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ASSESSMENT OF THE EFFECTS OF TEMPERATURE AND THERMAL TREATMENT DURATION ON THE STABILITY OF PHENOLIC CONSTITUENTS IN SWEET EXTRACTS PRODUCED FROM RARA NEAGRĂ GRAPE MARC

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Abstract. Sweet red grape pomace represents a valuable source of anthocyanins, polyphenols, and flavonoids. Heating affects the content of biologically active compounds. However, well-controlled thermal processing can contribute to their preservation. The aim of this study was to provide a detailed presentation of the degradation of biologically active compounds during heating, as well as to describe their thermal behavior. The study was based on the preparation and investigation of hydroalcoholic extracts acidified with citric acid obtained from unfermented sweet red grape pomace of the Rara Neagră grape variety, harvest year 2025. Following thermal treatment within the studied temperature range (80–140°C, 7–30 min), the total phenolic content of the acidified Rara Neagră extracts decreased from 50.67 mg GAE/g DW to 17.98 mg GAE/g DW. The total anthocyanin content remained almost constant. In contrast, the experimental results highlighted a decrease in the content of monomeric anthocyanins. Analysis of the absorption spectra revealed the highest stability at 80–100°C and rapid degradation at 120–140°C. The stability observed at 80–100°C indicates that these compounds can withstand moderate food-processing operations (pasteurization and short-term thermal treatments). Therefore, the obtained results are of direct importance for the food industry and for the valorization of wine industry by-products.

Keywords: *grape pomace, Rara Neagră, thermal treatment, anthocyanins, polyphenols, antioxidant compounds, by-product valorization, food processing.*

Rezumat. Tescovina dulce de struguri roșii reprezintă o sursă valoroasă de antociani, polifenoli și flavonoide. Încălzirea influențează asupra conținutului de compuși biologic activi. Însă o procesare termică bine controlată poate contribui la menținerea lor. Scopul acestui studiu a fost de-a oferi o prezentare detaliată a degradării compușilor biologic activi în timpul încălzirii, precum și de a descrie comportamentul termic al acestora. Studiile s–au bazat pe prepararea și studierea extractelor hidro-alcoolice acidulate cu acid citric din tescovina nefermentată dulce de struguri roșii Rara Neagră, anul recoltei 2025. În urma tratamentului termic în intervalul de temperaturi studiat (80–140°C, timp 7–30 minute),

conținutul total de substanțe fenolice din extractele acidulate de Rara Neagră a scăzut de la 50,67 mg EAG/g s.u. până la 17,98 mg EAG/g s.u. Conținutul de antociani a rămas aproape constant. În schimb, rezultatele experimentale au evidențiat o diminuare a conținutului de antociani monomeri. În urma realizării spectrelor de absorbție s–a observat o stabilitate maxima la 80–100 °C și o degradare rapida la 120–140 °C. Stabilitatea la 80–100°C indică faptul că acești compuși pot rezista unor procese tehnologice alimentare moderate (pasteurizare, tratamente termice de scurtă durată). Prin urmare rezultatele obținute au o importanță directă pentru industria alimentară și valorificarea subproduselor vitivinicole.

Cuvinte–cheie: *tescovină, Rara Neagră, tratare termică, antociani, polifenoli, compuși antioxidanți, valorificarea subproduselor, procesarea alimentelor.*

1. Introduction

Sweet grape pomace represents a valuable source of biologically active compounds, particularly anthocyanins, polyphenols, and flavonoids, recognized for their antioxidant properties [1]. These compounds contribute to the neutralization of many reactions involved in the onset of oxidative stress and numerous chronic diseases. The antioxidant activity of phenolic compounds is based on their ability to donate hydrogen atoms or electrons, stabilizing free radicals and interrupting oxidative chain reactions. They can also act through complementary mechanisms, such as chelation of metal ions or inhibition of oxidative enzymes. In the case of anthocyanins, for example, antioxidant efficiency is influenced by chemical structure, particularly by the number and position of hydroxyl groups, as well as by the stable molecular form in an acidic medium [1,2].

In recent times, diets rich in antioxidant compounds or based on functional foods have become increasingly promoted in public health sectors, due to their potential to reduce the incidence of cancer and other chronic diseases, considered leading causes of mortality. In addition to nutritional value itself, food contributes significantly to protecting the body against numerous pathologies, reinforcing the concept of functional food and nutraceuticals [3]. The development of an interdisciplinary link in food research integrating natural compound chemistry, food science, and nutrition allows for more detailed information on the chemical composition of food products, changes induced by industrial processing, and the bioavailability of bioactive compounds. Plant-based food sources are recognized as being extremely rich in substances with beneficial health effects [3]. For example, anthocyanins are natural pigments responsible for the red, violet, and blue hues of numerous plant products, recognized for their antioxidant properties and beneficial health effects [4]. The main challenge preventing the use of these compounds as alternatives to synthetic additives is their limited stability, particularly at high pH values and elevated temperatures. However, compared to other natural colorants (betalains, carotenoids, chlorophylls), anthocyanins show better thermal stability, which justifies the need for further applied research in this area. Academic interest in studying biologically active compounds is directed toward validating beneficial health effects, such as antioxidant, anti-inflammatory, anti-obesity, antidiabetic, antitumoral, anti-ulcer, and neuroprotective activities [5, 6].

Since most plant-based foods are subjected to heat treatments before consumption, to inhibit microbial growth, remove water, or inactivate enzymes, heating influences the content of biologically active compounds. Thermal stability experiments are generally carried out by treating extracts at different temperatures and for varying periods of time, after which the total or individual residual content is determined [6]. To reduce the degradation of

biologically active compounds during thermal processing of foods containing these compounds, appropriate formulation of thermal conditions is necessary so that product quality is not affected [7, 8]. Since the antioxidant activity of these compounds also depends on their total content, well-controlled thermal processing can contribute to maintaining or even improving antioxidant properties, due to the formation of other compounds with antioxidant activity [9] as a result of heat-induced reactions.

The aim of this study was to provide a detailed and updated overview of the molecular mechanisms and kinetics of degradation of biologically active compounds during heating, as well as to describe their thermal behavior.

2. Materials and Methods

2.1. Materials for research

The studies were based on the preparation and investigation of extracts from unfermented sweet red grape pomace of Rara Neagra, harvest year 2025. The red Rara Neagra grapes were brought from the Stefan Voda region, Purcari commune, and processed technologically in the micro-winemaking section of the Department of Oenology and Chemistry.

The pomace was dried to dryness at a temperature of 55 ± 2°C, in oven BOV-T30C ground with an electric mill Bosch KM13 and used for obtaining the study extract. The solvent used for extraction was ethyl alcohol at 60% acidified with citric acid. Acidification of the medium with citric acid is an efficient and eco-friendly technique for the recovery of bioactive compounds from pomace [10]. Citric acid, used to adjust the pH to acidic values (approximately pH 3.0), promotes the degradation of plant cell walls and increases the diffusion of phenolic compounds and anthocyanins [11] into the extraction medium.

The experimental extraction regime included 12 hours with periodic agitation at temperatures of 40_± 2°C. After extraction, the hydro-alcoholic samples were filtered and stored in the dark at 20 ± 1°C. Subsequently, the extracts were heat-treated at temperatures of 80, 100, 120 and 140 ± 5°C for 7, 14, 21 and 30 minutes at the Oenological Research Center, Faculty of Food Technology, UTM.

Reagents. The Folin–Ciocalteu reagent Merck (Darmstadt, Germany), gallic acid 98%, 2,2-diphenyl-1-picrylhydrazyl (DPPH) 90 %, sodium carbonate (Na_2CO_3) powder 99.5 %, ethanol (C_2H_5OH) 98%, sodium hydroxide (NaOH) solution 33% and hydrochloric acid (HCl) 37.5% were obtained from Sigma-Aldrich. All other chemicals were analytical reagent grade.

2.2 Research methods

For the pH value the InoLab pH 7110 was employed. Dry substance content was established using ATAGO Pocket refractometer PAL-1.

Evaluation of Chromatic Characteristics

Before spectrophotometric analysis, the extract samples were subjected to centrifugation at 2000 rpm for 15 min. The absorbance values were subsequently recorded at 420, 520, and 620 nm using a T80 UV–VIS spectrophotometer and 1 mm optical path cuvettes. These wavelengths are associated with the yellow, red, and blue-violet regions of the visible spectrum and provide information regarding the color characteristics of anthocyanin-containing extracts [12].

The absorbance measurements obtained at the three selected wavelengths were used to calculate the chromatic parameters according to equations (1) and (2). These parameters

allowed the evaluation of color intensity and hue, providing insight into the changes in pigment composition and color stability induced by thermal treatment.

$$\text{Color intensity: } I_c = A_{420} + A_{520} + A_{620} \quad (1)$$

$$\text{Hue color: } H_c = \frac{A_{420}}{A_{520}} \quad (2)$$

where: A_{420} – absorbance at 420 nm;

A_{520} – absorbance at 520 nm;

A_{620} – absorbance at 620 nm.

Summary appreciation of phenolic compounds

For the determination of total phenolic content, 1 cm³ of extract diluted at a ratio of 1:5 was transferred into a 100 cm³ volumetric flask. Subsequently, 50 cm³ of distilled water, 5 cm³ of Folin–Ciocalteu reagent, and 20 cm³ of 20% sodium carbonate solution were added. The mixture was brought to volume with distilled water and homogenized thoroughly. After an incubation period of 30 min, the absorbance was measured at 750 nm using 10 mm path-length cuvettes, with distilled water serving as the blank [12].

When the measured absorbance deviated significantly from the target value of approximately 0.3 absorbance units, the analysis was repeated using an adjusted dilution factor to obtain readings within the optimal analytical range. The concentration of total phenolic compounds was determined from a calibration curve prepared with gallic acid standards (0–500 mg/L). Results were expressed as milligrams of gallic acid equivalents per gram of dry weight of grape pomace extract (mg GAE/g DW).

Determining anthocyanin content

For the determination of anthocyanin content, 3 cm³ of clarified extract was transferred into a 25 cm³ pycnometer. Subsequently, 12.5 cm³ of rectified ethanol adjusted to pH 1.2 and three drops of concentrated hydrochloric acid were added. The mixture was then diluted to the calibration mark with distilled water and thoroughly homogenized by vigorous shaking. After centrifugation at 1500 rpm for 15 min, the absorbance of the content was recorded at 520 nm using a 1 mm path-length cuvette, with distilled water serving as the blank reference [12].

The concentration of anthocyanins was subsequently determined using the equation presented below:

$$C_{ant} = 1056.7\ A \quad (3)$$

where: A – absorbance of sample;

1056.7 – transfer coefficient, established for malvidin-3-glucoside isolated from grape skins.

Determination of monomeric pigments using the pH differential method (pH 1.0 and pH 4.5)

The concentration of monomeric anthocyanins was determined using the pH differential method, based on the reversible structural transformation of anthocyanin pigments under different pH conditions according [13]. The method is based on the structural transformation of anthocyanin-like monomeric pigments depending on pH. At pH 1.0, pigments exist predominantly in the colored flavylium cation form, while at pH 4.5, they are converted to a colorless hemiketal form. The difference in absorbance between the two pH values is

proportional to the concentration of monomeric pigments. For this method the preparation of two separate dilutions of the same sample is necessaire:

Sample Preparation: two dilutions of the same sample were prepared using buffer solutions of different pH values:

- pH 1.0 potassium chloride buffer;
- pH 4.5 sodium acetate buffer.

Typically, 0.5 mL of sample was mixed with 4.5 mL of the corresponding buffer solution, resulting in a 1:10 dilution ratio. The dilution factor was adjusted when necessary, according to the color intensity of the sample. The diluted samples were thoroughly mixed and allowed to equilibrate for 15–20 min at room temperature in the absence of light to ensure complete pigment stabilization.

Spectrophotometric Measurements: the absorbance of each solution was measured using a UV–VIS spectrophotometer at:

- 520 nm, corresponding to the maximum absorbance wavelength of anthocyanin pigments;
- 700 nm, used to correct for sample turbidity and light scattering effects.

Mathematical calculations: the absorbance values for each pH condition were calculated according to equations (4) and (5):

$$A_{pH1} = (A_{520} - A_{700})_{pH1} \tag{4}$$

$$A_{pH4.5} = (A_{520} - A_{700})_{pH4.5} \tag{5}$$

The net absorbance attributable to monomeric anthocyanins was then calculated as:

$$A = A_{pH1} - A_{pH4.5} \tag{6}$$

The concentration of monomeric anthocyanins, expressed as cyanidin-3-glucoside equivalents per liter of extract, was calculated using equation (7):

$$C = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l} \tag{7}$$

where: C - monomeric anthocyanin concentration (mg/L);

A -net absorbance of samples;

MW – molecular weight of cyanidin-3-glucoside (449.2 g/mol);

DF – dilution factor;

ε – molar absorptivity coefficient (26,900 $L \cdot mol^{-1} \cdot cm^{-1}$);

l – optical path length of the cuvette (cm).

3. Results and discussions

The dry matter content of the acidified hydroalcoholic extract of Rara Neagra, calculated in %, was 29.8 ± 0.05, and the pH value was 3.28 ± 0.01.

From the absorption spectra included in Fig.1, maximum stability was observed at 80 to 100 °C and rapid degradation at 120 to 140 °C. The relative stability in the temperature range 80 to 100°C (nearly constant absorbance) is explained by the fact that in this temperature range, most anthocyanins and polyphenols were in a zone of relative thermal stability, their degradation was slow. Additionally, pigmentation effects (interactions

between anthocyanins and other polyphenols) may have been present in the pomace extracts, stabilizing the color and absorbance. The decrease in absorbance at 120 and 140°C is explained by the degradation of anthocyanins, polymerization reactions, and degradation of flavonoids, resulting in a visibly decreased absorbance of the samples [14]. All at once the absorbance of the untreated control samples was lower, the most likely explanation is that heat treatment increased the content of anthocyanins in a soluble and detectable state in the extracts.

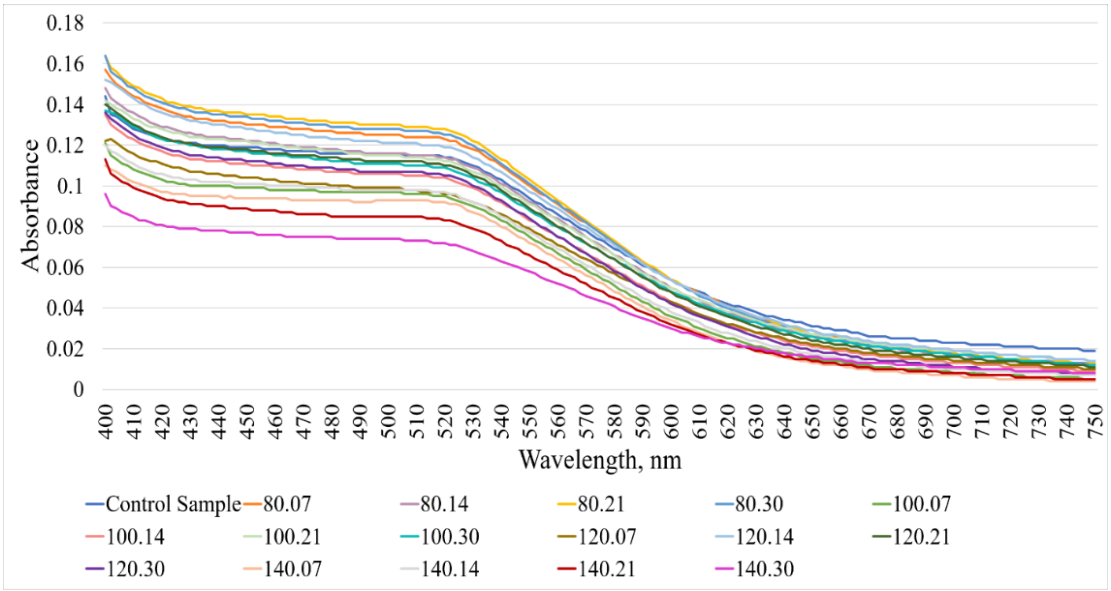

Figure 1. Absorption spectra of the studied extracts (dilution factor 50), temperature range (80–140°C), duration (7–30 minutes).

Following heat treatment in the studied temperature range (80–140°C, duration 30 minutes), the total phenolic content of the acidified Rara Neagra extracts decreased from 50.67 mg GAE/g DW to 17.98 mg GAE/g DW. As can be observed in Fig. 2, with the increase in temperature from 80°C to 140°C and treatment time from 0 to 30 minutes, a percentage reduction of total polyphenol content in the extract of up to 35.49% was observed. In the study conducted by Daniela Serea [15], a percentage reduction of total polyphenol content in the extract of up to 45.45% was observed, which is explained by the longer heat treatment duration and the different grape variety.

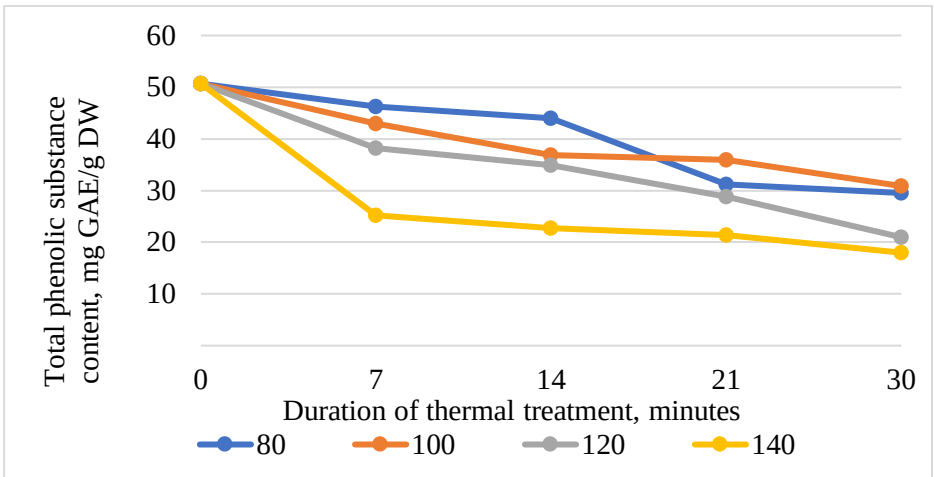

Figure 2. Influence of heat treatment (7, 14, 21 and 30 min) on total phenolic content of Rara Neagra extracts at different temperatures (80, 100, 120 and 140 °C).

The decrease in the total phenolic compound content observed in sweet red grape pomace extracts acidified with citric acid can be explained by thermal degradation processes, oxidation, and structural transformations of phenolic compounds [16]. High temperatures and prolonged treatment duration favored the formation of reaction products, such as quinones and polymeric compounds, which were no longer efficiently detected by the spectrophotometric methods used. Although the acidic medium contributed to partial stabilization of these compounds, it was unable to prevent their degradation under severe thermal conditions.

When determining the anthocyanin content, it was observed that they remained nearly constant (Fig. 3). This is explained by the fact that the hydroalcoholic extracts, being acidified with citric acid, created favorable conditions for anthocyanins. At pH acid (2.5– 3.5), the flavylium form dominated, being the most thermally and oxidatively stable [17]. Another important factor was the fact that pomace extracts are rich in polyphenols, sugars, and organic acids, which could interact with anthocyanins through pigmentation mechanisms. These interactions contributed to color stabilization and protection of anthocyanins against thermal degradation. In addition, the presence of acylated anthocyanins, known for their superior thermal stability, may have contributed to maintaining the total concentration under heating conditions.

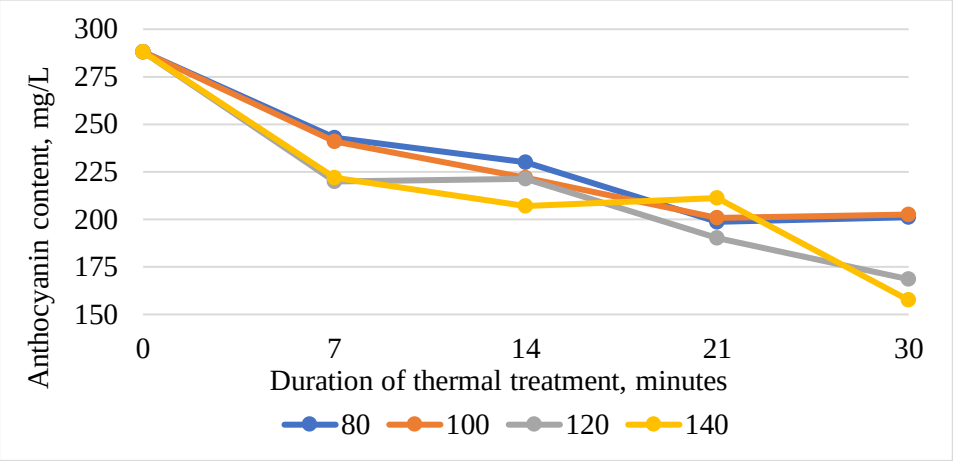

Figure 3. Anthocyanin content in Rara Neagra extracts at experimental condition.

Heating can increase the value determined by the pH differential method, as this method quantifies only anthocyanins that respond reversibly to pH change and exhibit characteristic absorption [13]. In untreated extracts, anthocyanins may be associated with tannins and polysaccharides, upon heating, these associations can be partially broken, making the anthocyanins more accessible.

Experimental results revealed a decrease in the monomeric anthocyanin content of sweet red grape pomace extracts acidified with citric acid, with increasing temperature from 80 to 140 °C and heating time from 7 to 30 minutes (Fig. 4). This behavior can be explained by the high sensitivity of monomeric anthocyanins to elevated temperatures, which accelerate their thermal degradation, hydration, and structural transformation processes [18]. Although the acidic medium contributed to stabilizing anthocyanins in the flavylium form, the protective effect of citric acid became insufficient at high temperatures and prolonged heating durations. In addition, polymerization and condensation reactions with other phenolic compounds may have led to a reduction of the monomeric anthocyanin fraction and to the formation of more complex and thermally stable pigments.

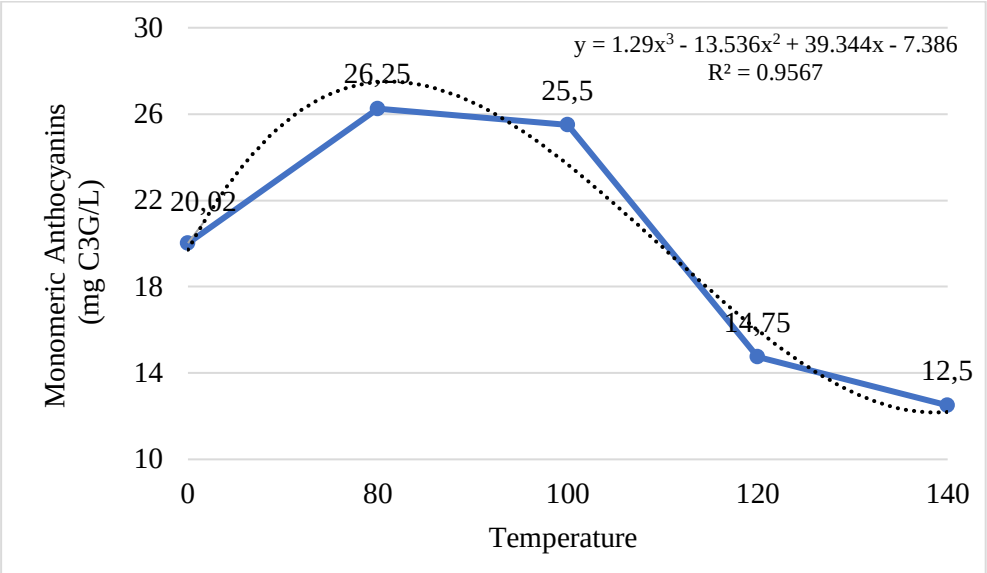

Figure 4. The dynamic of monomeric anthocyanin content in the studied experimental samples.

Based on the mean values calculated from all samples, a graph of the dependence of monomeric anthocyanin content on temperature was constructed (Fig. 4). It was observed that the maximum stability of anthocyanin monomers was between 80 °C and 100 °C, at 120°C, rapid degradation began, and at 140°C, significant destruction occurred. The relatively high stability of monomeric anthocyanins observed at 80–100°C may be attributed to the acidic conditions of the extract, which favor the predominance of the flavylium cation form, known to be the most stable anthocyanin structure.

The trend analysis revealed a temperature-dependent biphasic behavior of monomeric anthocyanins. Moderate heating (up to 80 °C) promoted anthocyanin release and increased their concentration, reaching a maximum value of 26.25 mg C3G/L. Further temperature elevation induced a progressive decline, suggesting thermal degradation, oxidation, and condensation reactions leading to the transformation of monomeric anthocyanins into more stable polymeric pigments. The high coefficient of determination (R^2 = 0.9567) confirms the strong relationship between temperature and anthocyanin content.

In addition, the presence of other phenolic compounds may contribute to pigment stabilization through copigmentation interactions. At 120°C, thermal energy became sufficient to accelerate the hydrolysis and structural transformation of anthocyanins, resulting in the formation of less stable chalcone and colorless degradation products. At 140°C, extensive degradation occurred due to the combined effects of thermal cleavage of anthocyanin molecules, oxidation reactions, and the breakdown of glycosidic bonds. These processes lead to substantial losses of monomeric anthocyanins and a significant reduction in their stability.

In the study conducted by Daniela Serea [15], the same pattern of monomeric anthocyanin degradation with increasing temperature and time is characteristic, from 25 mg C3G/L in the control to 5 mg C3G/L, while in our case from 20 mg C3G/L in the control to 12.5 mg C3G/L. Şelale Yalçınöz et al. [19], also demonstrated that the degradation of monomeric anthocyanins increases progressively during heating, meaning that significant reductions in monomeric anthocyanin content occur with increasing processing temperature, only they studied the anthocyanins in pomegranate juice.

The color hue remained relatively constant because the acidic medium created by citric acid acidification continued to stabilize the flavylium cationic form of the anthocyanins remaining in the system (Fig. 5). Under low pH conditions, the equilibrium between the different structural forms of anthocyanins was shifted toward the predominantly red forms, limiting major changes in chromatic tone.

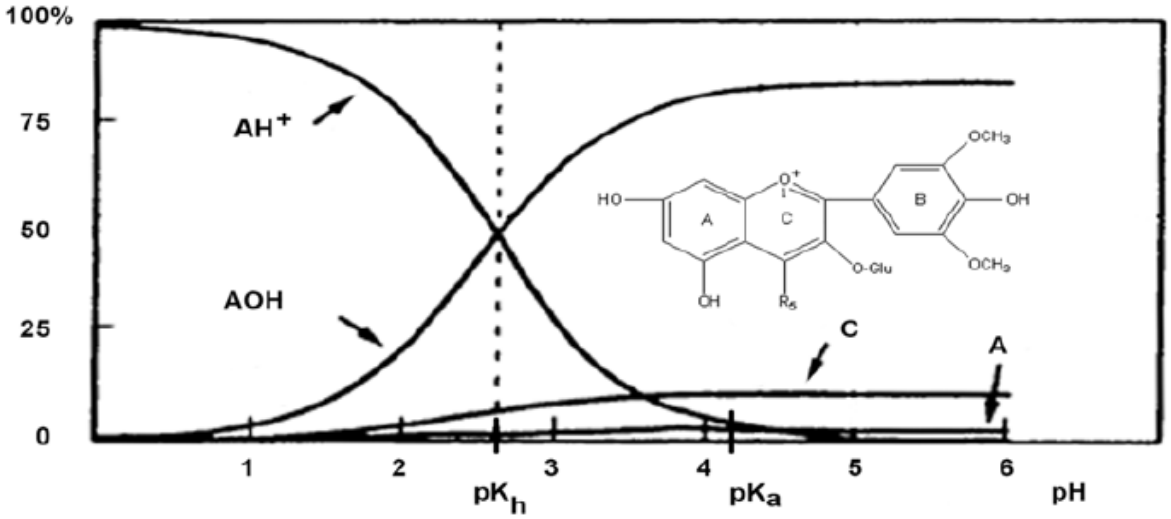

Figure 5. The distribution of anthocyanin structural forms as a function of pH.

The experimental pH value was 3.28, so the system was located close to the transition region around pK_h (~3.0). This indicates an intermediate equilibrium state: a significant fraction of anthocyanins remained in the colored forms (AH^+ and partially quinonoidal forms).

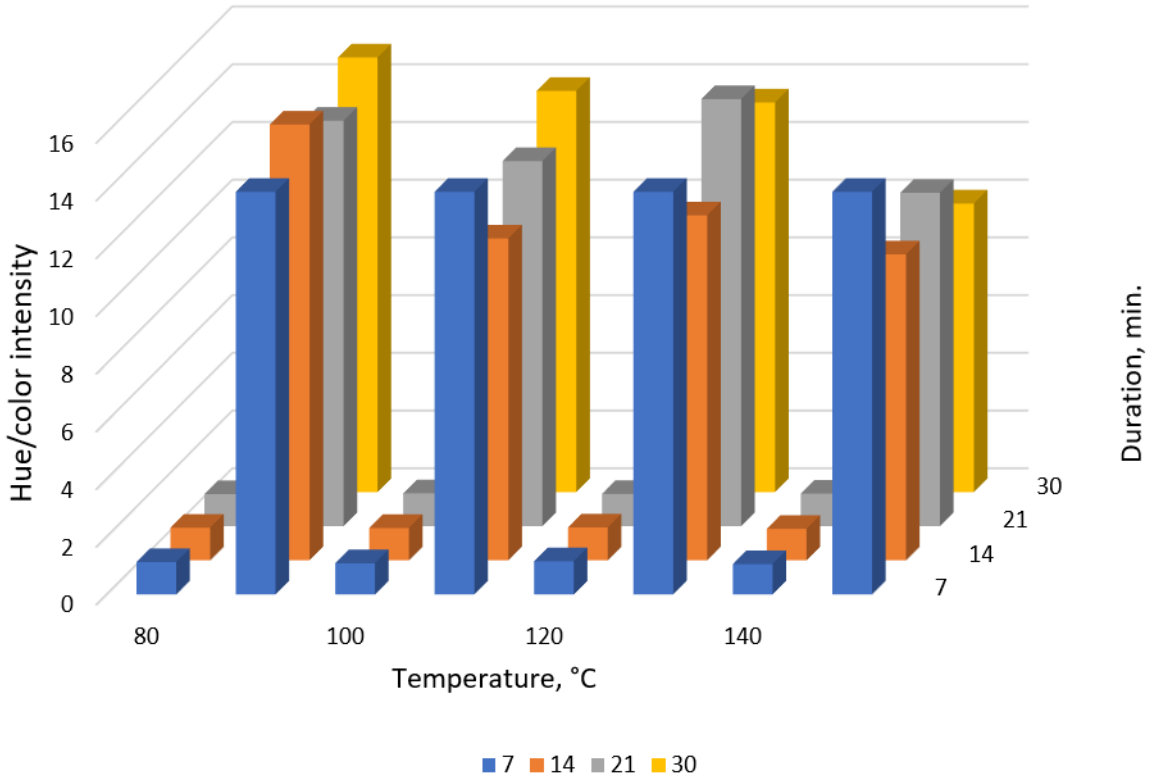

Figure 6. Influence of heat treatment (7, 14, 21 and 30 min) on color intensity and hue of Rara Neagra extracts at different temperatures (80, 100, 120 and 140 °C).

According to the anthocyanin structural equilibrium diagram (Fig. 5), the pH value of the investigated extracts (pH 3.28) was located slightly above pKh, where the flavylium cation (AH^+) and the hydrated hemiketal form (AOH) coexist in dynamic equilibrium.

Under these conditions, hydration of the flavylium cation becomes significant, leading to a partial conversion of the colored anthocyanin species into the nearly colorless hemiketal form. Consequently, the proportion of AH^+ decreases, resulting in a reduction of color intensity, while the hue remains relatively stable. At pH 3.28, the formation of quinonoidal bases and chalcone structures is still limited, since these transformations become more pronounced at higher pH values approaching pKa.

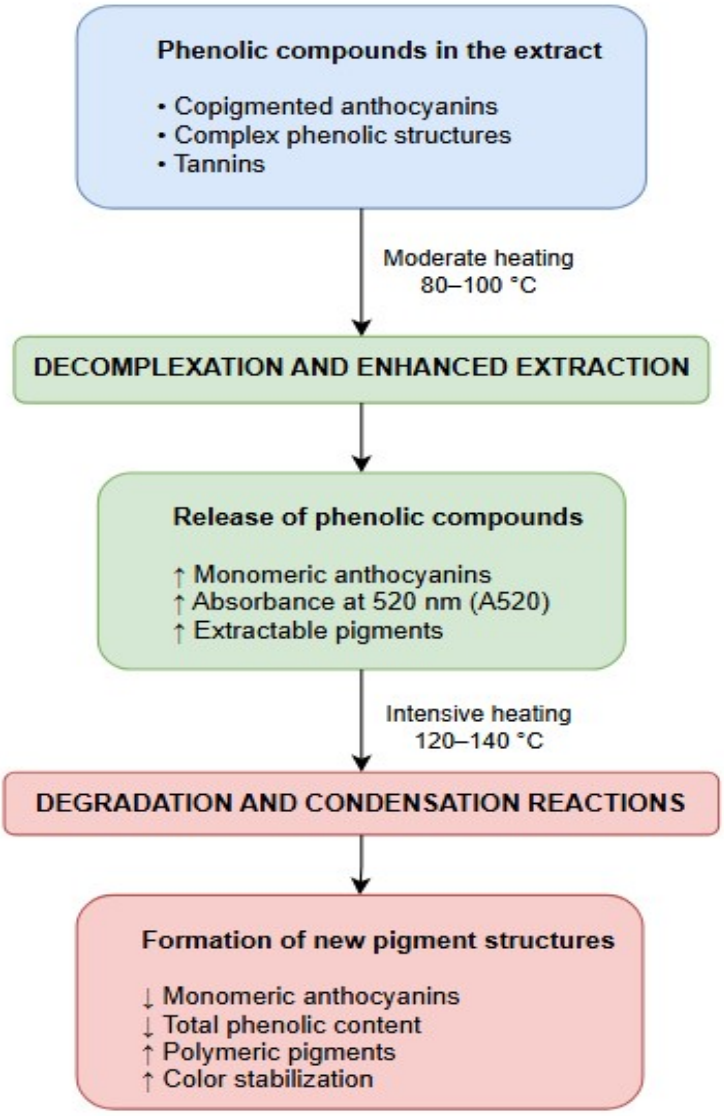

Figure 7. The comparative analyzes of the stages of Rara Neagra extracts.

Thus, even as the total concentration of coloring pigments decreases, the relative proportion of compounds responsible for the red color remained sufficiently stable so that the hue did not undergo significant variations (Fig. 6). The heat treatment simultaneously affected the release of anthocyanins and intensification of the red color as well as the appearance of minor chemical transformations of phenolic compounds that increased absorbance at 420 nm [20]. Therefore, although the color became more intense, the ratio

between yellow and red components increased slightly, leading to higher color hue values compared to the untreated control sample.

The reduction in color intensity indicated a decrease in the concentration of colored monomeric forms of anthocyanins, as a result of thermal degradation accelerated by increasing temperature and heating duration (Fig. 6). At high temperatures, anthocyanins underwent hydration reactions, opening of the flavylium nucleus, and conversion to less colored or even colorless forms, leading to a decrease in specific absorbance and consequently to a reduction in the chromatic intensity of the extract [21].

At 140°C, the decrease in color intensity was approximately 47%, a result consistent with the findings reported by Turturică et al. [18], who observed a reduction in color intensity of up to 60% at 120°C. These results indicate that the system under study falls within the same range of accelerated degradation, characterized by pronounced thermal breakdown of anthocyanin compounds at elevated temperatures.

Therefore, as a result of the experimental study, a conceptual model of the structural and functional transformations that occurred in the studied sweet red grape pomace extracts during heat treatment can be demonstrated (Fig. 7). In the initial state, the extract contained pigmented anthocyanins, complex phenolic compounds, and tannins, in a relatively stable molecular system. [22].

At moderate temperatures (80–100°C), heating favored decomplexation processes and the release of bioactive compounds from the plant matrix, leading to an increase in monomeric anthocyanin content and intensification of absorbance at 520 nm. The increase in temperature in the 120–140°C range resulted in predominance of thermal degradation and condensation processes of phenolic compounds. Under these conditions, a reduction in the monomeric anthocyanin fraction and total phenolic content was observed, concurrent with the formation of thermally more stable polymeric pigments [23].

4. Conclusions

Sweet red pomace represents a valuable source of natural bioactive compounds. The obtained results demonstrated that thermal processing of acidified hydroalcoholic extracts from sweet red grape pomace enhanced anthocyanin availability and improved chromatic characteristics, as evidenced by increased absorbance at 520 nm, color intensity, and monomeric anthocyanin content. The experimental results demonstrating the stability of anthocyanins in an acidified hydroalcoholic medium suggest the possibility of using the extracts as natural food colorants. Stability at 80–100°C indicates that these compounds can withstand moderate food processing conditions (pasteurization, short heat treatments). Degradation at 120–140°C shows that intense technological processes (severe sterilization, prolonged heat treatments) can reduce the functional and colorant value of the extracts.

The treatments temperature initiated the chemical reactions, while time only finalizes transformations already begun, this is why there are small differences between 7 and 30 minutes, but very visible differences between 80°C and 140°C. Overall, the combination of thermal treatment and acidic conditions proved effective in improving the technological and functional quality of grape pomace extracts intended for valorization as natural colorant. Therefore, the obtained results have direct importance for the food industry and the valorization of wine by-products.

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Contribution of Authors

Nadejda Botezatu: Conceptualization, Methodology, Investigation, Resources, Data curation, Formal analysis, Writing – original draft preparation.

Ecaterina Covaci: Software, Methodological guidance, Validation, Supervision, Conceptual discussion, Writing – review & editing, Project administration.

Alexandra Arseni: Software, Investigation, Visualization.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in designing the study; collecting, analyzing, or interpreting data; writing the manuscript; or deciding to publish the results.

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