 nm (figure 6.2.a). The λ_{max} ranged from 358 nm at 25°C to 361 nm at 70°C. The increasement of the temperature to 100°C resulted in a 2 nm blue-shift (λ = 356 nm) followed by a 3 nm red shift at 110°C (λ = 359 nm). When the extract was excited at 410 nm (figure 6.2.b), an increase of the fluorescence intensity occured in the temperature range of 70°C - 110°C, while the λ_{max} remained constant at wavelength λ = 676 nm.



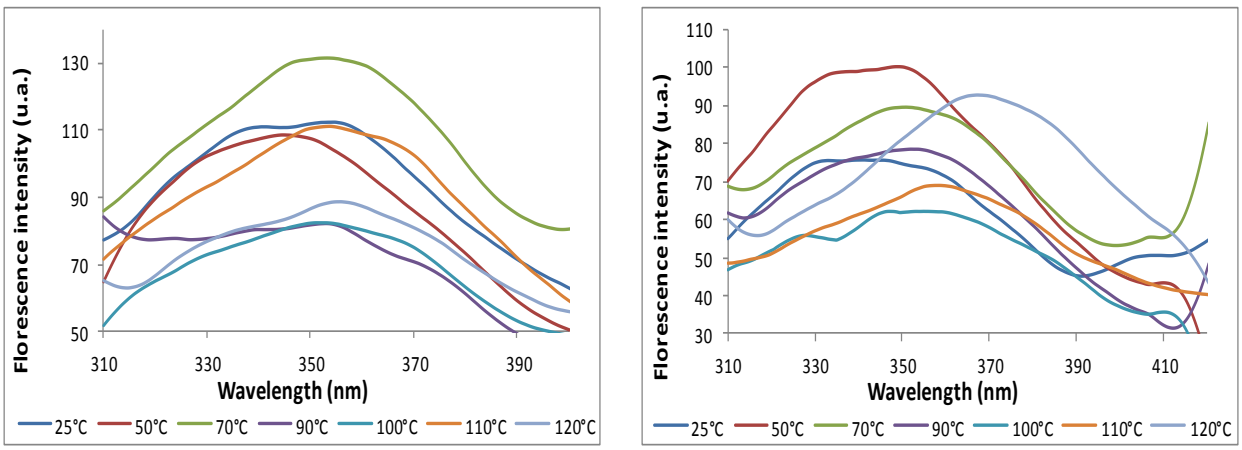
a) **b)**

Figure 6.2. The fluorescence spectra of the thermally treated plum skins extract at different temperatures. Excitation wavelength at 300 nm (a) and at 410 nm (b) (Turturică et al., 2016a)

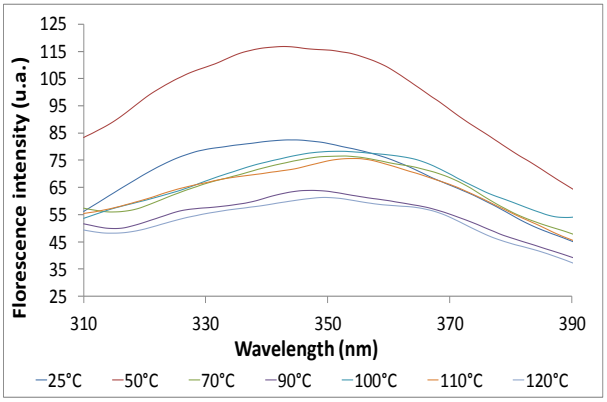
Figure 6.3. shows the fluorescence spectra of thermally treated juice after the excitation at 270 nm wavelength. For the PJW juice, the emission spectra presented emission bands at λ_{max} 342 nm at 25°C, while the heat treatment at temperatures between 110 -120°C caused a significant red-shift of 12-14 nm (figure 6.3.a).

In the case of the PJCA, the λ_{max} was around 341 nm in the case of the untreated sample, while after the heat-treatment at 110°C a 17 nm shift was highlighted, and at 120°C a 26 nm red-shift (Figure 6.3 b). For PJG, the spectra were characterized by emission bands up to 344 nm, while the thermal treatment at 90°C led to a 7 nm red shift. In the temperature range 100°C - 120°C, a 6 nm blue shift and a 3 nm red shift (figure 6.3.c) were observed.

Significant structural changes were observed for the PJM (figure 6.3 d). The fluorescence spectrum at 25°C showed the maximum emission at 340 nm. Rising the temperature to 70°C induced a 14 nm red shift. At temperatures between 90 and 100°C, blue-shift shifts of 6 nm and 4 nm were observed, followed by a 6-nm red shift and a 12 nm at higher temperature values (110°C - 120°C). The excitation of NPJ at the wavelength of 270 nm, the emission spectrum showed a peak at 357 nm, at 25°C. The increase of the temperature induced structural changes characterized by the occurrence of 3 nm blue-shifts at 50°C and 13 nm at 110°C.



a) **b)**



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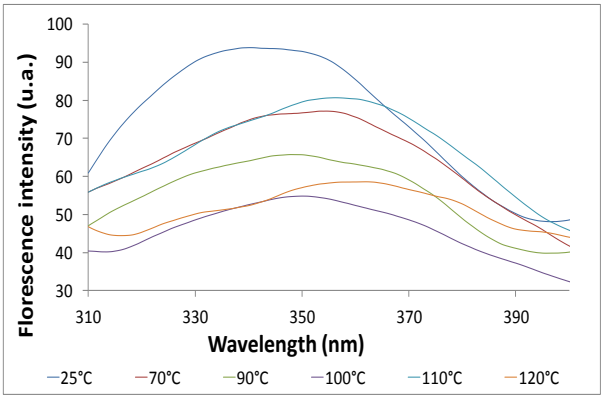

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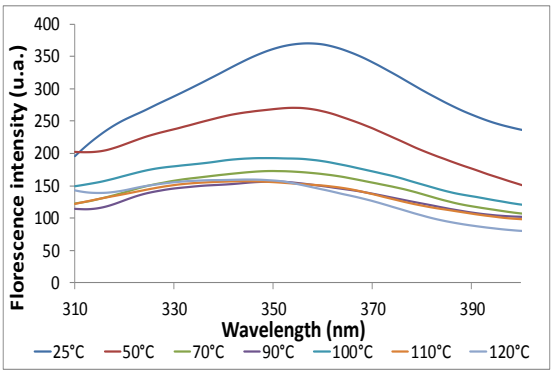


e)

Figure 6.3. The fluorescence spectra of PJW (a), PJCA (b), PJG (c), PJM (d) and NPJ (e) at different temperatures. The excitation took place at 270 nm. The experiments were performed in triplicate and the standard deviation was less than 3.5%

The significant variations of λ_{max} indicated the sequential structural changes induced in the structure of polyphenolic compounds by thermal treatment.

The results obtained in this study suggested that anthocyanins were unstable at high temperatures, which led an increase of the fluorescence intensity and the occurrence of red and blue shifts, phenomena that appear probably due to the processes of copolymerization and copigmentation of polyphenolic compounds.

6.3.1.2. The influence of the thermal treatment on the anthocyanin content and the antioxidant activity

Obviously, the quality of food is an important matter and is directly correlated to the satisfaction of the consumer's expectations; in other words, the quality experience delivered by a food product must meet the consumer's quality expectations.

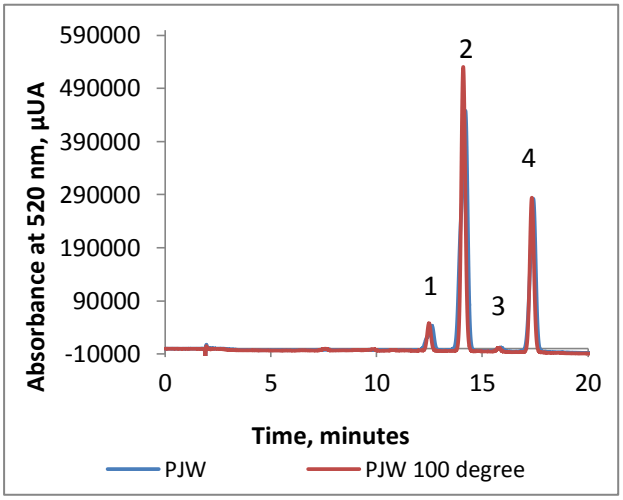
Chemical, biochemical, microbial and physical changes of quality can be assessed by kinetic studies. Kinetic models are useful to quantify the exact loss during processing. These models describe the degradation of targeted compounds, the formation of unwanted compounds, the aggregate formation kinetics, the enzymatic inactivation kinetics (polyphenoloxidase) and microorganisms, as well as the crystallization process kinetics. Food products are complex systems within which interactions can occur during processing and storage (Ahmed et al., 2012).

As described in Chapter 4, the total anthocyanin content (TMA) of the heat treated PJW, PJCA, PJG, PJM and NPJ juices varied as follows: 0.144, 0.052, 0.053, 0.051 and 0.038 mg

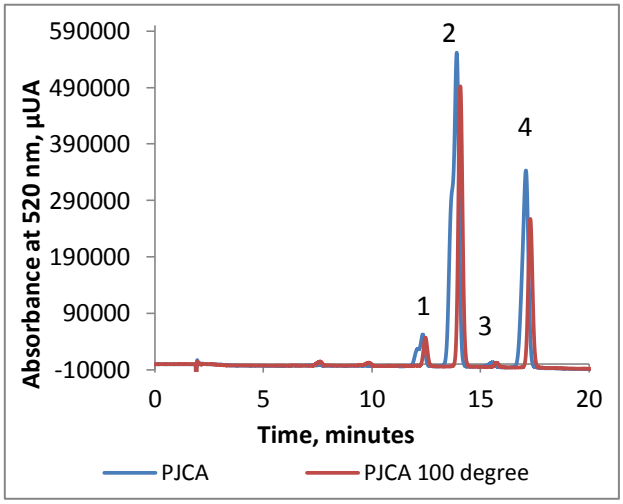
C3G/g, respectively. Thermal treatment of the PJCA juice in the temperature range 50-90°C, with a treatment time of 45 minutes, showed an increase of the TMA concentration. Thermal treatment had an impact on the anthocyanin content, as evidenced by a decrease of 85%, 53%, 61%, 50% and 86%, respectively, after 60 minutes of thermal treatment at 120°C. It has been shown that citric acid, glucose and the combination of these two have a stabilizing effect on anthocyanins during heat treatment, which is consistent with previous studies (Hubbermann et al., 2006, Kopjar et al., 2009).

The chromatographic analysis of the plum simulated juices (figure 6.4.) performed at 520 nm revealed the presence of four peaks corresponding to the next compounds: cyanidin 3-xyloside/cyanidin 3-glucoside (peak 1), cyanidin 3-rutinoside (peak 2), peonidin 3-glucoside (peak 3) and peonidin 3-rutinoside (peak 4). Regardless of the heat treatment, for each studied type of juice, the content of anthocyanins is presented in table 6.1.

The presence of eight anthocyanins in the untreated natural plum juice, mainly cyanidin 3-xyloside/cyanidin 3-glucoside (C3G) (peak 1) containing 87.05 µg/g, cyanidin 3-rutinoside (C3R) (peak 3) with 16.36 µg/g and peonidin 3-rutinoside (P3R) (peak 4) with a content of 415.54 µg/g, whereas the compounds corresponding to peaks 5 to 8 could not be identified.



a)



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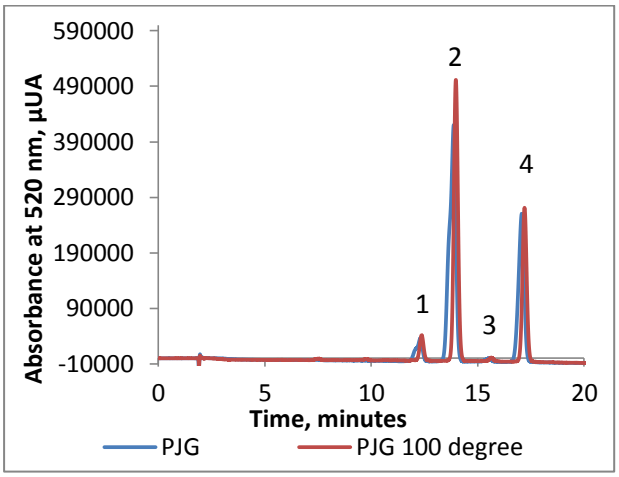

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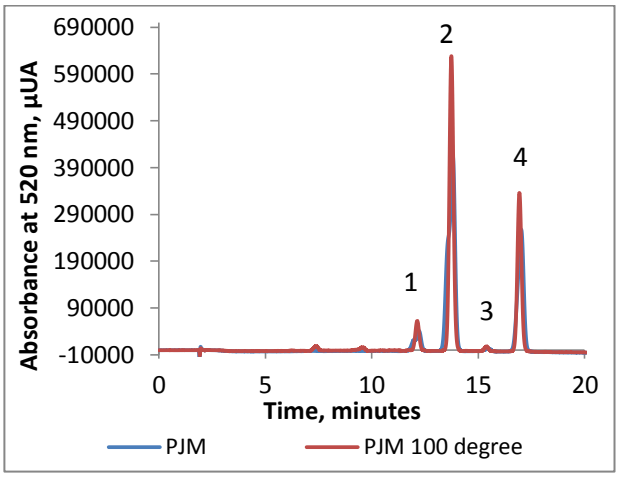

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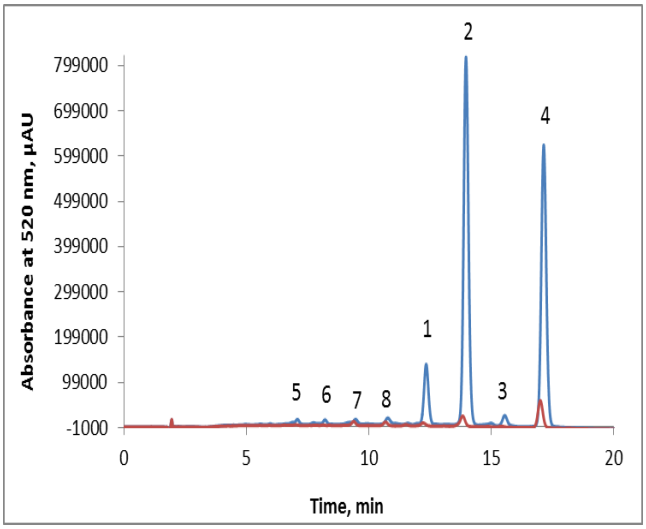

e)

Figure 6.4. Chromatographic separation (HPLC) of anthocyanins from plum juice (a) juice with added water, (b) juice with citric acid addition, (c) juice with sugars addition, (d) juice with a mixture based on the previous compounds, and (e) natural plum juice thermally treated (100°C, 20 minutes) and untreated at 520 nm. The peaks represent cyanidin 3-xyloside and cyanidin 3-glucoside (1); cyanidin 3-rutinoside (2); peonidin 3-glucoside (3) and peonidin 3-rutinoside (4).

It can be seen in table 6.1, that in all the studied plum juices, the predominant anthocyanin was cyanidin 3-rutinoside (C3R). After the thermal treatment at 100°C, for 20 minutes, the anthocyanin content decreased (table 6.1). The results highlighted an interesting fact that the C3G content increased by 8% in the case of the juice with sugars after the heat treatment at 100°C, while the concentration of the other two compounds decreased by 19-21%. A significant increase of the C3G and P3G content of about 44% and 162% occured in the juice with the mixture of compounds, while the content of C3R and P3R decreased by 5.72% and 4.33%, respectively. Thus, it can be concluded that the mixture of citric acid, glucose and fructose had the highest protective effect against the thermal degradation of anthocyanins.

Only four anthocyanins were identified in the natural plum juice after the heat treatment at 100°C, namely cyanidin 3-xyloside/cyanidin 3-glucoside (peak 1), cyanidin 3-rutinoside (peak 2) and peonidin 3-rutinoside (peak 4), whereas the compounds corresponding to peaks 3, 5, 6, 7 and 8 were totally degraded. In the case of cyanidin 3-xyloside/cyanidin 3-glucoside, cyanidin 3-rutinoside and peonidin 3-rutinoside, the rate of degradation was 91.67%, 96.52% and 91.22%, respectively.

Table 6.1. Total anthocyanin content from simulated plum juices PJW, PJCA, PJG, PJM and natural plum juice (NPJ) expressed in μg/mL

Juice	**PJW**		**PJCA**		**PJG**		**PJM**		**NPJ**	
Anthocyanin	**25^{0}C**	**100^{0}C**	**25^{0}C**	**100^{0}C**	**25^{0}C**	**100^{0}C**	**25^{0}C**	**100^{0}C**	**25^{0}C**	**100^{0}C**
C3G	37.89±2.87	28.17±1.78	32.30±1.25	27.02±1.78	21.47±1.99	23.16±1.54	24.62±1.41	35.40±1.24	87.05±4.25	7.25±0.6
C3R	434.61±11.54	336.42±15.98	559.55±52.21	309.08±11.78	403.36±14.32	316.47±17.56	421.07±16.27	396.99±5.01	525.89±13.06	18.29±1.11
P3G	Nd	Nd	0.93±0.12	0.65±0.10	Nd	Nd	1.58±0.45	4.16±1.01	16.36±0.8	Nd
P3R	236.78±14.54	183.36±10.24	300.71±11.24	166.22±2.36	217.21±10.87	174.65±9.47	224.87±11.47	215.12±14.21	415.54±10.52	36.47±0.98

C3G - cyanidin 3-xyloside/ cyanidin 3-glucoside; C3R - cyanidin 3-rutinoside; P3G - peonidin 3- glucoside; P3R - peonidin 3-rutinoside.
Nd – not determined

The values for the antioxidant activity of the five untreated juices were as follows: 66.07%, 25.00%, 30.31%, 26.34% and 52.57% for the PJW, PJCA, PJG, PJM and NPJ, respectively. Thermal treatment led to an increase of the inhibition value up to 83.14%, 39.70% and 45.02%, after a heat treatment of 15 minutes, at 50°C. The increase of the antioxidant activity may be due to the degradation of anthocyanins into floroglucinaldehyde and protocatehuic acid, the latter presenting the highest antioxidant activity (Sadilova et al., 2007). At high temperatures, the heat treatment induced a decrease of the concentration of anthocyanins, which had a negative impact on the antioxidant activity. However, at a high temperature, a decrease of 8% in the case of the PJW coded juice and 94.38% in the case of PJM coded juice was recorded after 60 minutes of thermal treatment, at 120°C. A protective effect of the food matrices was observed for the NPJ juice, in which case the DPPH RSA reduction was only 24%.

6.3.1.3. Thermal degradation kinetic of bioactive compounds from plum derived matrices

After the heat treatment, in the studied temperature range (70-110°C), after 5 minutes of treatment, a significant loss of the phenolic compounds was observed. Therefore, between 70-90°C, a decrease of the total polyphenolic compounds (TPF) was observed somewhere around 4-23%, while between 100-110°C the degradation was achieved around 43 - 72% (figure 6.5.a). By increasing the time of the thermal treatment at 20 minutes, the TPF content decreased by 29-41% in the temperature range of 70-90°C and by 48-77% in the temperature range of 100 - 110°C, respectively, compared to the untreated extract. The results suggested that the thermal treatment facilitated the solubilization of the polyphenolic compounds, which led to the TPF content reduction.

The results obtained showed that, after 5 minutes of thermal treatment, the degradation process starts and intensifies rapidly, with a rapid decrease of the TMA content from 47% at 70°C to 91% at 110°C, after 20 minutes of treatment (figure 6.5.b).

TF content showed a similar trend across the studied temperature range. Thus, a reduction of 36%, 46%, 59%, 64% and 67% was assessed as a result of the heat treatment in the temperature range 70-110°C, after 15 minutes of treatment. The degradation process continued for up to 20 minutes of treatment, totaling a loss between 43% and 71% of the original TF content (Figure 6.5.c).

The effect of the heat treatment on the antioxidant activity of plum skins extract is shown in Figure 6.5 (d). As it can be seen, the heat treatment influenced the antioxidant activity. Similar to the phytochemical content, in the temperature range of 70-90°C, after 5 minutes of thermal treatment, a decrease of 3% to 12% of the antiradical activity was also observed.

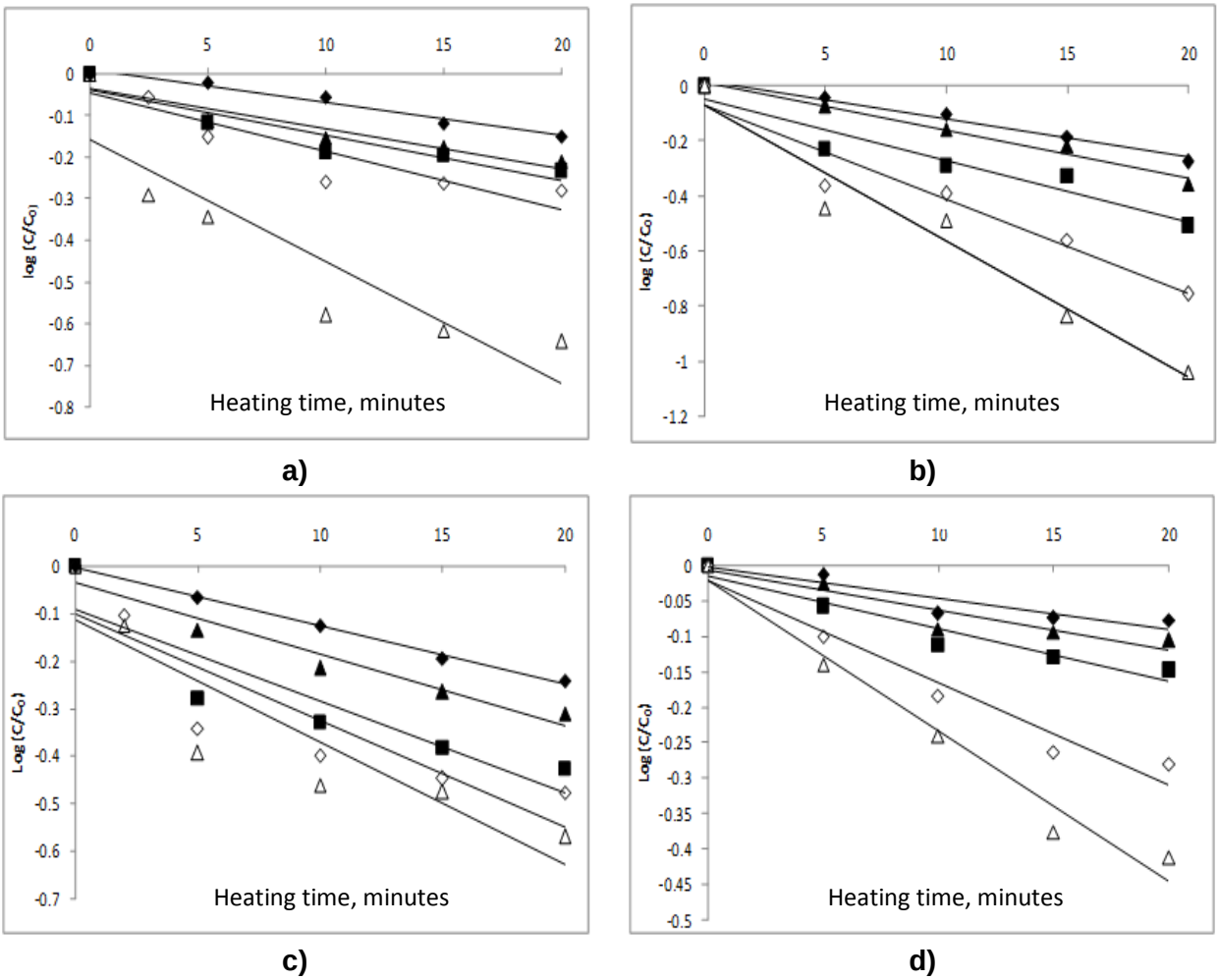

Figure 6.5. The influence of heat treatment on the stability of bioactive compounds (TPF-a; TMA-b; TF-c) and antioxidant activity (d) in the plum skins extract at different temperatures (◆ 70°C, ▲80°C, ■ 90°C, ◇100°C and △110°C) (Turturică et al., 2016a)

By linear regression analysis, it was confirmed that the thermal degradation of the biologically active compounds from the plum skin extract followed a first order kinetic model.

The results suggested that TF exhibited the highest thermal stability. Given the obtained *k* values, it could be appreciated that monomeric anthocyanins degraded at the highest degree of rapidity due to the oxidation process, the breakage of covalent bonds or the intensification of the oxidation reactions correlated to thermal processing (Zhang et al., 2012).

After the thermal treatment at 70°C, the TPF, TMA, TF and the antioxidant activity levels were 99.02 ± 5.60, 53.31 ± 4.30, 57.76 ± 3.90 and 173, 28 ± 11.23 minutes, respectively. The data from table 6.2. revealed that the degradation of polyphenolic compounds occured at temperatures higher than 90°C, and also that anthocyanins were more susceptible to thermal degradation compared to the flavonoidic compounds, while the highest decrease of the $t_{1/2}$ had been observed in the case of the antioxidant activity.

Table 6.2. The estimated kinetic parameters (degradation constant – *k*, the energy of activation - E_a and half time – $t_{1/2}$) of the phytochemical compounds following the thermal treatment of the plum skin extract (Turturică et al., 2016a)

Parameter	Temperature°C	*k* (min^{-1})	R^2	$t_{1/2}$	*E*a (kJ/mol)	R^2
TPF	70	0.007 ± 0.001[a]	0.97	99.02 ± 5.60	35.50 ± 7.77	0.88
	80	0.009 ± 0.001	0.90	77.01 ± 3.46		
	90	0.01 ± 0.005	0.88	69.31 ± 4.87		
	100	0.014 ± 0.003	0.96	49.51 ± 2.65		
	110	0.029 ± 0.007	0.98	23.90 ± 3.23		
TMA	70	0.013 ± 0.003	0.98	53.31 ± 4.30	36.42 ± 2.89	0.98
	80	0.017 ± 0.001	0.98	40.77 ± 4.50		
	90	0.022 ± 0.007	0.93	31.50 ± 3.67		
	100	0.034 ± 0.006	0.94	20.38 ± 2.56		
	110	0.049 ± 0.008	0.96	14.14 ± 2.34		
TF	70	0.012 ± 0.001	0.99	57.76 ± 3.90	17.99 ± 1.92	0.97
	80	0.015 ±0.002	0.95	46.20 ± 4.51		
	90	0.019 ±0.004	0.81	36.48 ± 3.49		
	100	0.022 ±0.008	0.80	33.00 ± 3.45		
	110	0.025 ±0.006	0.80	30.12 ± 2.23		
Antioxidant activity	70	0.004 ± 0.002	0.85	173.28 ± 11.23	47.22 ± 5.78	0.96
	80	0.005 ± 0.002	0.88	138.62 ± 10.97		
	90	0.007 ± 0.001	0.93	99.02 ± 7.89		
	100	0.014 ± 0.002	0.96	49.51 ± 6.78		
	110	0.021 ± 0.001	0.97	33.00 ± 2.56		

[a] Standard error

The thermal degradation kinetics of TMA has also been studied in the case of the simulated plum juices (PJW, PJCA, PJG and PJM), that also corresponded to a first-order kinetic model (eq. 6.1) (figure 6.6). In the case of NPJ juice, the thermal degradation followed a fractional degradation kinetic model (eq. 6.6) (figure 6.7).

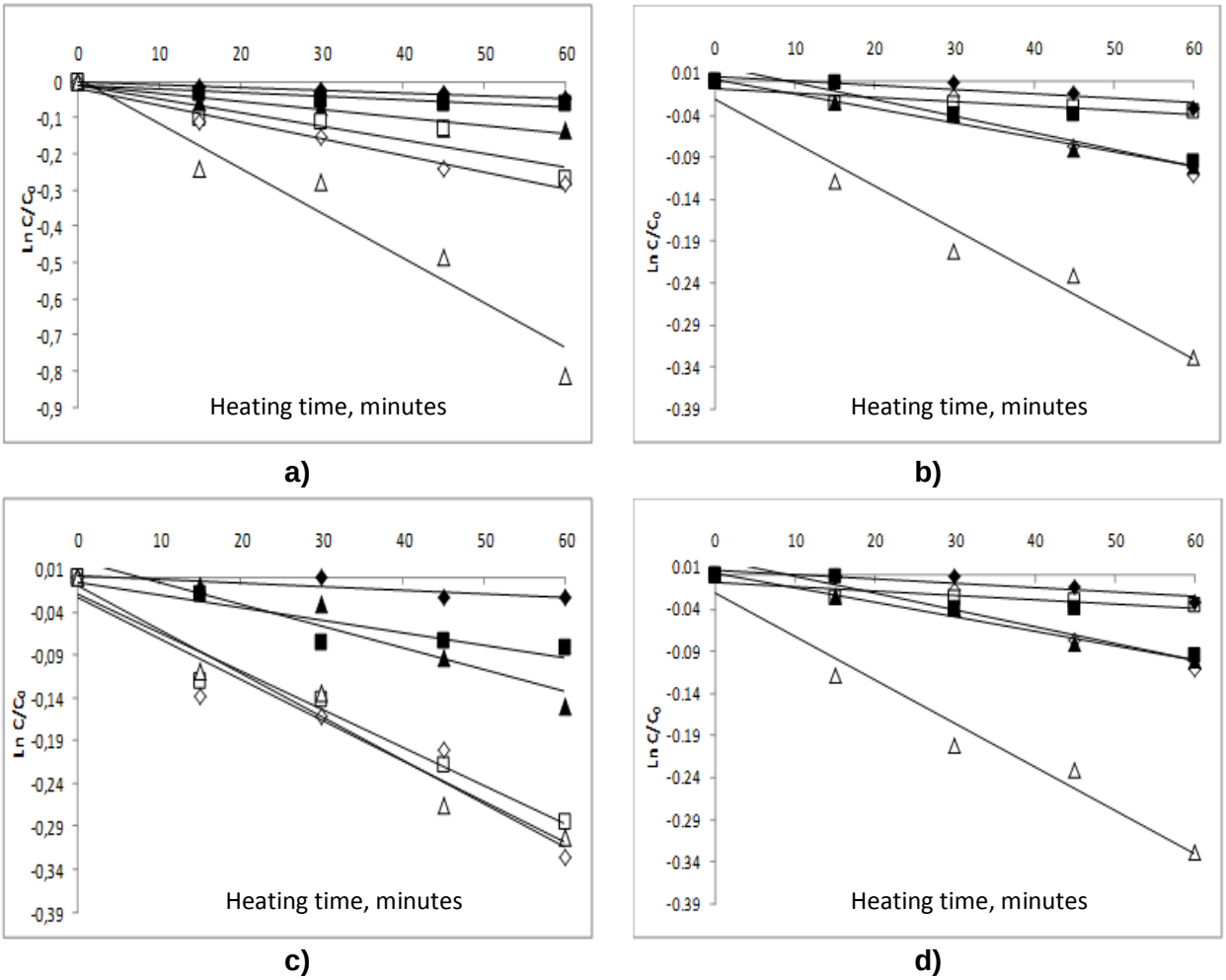

Figure 6.6. Isothermal degradation of anthocyanins from the PJW (a), PJCA (b), PJG (c) and PJM (d) juices at different temperatures ♦ 50 ºC, ■ 70 ºC, ▲ 90 ºC, □ 100 ºC, ◇110ºC and Δ 120°C

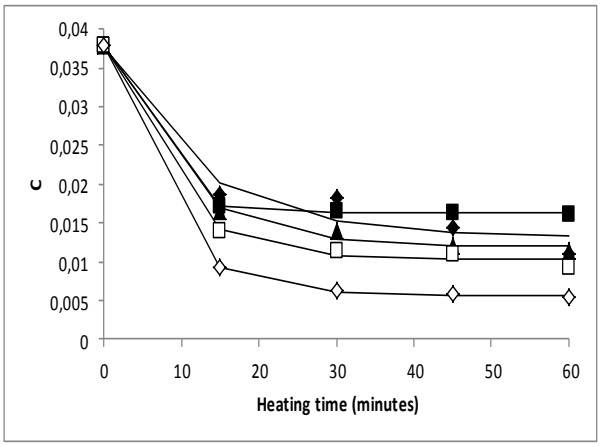

Figure 6.7. Isothermal degradation of anthocyanins from the NPJ juice at different temperatures ♦ 50 ºC, ■ 70 ºC, ▲ 90 ºC, □ 100 ºC and ◇110ºC. The lines represent the

predictive data that fits the experimental ones.

The kinetic parameters describing the thermal degradation of anthocyanins from the analysed plum juice are presented in table 6.3.

In the case of the NPJ juice, the use of the fractional conversion kinetic model allowed the prediction of the anthocyanin content and antioxidant activity after increasing the heat treatment at different temperatures (C_∞). Furthermore, as previously stated the final degree of the thermal degradation was temperature dependent (figure 6.9.).

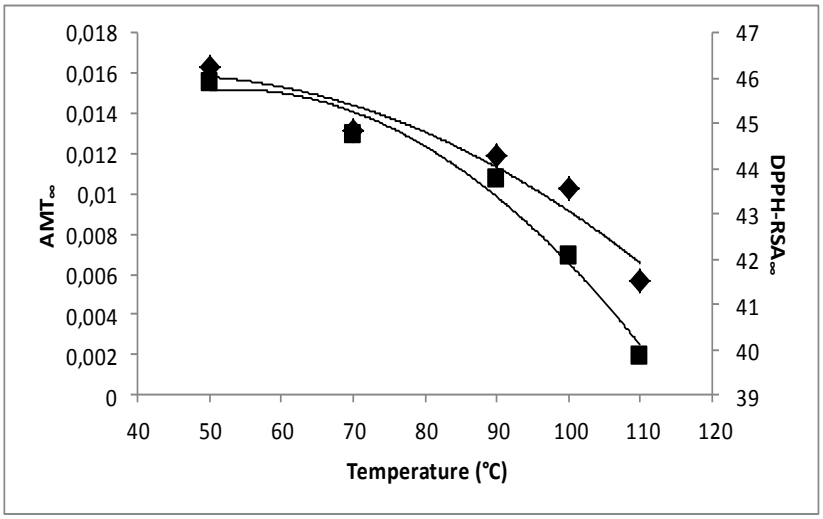

Figure 6.9. Correlations between TMA and DPPH after the prolongation of the thermal treatment time (TMA_∞) (in the NPJ juice)

The kinetic parameters estimated in this study for the simulated plums juice revealed an increased sensitivity to temperature of the anthocyanins present in the natural juice from plums. Since a high activation energy value indicates a greater sensitivity of the reaction rate depending on the temperature, the anthocyanins from the PJCA juice appeared to be less susceptible to degradation. This matter indicated the protection against the degradation of anthocyanins in the juice with citric acid.

Table 6.3. The estimated kinetic parameters (degradation rate constant – *k,* activation energy E_a and half time – $t_{1/2}$) that describe the thermal degradation of anthocyanins from simulated and natural plum juice

Juice	Temperature °C	$k \cdot 10^{-2}$ (min^{-1})	R^2	$t_{1/2}$ (h)	E_a (kJ/mol)	R^2
PJW	50	0.18 ± 0.01[a]	0.99	6.27 ± 1.15	42.40 ± 6.87	0.90
	70	0.23 ± 0.02	0.85	5.01 ± 0.57		
	90	0.52 ± 0.05	0.94	2.18 ± 0.23		
	100	0.87 ± 0.11	0.85	1.32 ± 0.10		
	110	1.08 ± 0.07	0.98	1.06 ± 0.16		
	120	2.87 ± 0.47	0.94	0.40 ± 0.02		
PJCA	50	0.04 ± 0.01	0.75	25.08 ± 1.28	40.70 ± 4.25	0.99
	70	0.11 ± 0.01	0.97	10.03 ± 0.98		
	90	0.23 ± 0.02	0.87	5.01 ± 0.57		
	100	0.34 ± 0.05	0.76	3.34 ± 1.04		
	110	0.46 ± 0.04	0.89	2.50 ± 0.69		
	120	1.17 ± 0.11	0.9	0.98 ± 0.15		
PJG	50	0.36 ± 0.01[a]	0.93	3.13 ± 0.15	23.03 ± 3.53	0.91
	70	0.46 ± 0.08	0.92	2.50 ± 0.15		
	90	0.59 ± 0.11	0.85	1.92 ± 0.10		
	100	1.05 ± 0.12	0.96	1.09 ± 0.09		
	110	1.35 ± 0.17	0.93	0.85 ± 0.06		
	120	1.54 ± 0.12	0.88	0.74 ± 0.09		
PJM	50	0.11 ± 0.08[a]	0.74	10.03 ± 0.38	35.99 ± 3.60	0.96
	70	0.34 ± 0.07	0.83	3.34 ± 0.16		
	90	0.57 ± 0.05	0.91	2.00 ± 0.23		
	100	1.03 ± 0.12	0.96	1.11 ± 0.09		
	110	1.10 ± 0.14	0.93	1.04 ± 0.08		
	120	1.17 ± 0.25	0.96	0.98 ± 0.04		
NPJ	50	8.21 ± 3.49	0.96	0.14 ± 0.003	14.19 ± 2.39	0.92
	70	10.93 ± 1.69	0.99	0.10 ± 0.006		
	90	13.04 ± 1.39	0.99	0.08 ±0.001		
	100	14.64 ± 2.09	0.99	0.07 ±0.005		
	110	20.92 ± 0.50	0.99	0.05 ± 0.002		

[a] Standard deviation

6.3.2. Chemical and kinetic stability of cherry derived matrices

6.3.2.1. The influence of the thermal treatment on the spectral properties of biologically active compounds in cherry skins

Similarly, in order to study the structural properties of polyphenolic compounds from cherry skins, combined studies were carried out which focused on the influence of thermal treatment upon the spectral properties and biologically active compounds concentration on a kinetic basis.

Firstly, the studies focused on assessing the fluorescence properties of the cherry skins extract and assessing the effect of the heat treatment at different temperatures, for 30 minutes (figure 6.10).

From figure 6.10. it can be seen that the cherry skins extract had well-defined spectra with a maximum value at the wavelength of 356 nm. These bands suggested the presence of no more than two fluorescent molecular species.

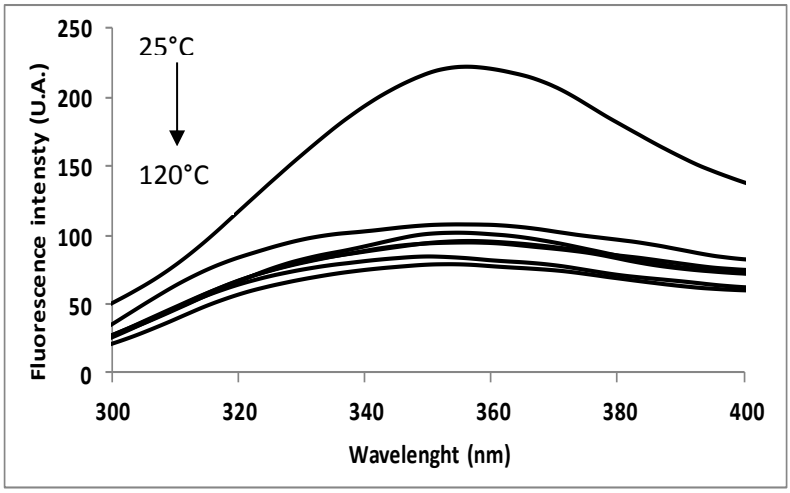

Figure 6.10. Fluorescence spectra of anthocyanins from cherry skins extract at different temperatures (excitation at λ = 250 nm, emission λ = 270 nm - 600 nm) (Turturică et al., 2016b)

Figure 6.10. illustrates the fluorescence spectra of the thermally treated cherry skins extract in the range 25 - 120^{0}C obtained after the excitation at wavelength λ = 250 nm. The thermal treatment of cherry skins extract (figure 6.10) revealed structural changes that led to a decrease of the fluorescence intensity. The maximum fluorescence intensity (λ_{max}) ranged from 356 nm at 25°C to 353 nm at 70°C. The rise of the temperature led to a red-shift at 90°C and 100°C (λ_{max} = 356 nm), followed by a 6 nm blue shift at 110°C (λ = 350 nm) and a red shift of 4 nm at 120°C. The λ_{max} variations indicated the structural changes of anthocyanins induced by thermal treatment.

The second step consisted in assessing the fluorescence properties of the natural cherry juice and the effect of the heat treatment at different temperatures, for 30 minutes (figure 6.11).

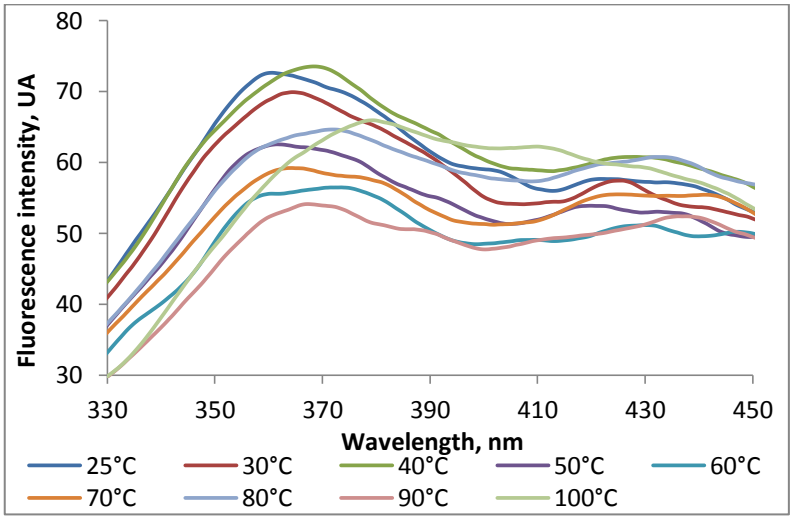

Figure 6.11. The fluorescence spectrum of natural cherry juice at various temperatures after an excitation at λ = 250 nm, with the emission being collected between λ = 270 nm - 600 nm

From figure 6.11. it can be seen that the natural cherry juice presented well-defined spectra with two maximum values, one at 368.5 nm, with a higher intensity, and the second one at 428 nm, with a lower intensity. The two peaks suggested the presence of at least two fluorescent molecular species.

6.3.2.2. Chemical and kinetic stability of biologically active compounds from cherries

The changes induced by the heat treatment by increasing the treatment time up to 60 minutes showed a reduction of the TMA content by 46% in the 70-90°C range and 47% to 63%, respectively, in the 100-120°C range, results that were compared to the untreated thermal extract (figure 6.14. b).

The changes induced by the heat treatment in the case of TF content in the cherry skin extract are shown in figure 6.14 (c). The values obtained for the TF illustrated a quantitative decrease that ranged from 39% to 48%, after a thermal treatment of 60 minutes in the temperature range of 50-120°C. In the temperature range 50-100°C there was a decrease of 22-27% concerning the antioxidant activity (DPPH RSA), after a thermal treatment of 15 minutes (figure 6.14. d). The degradation rate increased with the increase of the heat treatment period. This may be due to the loss or degradation of certain types of phenolic compounds or other compounds that are responsible for the antioxidant activity during the heat treatment.

Thermal treatment induced a decrease of the TPF content throughout the studied temperature range as follows: starting from 28% after 5 minutes of treatment at 50°C and up to 56% after 60 minutes at the temperature of 120°C (figure 6.14.a).

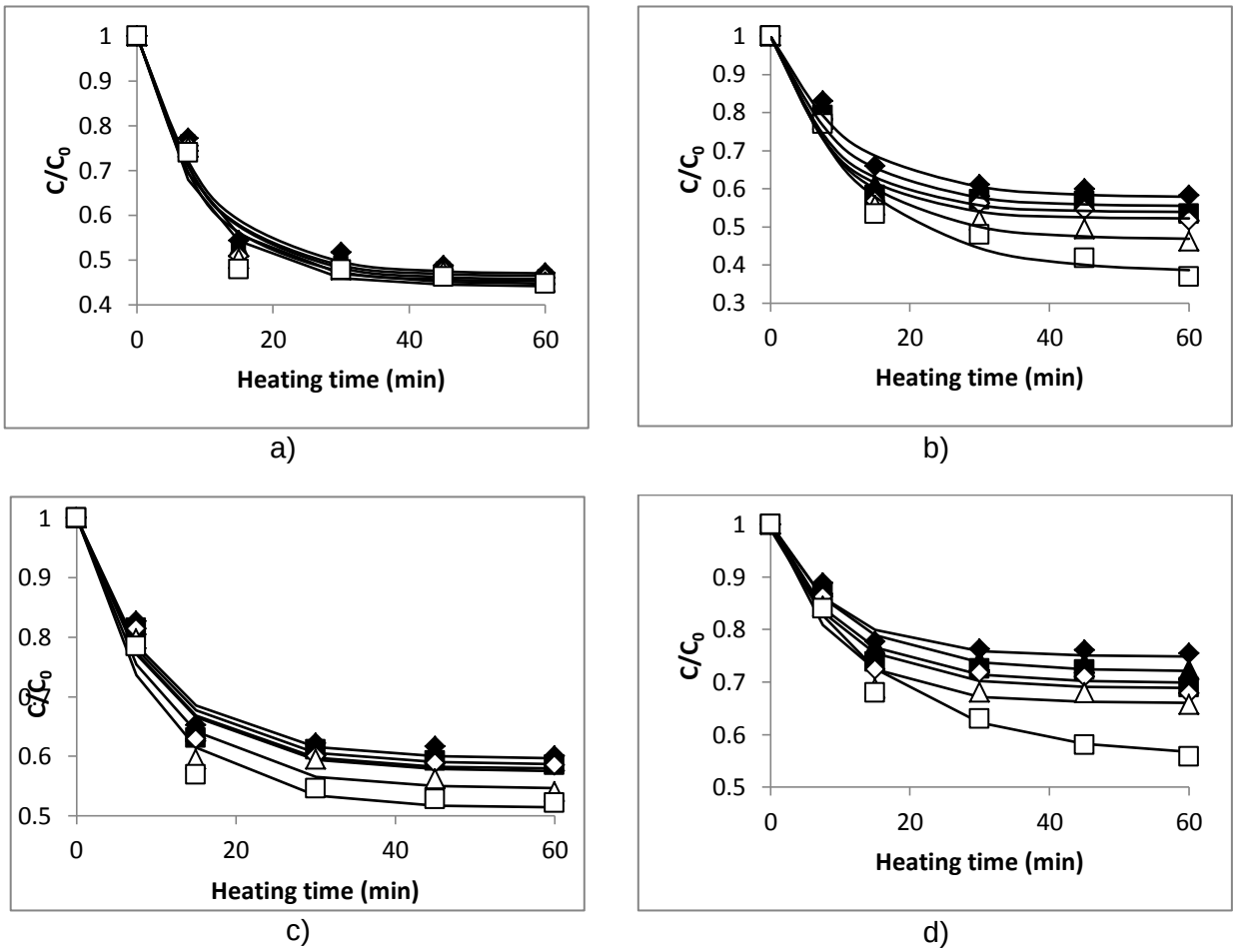

Figure 6.14. Isothermal degradation of TPF (a), TMA (b), TF (c) și DPPH RSC (d) from the cherry skins extract, treated at different temperatures (◆ 70°C, ▲80°C, ■ 90°C, ◇100°C and △110°C) (Turturică et al., 2016b)

The structural changes induced by heat treatment on TPF, TMA, TF, and DDPH RSA were described in accordance to the thermal degradation rate (min^{-1}) and degradation energy (E_a). For the TMA, the *k* value was 1.4 times higher at 120°C, suggesting a low thermostability of anthocyanins at high temperatures. The TF degradation constants did not undergo any significant change in the studied temperature range. The values of *k* corresponding to the DPPH RSA increased with the increasing temperature.

Thus, on the basis of the *k* values, it can be stated that the most thermolabile compounds in the Romanian cherry skins extract are anthocyanins, while the most thermostable are the flavonoids. Thermal treatment led to a decrease of both the anthocyanins content and the polyphenolic compounds content, hence having a negative impact on the antioxidant activity. The process of monomeric anthocyanins degradation happens because of the oxidation process, covalent bonds breakage or the acceleration of the oxidation reactions during the thermal processing.

As it can be seen from table 6.6, the prolongation of the thermal treatment at different temperatures (C_∞) determined the variation of the phytochemical content and antioxidant capacity, both being temperature dependent.

Table 6.6. The estimated kinetic parameters (degradation rate constant – *k,* activation energy E_a and half time – $t_{1/2}$) that describe the thermal degradation of bioactive compounds and the antioxidant activity from cherry skins extract (Turturică et al., 2016b)

Compound	Temperature °C	$k \cdot 10^{-2}$ (min^{-1})	$t_{1/2}$	C_∞	Ea (kJ/mol)	R^2
TPF	50	9.86 ± 2.12[a]	7.02 ± 0.63	0.94 ± 0.05	1.95 ± 0.39	0.86
	70	10.14 ± 2.27	6.83 ± 0.97	0.93 ± 0.05		
	90	10.27 ± 2.17	6.74 ± 1.02	0.92 ± 0.05		
	100	10.74 ± 2.48	6.45 ± 0.63	0.91 ± 0.06		
	110	10.76 ± 2.52	6.44 ± 0.46	0.90 ± 0.06		
	120	11.41 ± 2.92	6.07 ± 0.53	0.89 ± 0.06		
TMA	50	7.56 ± 1.64	9.16 ± 1.23	0.10 ± 0.003	5.35 ± 0.58	0.95
	70	9.00 ± 2.58	7.70 ± 1.09	0.10 ± 0.005		
	90	9.26 ± 2.68	7.48 ± 0.98	0.09 ± 0.004		
	100	9.89 ± 2.64	7.00 ± 0.75	0.09 ± 0.005		
	110	10.80 ± 1.95	6.41 ± 0.58	0.08 ± 0.005		
	120	10.87 ± 1.57	6.37 ± 1.37	0.06 ± 0.006		
TF	50	10.02 ± 2.12	6.91 ± 1.97	1.52 ± 0.05	0.62 ± 0.06	0.96
	70	10.10 ± 2.16	6.86 ± 0.88	1.49 ± 0.05		
	90	10.20 ± 2.25	6.79 ± 0.79	1.47 ± 0.05		
	100	10.29 ± 2.21	6.73 ± 1.04	1.46 ± 0.05		
	110	10.37 ± 2.57	6.68 ± 0.94	1.39 ± 0.06		
	120	10.44 ± 2.31	6.63 ± 0.73	1.30 ± 0.06		
DPPH RSA	50	6.31 ± 2.42	10.98 ± 1.23	63.58 ± 1.15	8.56 ± 1.42	0.90
	70	9.27 ± 1.76	7.47 ± 0.71	61.13 ± 1.14		
	90	9.98 ± 2.46	6.94 ± 0.65	59.34 ± 1.54		
	100	10.29 ± 2.63	6.73 ± 0.57	58.45 ± 1.64		
	110	10.65 ± 2.83	6.50 ± 0.43	56.04 ± 1.73		
	120	12.13 ± 2.84	5.71 ± 0.56	47.36 ± 4.90		

[a] Standard deviation

The polyphenolic content after prolonging the treatment time was correlated to the antioxidant activity values (figure 6.15).

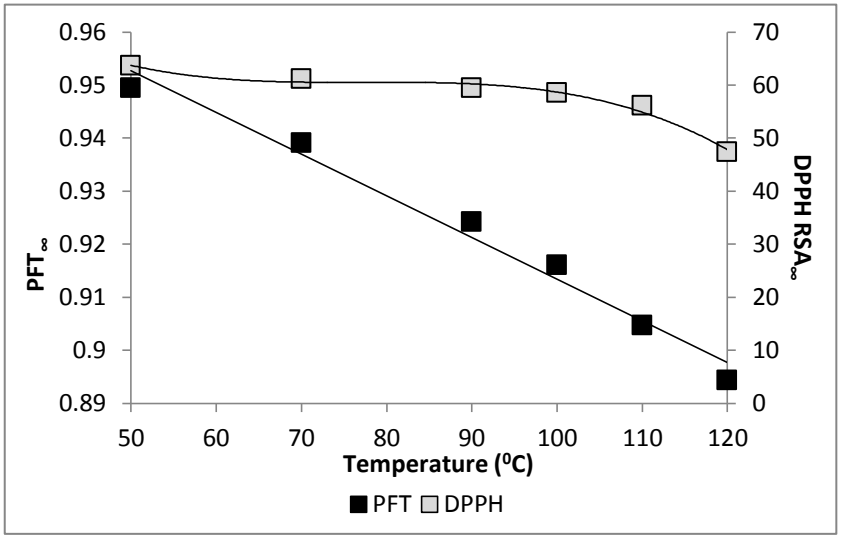

Figure 6.15. Correlations between TPF after the prolonged thermal treatment (TPF_{∞}) and the corresponding antioxidant activity ($DPPH\ RSA_{\infty}$) (Turturică et al., 2016b)

The obtained values are much lower compared to those reported by the literature, which demonstrates a thermal stability of the phytochemical compounds during the thermal processing of the cherry skins extracts.

After studying the thermal degradation kinetic behavior of Romanian cherry skins extracts, it was observed that the parameters indicated an increased thermal sensitivity in the case of the monomeric anthocyanin content and the antioxidant activity compared to the total polyphenols and total flavonoids contents.

In figure 6.16., the degradation curves, at temperatures between 50 -120^{0}C, for the total monomeric anthocyanins content from the natural cherry juice are presented. A rapid degradation process could be observed that followed a first-order kinetic model.

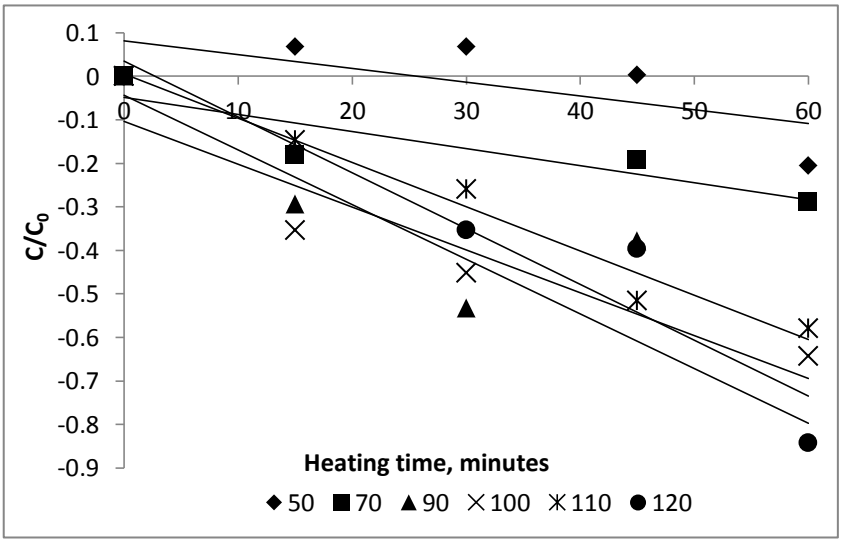

Figure 6.16. Thermal degradation kinetics of the natural cherry juice at different temperatures (C is the concentration of the juice at t time, C_0 is the initial concentration of the juice)

The chromatographic analysis of the untreated natural cherry juice (figure 6.17) revealed the presence of six peaks corresponding to seven anthocyanins: cyanidin 3-xyloside/cyanidin 3-glucoside (peak 1 - 0.393 mg/mL), cyanidin 3-rutinoside (peak 2 - 0.124 mg/mL), peonidin 3-glucoside (peak 3 - 0.009 mg/mL), peonidin 3-rutinoside (peak 4 - (not determined - 0.006 mg/mL) and peak 6 (not determined - trace). The chromatogram of the thermally treated cherry juice (figure 6.17) revealed the presence of six anthocyanins: cyanidin 3-rutinoside (peak 2 - 0.527 mg/mL), peonidin 3-rutinoside (peak 4 - 0.565 mg/mL), peak 5 (not determined - 0.0004 mg/mL) while the other three were traces.

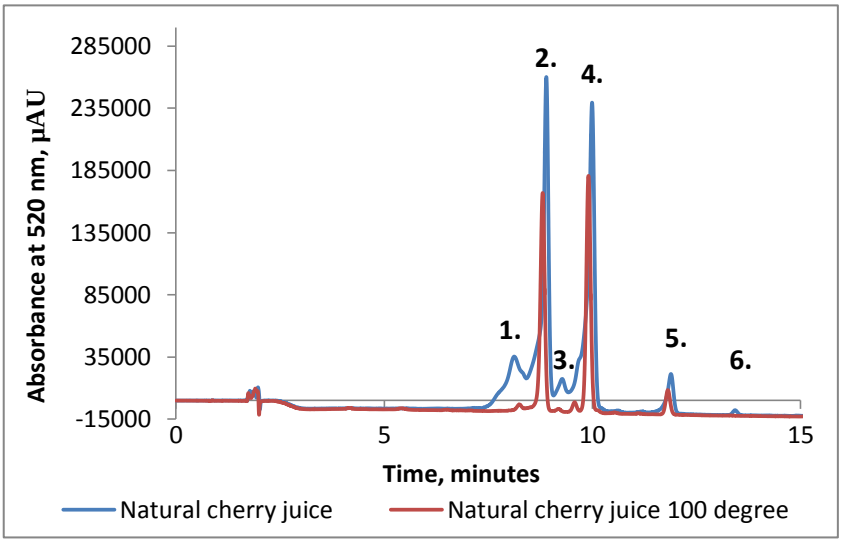

Figure 6.17. Chromatographic separation (520 nm) of anthocyanins from the treated and untreated natural cherry juice. The peaks correspond to (1) cyanidin 3-xyloside and cyanidin 3-glucoside; (2) cyanidin 3-rutinoside; (3) peonidin 3-glucoside, (4) peonidin 3-rutinoside, (5) and (6) undetermined

Following the heat treatment applied to the natural cherry juice, the obtained results for the total polyphenolic content could not be modeled on any mathematical model. As it can be seen in table 6.7., the total polyphenolic content had an increase in the studied temperature range.

Table 6.7. Quantitative variation of total polyphenols (mg GA/mL) from the natural cherry juice (NCJ) during the heat treatment

T/t	0 min	15 min	30 min	45 min	60 min
25°C	0.94±0.021	0.94±0.021	0.94±0.021	0.94±0.021	0.94±0.021
50°C	0.94±0.021	1.28±0.006	1.26±0.097	1.21±0.032	1.18±0.058
70°C	0.94±0.021	1.27±0.091	1.21±0.078	1.19±0.065	1.1±0.052
90°C	0.94±0.021	1.22±0.019	1.2±0.071	1.18±0.013	1.09±0.039
100°C	0.94±0.021	1.21±0.084	1.15±0.006	1.11±0.039	1.08±0.052
110°C	0.94±0.021	1.18±0.058	1.12±0.039	1.11±0.006	1.06±0.071
120°C	0.94±0.021	1.12±0.032	1.1±0.019	1.07±0.019	1.04±0.006

The same behavior could also be observed in the case of flavonoids from natural cherry juice after the heat treatment. The behavior of these compounds did not fit into any kinetic model since an increase in the range of 50-70°C has been observed. In contrast, the thermal

treatment at temperatures above 90°C produced a decrease of the flavonoidic content (Table 6.8).

Table 6.8. Quantitative variation of flavonoids (mg CE/mL) from the natural cherry juice (NCJ) during heat treatment

T/t	0 min	15 min	30 min	45 min	60 min
25°C	0.63±0.080	0.63±0.080	0.63±0.080	0.63±0.08	0.63±0.080
50°C	0.63±0.080	0.68±0.004	0.66±0.023	0.65±0.03	0.60±0.061
70°C	0.63±0.080	0.68±0.011	0.65±0.011	0.61±0.015	0.59±0.019
90°C	0.63±0.080	0.61±0.004	0.57±0.084	0.56±0.069	0.48±0.034
100°C	0.63±0.080	0.53±0.034	0.51±0.072	0.48±0.110	0.42±0.100
110°C	0.63±0.080	0.52±0.038	0.51±0.091	0.46±0.011	0.41±0.023
120°C	0.63±0.080	0.51±0.046	0.47±0.004	0.44±0.061	0.39±0.053

In figure 6.18., the thermal degradation curves of the natural cherry juice antioxidant activity at different temperatures are presented.

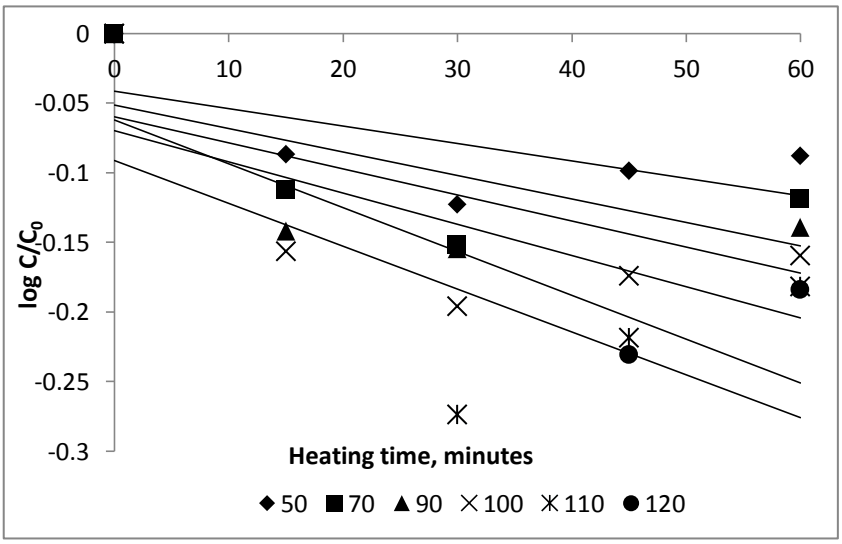

Figure 6.18. Kinetic thermal degradation of the cherry natural juice antioxidant activity at different temperatures (C is the concentration of the juice at t time while C_0 is the initial concentration of the juice)

According to the first order kinetic model, the antioxidant activity (DPPH RSA) rate of degradation (*k*) showed higher values at 120°C, compared to the values recorded at the temperature of 50°C. The calculated activation energy presented low values compared to similar data from the literature. This value indicated an increased thermostability in the antioxidant activity.

6.4. Partial conclusions

1. The study aimed the investigation of the temperature stability of biologically active compounds from the simulated plum juice and natural juice using fluorescence spectroscopy, chromatographic analysis, and the modeling of thermal degradation kinetics.

2. Regardless of the complexity of the studied matrix, the thermal treatment induced significant changes in the fluorescence intensity, hence indicating the degradation of these biologically active compounds.
3. After the heat treatment at 100°C, for 20 minutes, the anthocyanin content decreased as follows: in the juice with the addition of water, the anthocyanin content decreased by approximately 22-25%, while for the juice with the citric acid addition, the concentration of cyanidin 3-glucoside decreased by 17% compared to the other three anthocyanins, which presented a degradation degree more than 45%.
4. In the plum natural juice after the heat treatment at 100°C, for 20 minutes, the presence of 4 anthocyanins was revealed: cyanidin 3-xyloside/cyanidin-3-glucoside, cyanidin 3-rutinoside and peonidin 3-rutinoside. In the case of cyanidin 3-xyloside/cyanidin 3-glucoside, cyanidin 3-rutinoside and peonidin 3-rutinoside, the degree of degradation was 91.67%, 96.52% and 91.22%, respectively.
5. The antioxidant activity value of the tested juices (juice with added water, juice with citric acid, juice with sugars), that were thermally treated at 50°C for 15 minutes, was as follows: 83.14%, 39.70% and 45.02%. The increase of the antioxidant activity may be due to the degradation of anthocyanins into floroglucinaldehyde and protocatehuic acid, the latter presenting the highest antioxidant activity.
6. After 5 minutes of thermal treatment, the TMA degradation process began and intensified promptly with a rapid drop to 47% at 70°C and 91% at 110°C, respectively, after a thermal treatment for 20 minutes.
7. The half-lives required to degrade 50% of the AMT content at temperatures of 70°C, 80°C, 90°C, 100°C and 110°C were 21.31 minutes, 17.87 minutes, 14.53 minutes, 12.13 minutes and 11.00 minutes, respectively.
8. From the kinetic point of view, after the heat treatment, the plum extract anthocyanins followed a first order kinetic model.
9. The estimated activation energy for the plum skin extract was E_a = 47.22 ± 5.78 kJ/mol, indicating that the antioxidant activity exhibited the highest temperature dependence. For the bioactive compounds, the calculated E_a values were: 36.42 ± 2.89 kJ/mol for anthocyanins, 35.50 ± 7.77 kJ/mol for total polyphenols and 17.99 ± 1.98 kJ/mol, for flavonoids.
10. Thermal degradation of anthocyanins from plum juices, both simulated and natural, has been described by a first order kinetic model for the juices with different added components and a fractional conversion kinetic model for the natural juice from plums.
11. The calculated kinetic parameter indicated an increased temperature sensitivity of the anthocyanins from the natural plum juice. The degradation of anthocyanins, in the presence of citric acid, is less sensitive to high temperature than the other juices, since the addition of citric acid has a protective effect on anthocyanins.
12. The stabilizing effect highlighted by the addition of sugars, after the heat treatment, was also evidenced by the chromatographic analysis. Thus, in the case of the juice with sugars, five anthocyanins could be identified: cyanidin 3-xyloside/cyanidin 3-glucoside (0.003 mg/mL), cyanidin 3-rutinoside (0.099 mg/mL), peonidin 3-glucoside (as a trace)

and peonidin 3-rutinoside (0.064 mg/mL), in the case of the juice with water addition, four anthocyanins were present, whereas in the case of the juice with citric acid, only two anthocyanins were assessed.

13. The thermal degradation of anthocyanins from cherry skin extract was modeled using the fractional conversion kinetic model.

14. In the case of the thermally treated cherry extract, it was observed that the antioxidant activity exhibited the highest temperature dependence (E_a = 8.56 ± 1.42 kJ/mol), and the lowest temperature dependence was highlighted for the content of flavonoids (E_a = 0.62 ± 0.06 kJ/mol).

15. The effect of the heat treatment on the total anthocyanins content from the natural cherry juice was described by a first order kinetic model, whereas the values obtained for the degradation process of polyphenols and flavonoids did not correspond to any mathematical model.

16. The HPLC technique provided the means to identify that only three anthocyanins could be found as traces after the heat treatment while cyanidin 3-xyloside/cyanidin-3-glucoside was completely degraded.

17. As a conclusion, the thermal treatment in the temperature range between 70-90°C, for 30 minutes, can facilitate the biologically active compounds extraction from the cherry skins. Nonetheless, after a longer period of thermal treatment combined with high temperatures, an oppsite and destructive effect was observed upon these compounds.

18. According to the first-order kinetic model, the rate of degradation (k) of the natural cherry juice antioxidant activity (DPPH RSA) was 2.81 ± 0.019x10^{-2} min^{-1} at 120°C, compared to 0.37 ± 0.04x10^{-2} min^{-1} at 50°C, with an activating energy of E_a = 29.02 ± 3.43 kJ / mol.

19. The detailed and complete understanding of the action mechanism of anthocyanins and polyphenolic compounds from plant sources is still very difficult because of the different forms of anthocyanins and also of their behavior that depends on the applied heat treatment.

20. The obtained results can be considered as reference data for the future research that highligths the understanding of the structural changes of bioactive compounds during processing so that high quality products can be obtained.

General conclusions

The aims of the Ph.D. thesis were the study of polyphenolic compounds found in fruits (plums and cherries), from the chemical and biochemical characterization perspective, the stability in natural matrices and food systems, as well as the evaluation of their kinetic and molecular behavior under similar conditions as the industrial processing. Based on the obtained experimental results and the partial conclusions presented at the end of each chapter from the experimental part, a series of general conclusions are summarized as follows:

The Romanian fruit varieties, plums (*Prunus domestica* var. *Vanette*) and sweet cherries (*Prunus avium* var. *Giant of Bistriţa*) are very rich in phenolic compounds with an important physiological impact. It is important that the physiological activity of these compounds is also maintained after the separation from the natural matrix or after processing. Thus, knowing the bioactive potential and to preserve it during processing, is of particular importance for the quality of the commercial fruit products.

The extraction and the biochemical characterization of the bioactive phenolic compounds from the studied fruits demonstrated their potential to be used as natural resources with a great impact on the quality of life. With their high content of polyphenolic compounds (mainly flavonoids and anthocyanins) and a high antioxidant activity, local plums and sweet cherries can be considered as very nutritional fruits and they must be further promoted in this regard through publications, patents and derivative products that should have a greater impact on the market.

In order to demonstrate the stability of the bioactive compounds from plums skins and sweet cherry skins, in the natural matrix and in different simulated food systems, modern methods of investigation such as fluorescence spectroscopy and high performance liquid chromatography were used to monitor the structural changes. Moreover, it was demonstrated that a series of molecular transformations that involve a variety of processes such as deglycosylation and cleavage happen within the anthocyanins, thus highlighting the presence of several molecular species that are sensitive to pH and temperature variations.

The addition of certain compounds, such as citric acid, glucose and fructose can help to increase the stability of the biologically active compounds contents, especially monomeric anthocyanins. Thus, it was shown that the degradation of anthocyanins in the presence of citric acid is much slower, these compounds exerting a protective effect. The stabilizing effect induced by the addition of sugars can be explained by the water activity reduction, as evidenced by the literature, some studies reporting that water activity influences the stability of the anthocyanins.

Fruit processing can substantially influence the stability of bioactive compounds and the functional quality of the obtained products. Thus, the studied bioactive polyphenols, especially anthocyanins, were shown to have the highest stability in the envinronments/matrices with an acidic pH. At pH 4.0, these compounds exhibit the highest stability and can be found under all the four forms: flavylium cation, quinoidal base, chalcone and pseudobase. Thermal degradation of anthocyanins extracted from natural matrices and simulated systems follows both the first order kinetic model and the fractional conversion model, being correlated to the chemical composition of the environment in which the bioactive compound is found.

To analyze the structural changes that the bioactive compounds pass through under different pH and temperature conditions, a series of specific kinetic parameters were evaluated so that

two types of models were identified and validated. The values obtained for the activation energy shown that the anthocyanins have a high thermostability in the temperature range between 50 and 120°C. At the same time, thermal treatment at temperatures between 50-120°C can lead to an increase of the polyphenolic compounds and flavonoids extractability in the reaction medium. The thermal degradation process intensified with the increase of the temperature and the heat treatment period, so that the highest degradation degree was observed after 20 minutes of treatment, at 110°C.

The obtained data are important for fruit processors in order for them to establish the technological parameters that guarantee the quality of products as well as their nutritional and functional properties.

Although many specialists are currently studying the structural and functional behavior of bioactive compounds from plant sources, as a complex form or in combination with other natural compounds that have a wide variability depending on the species, genetic character, geographical area, etc. Furthermore, the elucidation of these aspects requires a thoroughgoing study mainly due to the natural matrices diversity but also due to the structural and compositional characteristics.

7. Original contributions and prospects for further research

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

1. The biologically active compounds composition and the antioxidant activity of two categories of local fruits, plums and cherries, which are of major importance for the health and quality of life, were analyzed. The originality derives from the fact that so far no such fruits varieties have been characterized in our country.

2. The spectrofluorimetrical analysis was used to evaluate the molecular behavior of polyphenolic compounds from Romanian varieties of plum and sweet cherry skins. The study of kinetic degradation parameters with regards to the structure-function-process correlation was undertaken in order to maintain the biochemical properties of the processed products. This type of technique, although until now was little used for this type of evaluations, offers many advantages such as speed, reproducibility and economic efficiency.

3. The effect of the processing conditions on the bioactive properties of phenolic compounds was assessed so that practical recommendations could be developed with reference to pH, temperature and maintenance time conditions that would not alter the bioactive potential of the phenolic compounds, mainly regarding the anthocyanins, in terms or their use in commercial fruit products.

4. From an applicative point of view, the obtained data presents a high scientific value that can contribute to the development of fundamental knowledge regarding the *in vitro* biochemistry of phenolic compounds so that to increase the functional *in vivo* value.

Into perspective, similar studies can be developed for other insufficiently studied plant resources, with major benefits for improving the life quality. Also, the kinetic modeling studies can be undertaken both in simulated environments and in food matrices with a simple or complex composition.

These studies will also contribute to increase the level of knowledge both in the field of biotechnology and in the field of food science and engineering, for processors and consumers alike, with major impact in the strategic research, development and innovation fields that are currently promoted in the Bioeconomy and Health domain.

8. Dissemination of research results

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

A. Articles published in ISI rated journals

1. **Turturică, Mihaela**, Stănciuc, Nicoleta, Râpeanu, Gabriela, 2017. *Thermal degradation of plum anthocyanins: comparasion of kinetics from simple to natural systems*. Submitted to Chemical Paper, CHPA-D-16-00290.
2. **Turturică, Mihaela**, Stănciuc, Nicoleta, Bahrim, Gabriela, Râpeanu, Gabriela, 2016. *Effect of thermal treatment on phenolic compounds from plum (Prunus domestica) extracts – A kinetic study*. Journal of Food Engineering, 171, 200-207. Impact factor: 3.199.
3. **Turturică, Mihaela**, Stănciuc, Nicoleta, Bahrim, Gabriela, Râpeanu, Gabriela, 2016. *Investigations on Sweet Cherry Phenolic Degradation During Thermal Treatment Based on Fluorescence Spectroscopy and Inactivation Kinetics*. Food and Bioprocess Technology, 9(10): 1706-1715. Impact factor: 2.574.

B. Articles published in international databases journals

1. **Turturică, Mihaela**, Oancea, Ana Maria, Râpeanu, Gabriela, Bahrim, Gabriela, 2015. *Anthocyanins: naturally occuring fruit pigments with functional properties*. The Annals Of The University Dunarea de Jos of Galati, Fascicle VI – Food Technology, 39(1), 9-24.

C. Papers communicated at international scientific events

1. **Turturică Mihaela**, Stănciuc Nicoleta, Bahrim Gabriela, Râpeanu Gabriela, 2016. *Thermal stability of anthocyanins from red plums (Prunus domestica)*, 18th IUFoST – ”World Congress of Food Science and Technology”, 21 - 25 August, Dublin, Irlanda.
2. **Turturică Mihaela**, Stănciuc Nicoleta, Bahrim Gabriela, Râpeanu Gabriela, 2016. *Characterisation and thermal degradation of anthocyanins from red plums*, 8th Congress Pigments in Food ”Coloured food for health benefits”, 28 Iunie – 1 Iulie, Cluj-Napoca, România.
3. **Turturică Mihaela**, Stănciuc Nicoleta, Bahrim Gabriela, Râpeanu Gabriela, 2016. *Thermal degradation kinetics of anthocyanins extracted from sweet cherries*, 8th Congress Pigments in Food ”Coloured food for health benefits”, 28 Iunie – 1 Iulie, Cluj-Napoca, România.
4. **Turturică Mihaela**, Stănciuc Nicoleta, Bahrim Gabriela, Râpeanu Gabriela, 2016. *Degradation of phenolic compounds from cherries during thermal treatment - a kinetic study*, European Biotechnology Congress, 5 – 7 mai, Riga, Latvia.

5. **Turturică Mihaela**, Cazacu Gabriela, Râpeanu Gabriela, Stănciuc Nicoleta, Aprodu Iuliana, Bahrim Gabriela, 2015. *Thermal degradation kinetics of polyphenols extracted from cherries*, International Symposium EuroAliment, 24-26 septembrie, Galați, Romania.
6. **Turturică Mihaela**, Râpeanu Gabriela, Stănciuc Nicoleta, Bahrim Gabriela, 2015. *Fluorescence spectroscopy investigation on pH and heat changes of cherries anthocyanin extracts*, "European Biotechnology Congress", 7 – 9 mai, București, România.
7. **Turturică Mihaela**, Bahrim Gabriela, Râpeanu Gabriela, Stănciuc Nicoleta, Aprodu Iuliana, 2014. *Effect of thermal treatment on phenolic compounds from plums*, 13th International Symposium Prospects for the 3rd Millennium Agriculture, 25-28 septembrie, Cluj Napoca, Romania.

D. Papers communicated at national scientific events

1. **Turturică Mihaela**, Bahrim Gabriela, Stănciuc Nicoleta, Râpeanu Gabriela, 2017. *The Analysis of Anthocyanins from Simulated Plum Juices*. Scientific Conference of Doctoral Schools from UDJ – Galaţi CSSD-UDJG, 2017, 8th-9th June, Galaţi, România.
2. **Turturică, Mihaela**, Râpeanu, Gabriela, Stănciuc, Nicoleta, Aprodu, Iuliana, Bahrim, Gabriela, 2016. *Kinetic and fluorescence spectroscopy investigations on heat induced changes of sweet cherries phenolic extracts*. Scientific Conference of Doctoral Schools from UDJ – Galaţi CSSD-UDJG, 2015, 2th-3th June, Galaţi, România.
3. **Turturică, Mihaela**, Oancea, Ana Maria, Bahrim, Gabriela, Râpeanu, Gabriela, Stănciuc, Nicoleta, Aprodu, Iuliana, 2014. *Evaluation of phenolic potential from regional fruits*. Scientific Conference of Doctoral Schools from UDJ – Galaţi CSSD-UDJG, 2014, 15th-16th May, Galaţi, România.