Journal of Engineering Science *Vol. XXXI, no. 3 (2024), pp. 117 - 129*
Fascicle *Food Engineering* *ISSN 2587-3474*
Topic *Food Technologies and Food Processes* *eISSN 2587-3482*

https://doi.org/10.52326/jes.utm.2024.31(3).10
UDC 663.257:665.939.14

INFLUENCE OF POTATO AND PEA PROTEIN FINING ON THE CHROMATIC PROFILE FEATURES OF RARA NEAGRA WINE

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Received: 07. 28. 2024
Accepted: 08. 31. 2024

Abstract. Due to food security issues associated to use of animal proteins and the rising demand for non-animal-based fining agents, the wine industry is becoming more interested in developing alternatives to conventional protein fining. This study evaluated the effects of several protein fining agents on the color and phenolic content of Rara Neagra red wine. Wines that were fined with proteins from potatoes and peas were contrasted with gelatin treated wines and untreated control wines. Variations in color and phenolic content revealed that plant and animal proteins had different capacities for clarification and interaction with colorless phenolics and anthocyanins, which had significant effects on color characteristics. The experimental study revealed that, in comparison to gelatin, potato and pea protein extracts were more protective in lowering the overall polyphenol level of Rara Neagra wines. Similar to other proteinaceous fining agents, the acquired data demonstrated a minor decrease in color intensity and a low decrease in the quantity of total anthocyanins. Overall, results showed that potato protein and pea protein could be used as effective fining alternatives to animal proteins, and their effectiveness should be researched in different variations depending on the chemical composition or variety of the wines.

Keywords: *clarification, chromatic indices, plant protein, stabilization.*

Abstract. Preocuparea tot mai mare a industriei vinului cu privire la dezvoltarea alternativelor de tratare complexa a vinurilor se datorează problemelor de siguranță alimentară asociate utilizării proteinelor animale și a cererii pentru agenți de tratare de origine ne animalieră. Acest studiu a evaluat efectele mai multor agenți de tratare proteici asupra culorii și conținutului de compuși fenolici al vinului Rara Neagră. Vinurile tratate cu proteine din cartofi și mazăre au fost comparate cu vinurile tratate cu gelatină și vinurile martor netratate. Variațiile de culoare și conținutul de polifenoli au arătat că proteinele vegetale și animale au capacități diferite de limpezire și interacțiune cu polifenolii incolori și antocieni, având efecte semnificative asupra caracteristicilor culorii. Studiul experimental a arătat că, în comparație cu gelatina, extractele proteice de cartofi și mazăre au fost mai protectoare în scăderea nivelului general de polifenoli din vinurile Rara Neagră. Similar altor agenți de limpezire proteici, datele obținute au demonstrat o scădere minoră a intensității culorii și o scădere nesemnificativa a cantității de antocieni. În ansamblu, rezultatele au arătat că proteina din cartofi și proteina din mazăre ar putea fi folosite ca alternative eficiente de limpezire, iar eficacitatea acestora ar trebui cercetată în diferite variații, în funcție de compoziția chimică sau soiul de struguri.

Cuvinte-cheie: *limpezire, caracteristici cromatica, proteine vegetale, stabilizare.*

1. Introduction

Wine is an extremely complex medium, where polyphenols have a crucial impact on its final physicochemical and sensory properties [1]. The chemical diversity of polyphenols in grapes and wines is countless. With varying degrees of hydroxylation, substitutions, and even the formation of adducts between them, any family can exist in free or conjugated forms [2,3]. The primary chemical components that give wines their organoleptic qualities—such as color, bitterness, and astringency—especially in red wines, are phenolic compounds [4]. Since red wine's color is the first thing people notice about it, it has a significant impact on consumer preferences and purchasing decisions, making it one of the most crucial factors in determining its quality [5].

Anthocyanins are responsible of the diversity of red wines color, and their profile can be utilized as an analytical method to verify authenticity. Condensed tannins and flavan-3-ols, on the other hand, are important substances because of their effects on color stabilization as well as their astringent and bitterness qualities [6,7]. The primary function of other phenolics, such as flavonols and hydroxycinnamic acids, may be considered that of co-pigmentation, which aids in color evolution and stabilization, is directly tied to the anthocyanin composition of wine and the interactions between them or with other wine components (mostly colorless phenolics) [8,9].

Numerous factors influence the types and quantities of the various phenolic compounds found in grapes [1−4]. In particular, viticulture methods, environmental factors (soil, climate), and disease diseases all have a significant impact on the polyphenolic composition of grapes [10]. However, the winemaking technique and varietal or genetic variances [11] are certainly among the most significant variables. Controlling and regulating the phenolic composition during vinification is therefore essential to producing full-bodied red wines with stable, rich colors and balanced bitter and astringent characteristics [9,12].

The presence of microorganisms (bacteria and yeast), tartrate crystals, remaining grape peel and pulp, and aggregation of macromolecules (mostly pectin and protein components) produced during the fermentative maceration process are the main causes of wines' turbidity and instability after fermentation [13]. Wine's natural slow precipitation and sedimentation are determined by the presence of colloidal unstable molecules at advanced stages of vinification, which are linked to the formation of less soluble phenolic compounds that tend to co-aggregate gradually over time [14]. In order to prevent changes in taste, flavor, or color before bottling and consumption, the variety of particles that cause hazes and deposits must be eliminated or stabilized. These particles can aggregate coloring matter and uncolored phenolics, affecting the wines' sensory quality [13,14].

In oenology, fining agent clarification is a widespread procedure that involves adding an exogenous material to a turbid wine that uses flocculation or adsorption to precipitate other suspended particles [15]. By removing or lowering content of certain phenolic components of colloidal type linked to oxidation processes or harsh taste sensations, the primary advantages focus on improving the wine's limpidity, color stability, and mouthfeel perception [16,17]. Nevertheless, this is a significant problem for red wines with low phenolic content since excessive clarifying might harm the stabilizing processes linked to tiny solutes and macromolecules that mostly affect color [18-20].

The tendency of protein fining agents to interact with wine phenolics and their varied affinity for various phenolic classes make them highly desirable among clarifying additives for wine fining [13,14,17,21,22]. Different studies show that certain phenolic compounds may be significantly reduced by commonly used fining agents in the wine sector, such as bentonite, gelatine, casein, egg albumin, and PVPP [5,15−22]. However, it has also been shown that gelatine fining treatments had no noticeable effect on the phenolic level and composition of wine. Yet due to their potential for causing allergies or food intolerance, conventional animal-derived fining agents, such as milk and egg proteins, have been more strictly regulated by the European Union in the past ten years, even though their effectiveness [14,23,24]. This is why alternative approaches for clarifying white, rose, and red wines have been recently suggested as plant-derived macromolecules including proteins, cell wall material, or fiber from various vegetal sources [13,14,19,23−27].

In the last decades, socioeconomic changes and globalization have led the industrial environment to develop products adapted to the changing interests of consumers. This new generation has more options and access to information, which means they have access to good products with a higher value. Growing consumers concern about health and environmental issues is leading to increased interest in the use of alternative food additives. In the wine industry, the preference for ecological, "vegan", bio-dynamic, alternative and more sustainable wines is getting popular [24,27].

The International Organization of Vine and Wine reports that currently around 53 million tons of grapes are processed globally, and global wine production is estimated at an average value of 244.1 million hectoliters [24,28]. According to the data of the National Vine and Wine Office of the Republic of Moldova, in 2022, 280 thousand tons of grapes were processed, which were converted into about 1.9 million hectoliters of wine, and about 55% were destined for export [29]. These data demonstrate that the wine industry is a primary one with potential for development, therefore it becomes even more important to connect the quality of wine to the demands of consumers [30], thus contributing to the harmlessness and sustainability of the food industry. For this reason, there is a growing interest in finding alternative wine fining agents to replace potentially allergenic animal-derived proteins and to avoid the legal obligation of indicating their presence on the label [27,30]. Moreover, some diets, such as vegetarians or vegans, do not accept foods or beverages treated with animal products [27].

The innovative solutions currently proposed for the fining of wines with materials of plant origin are in the sights of wine producers and consumers for various reasons. They certainly solve some of the problems that have led to the need for plant-based treatment agents, such as the lack of labeling requirements for animal-based adjuvants, and provide a solution to consumer demands for products that are vegetarian/vegan friendly [24,30].

Considering the allergenic issues with using animal proteins in order to clarify the wines and the increased demand for vegan wines, new researches are needed to promote alternative wine fining agents like plant extracted proteins.

The purpose of this research was to investigate the effects of alternative oenological additives: use of plant-based fining agents (patatin and pea protein) compared to gelatin (pork origin) and their action on the phenolic compounds content and color indices of Rara

Neagra red dry wine using spectrophotometric method, as well as the physical-chemical and sensorial assessment.

2. Materials and Methods

The Rara Neagra variety belongs to the group of autochthonous (Moldovan-Romanian) varieties and represents a major interest for oenologists [31].

Grapes of Rara Neagra variety from Purcari region, harvest 2023, were technologically processed according to the classic vinification scheme under micro winery conditions at the Department of Oenology and Chemistry, Technical University of Moldova. At the completion of the fermentation-maceration process, the must was pressed in a pneumatic press and the young wine was directed to post-fermentation. After the post-fermentation period, the dry red wines were added 40 mg/L of SO_2 and subjected to physicochemical analyses.

The research focused on the influence of protein fining agents of vegetable origin from potatoes (Vegecoll, Laffort) and from peas (Clear V, Perdomini IOC) on the evolution of the phenolic complex of the studied wine. For comparison, a preparation of animal origin was also used (gelatin – ErbiGel, Erbslöh Geisenheim GmbH). The principle of the method consisted in the fact that the protein adjuvant interacts with the tannins present in the wine, canceling the electric charge of these substances [13,32]. This process causes the formation of insoluble flocs, for the efficient sedimentation of which bentonite (GranuBent, Erbslöh Geisenheim GmbH) was used. The optimal doses of administered adjuvants were determined empirically, the samples were refrigerated and analyzed for the presence of disorders, as well as subjected to the tannin test [13].

The physico-chemical and quality indices of the grapes and raw material wines were established by modern analysis methods recommended in the national [33] and international official International Organization of Vine and Wine (OIV) practices [34].

The sensory analysis of the samples was carried out by a group of 12 tasters, which provided the description of the sensorial profile. Each descriptor was scored by points between 1 (least felt) and 10 (most felt) and then recorded in a special descriptive evaluation sheet [35].

The content of total phenolic substances was determined by the UV−Vis spectrophotometry method with the Folin-Ciocalteu reagent [36], gallic acid (Sigma-Aldrich) being used as a calibration substance. Prior to determination of phenolic compounds, the red wines were centrifuged at 8000 rpm for 15 min. The calibration curve and the regression equation ($y = 1.378x + 0.0423$) were used to determine the concentrations of total phenolic substances in the studied wines. There were no essential deviations from the calibration curve, which is also demonstrated by the coefficient R^2 =0.9898.

The total content of anthocyanins was determined spectrophotometrically by the method of dilution in ethyl alcohol acidified with HCl and measuring the absorbance at wavelength 520 nm. The method is based on the principle of balance between the colored and colorless forms of anthocyanins present in an acidic environment. Anthocyanin content was calculated based on the difference in optical absorbance at a wavelength of 520 nm, which is obtained by adding two buffer solutions (pH 0.6 and 3.5) and an alcoholic solution of HCl 0.1 % in the sample [37,38].

Chromatic features were measured spectrophotometrically using quartz cuvettes with 1 mm optical length. The UV-Vis spectrophotometer was used to measure the absorption and transmission of light in the UV spectrum and the visibility of samples. The phenomenon of light radiation adsorption by its passage through adsorbent media is governed by the fundamental Lambert-Beer law expressed as follows: the intensity of a monochromatic flux of a certain wavelength passing through a colored solution decreases proportionally with the concentration and the thickness of the layer of liquid [39].

The analysis of the color parameters of Rara Neagra red wines samples subjected to treatment with protein additives of different origin was carried out by the usual spectrophotometric method recommended by the OIV [34]. The intensity of the color results from the summation of the absorbances of the red, yellow and blue pigments which have a maximum absorption at the wavelengths 520 nm, 420 nm and 620 nm, respectively Eq. (1). The color hue results from the overlap of the red color measured at 520 nm over the yellow color measured at 420 nm Eq. (2). The color composition represents the percentage contribution of each of the three components and is given by A_{420}, A_{520} and A_{620} Eq. (3-5) from the coloring intensity (I_c). The proportion of the red color, produced by free and bound anthocyanins in the spectrum form of flavylium cations (dA) Eq. (6), was calculated using the formula described by Glories [10]:

$$I_c = A_{420} + A_{520} + A_{620} \tag{1}$$

$$H_c = \frac{A_{420}}{A_{520}} \tag{2}$$

$$Y = \frac{A_{420}}{I_c} \times 100\% \tag{3}$$

$$R = \frac{A_{520}}{I_c} \times 100\% \tag{4}$$

$$B = \frac{A_{620}}{I_c} \times 100\% \tag{5}$$

$$dA = [1 - \frac{A_{420}+A_{620}}{2 \times A_{520}}] \times 100\% \tag{6}$$

where:

A420 – the absorbance value at 420 nm, characterizes the yellow (Y) component of the colour;

A520 – the absorbance value at 520 nm, characterizes the red (R) component of the colour;

A620 – the absorbance value at 620 nm, characterizes the blue (B) component of the colour.

The statistical processing and mathematical modeling of the experimental results was carried out to exclude the results with accidental errors and those with a high level of uncertainty [40]. For this purpose, 3-4 parallel measurements were performed, the results were subjected to dispersion and correlative statistical processing, which was carried out through the MS Excel and ANOVA programs with the statistical significance threshold of $p < 0.05$, and the Q test was used to establish the degree of conformity of the experimental results.

3. Results and Discussion

The experimental study carried out allowed determining the influence of fining treatment operations on specific physicochemical indices. The results of the physicochemical analyzes of the investigated wines obtained under micro vinification conditions and with the addition of fining agents of different origins are presented in Table 1.

Table 1

The variation of the physicochemical parameters of the Rara Neagra wine samples

Physicochemical parameter	Control	Vegecoll	Clear-V	ErbiGel
Concentration of alcohol, % vol.	13.95± 0.11	13.90±0.12	13.90±0.11	13.85±0.11
Mass concentration of titratable acids, g/L	5.78±0.12	5.33±0.10	5.39±0.08	5.6±0.12
Mass concentration of volatile acids, g/L	0.64±0.08	0.61±0.08	0.66±0.11	0.62±0.06
pH	3.91±0.01	3.901±0.01	3.901±0.01	3.904±0.01

Thus, it was found that depending on the fining agent used, the physicochemical parameters of the Rara Neagra wine also vary. The concentration of the volume alcohol, expressed in %vol in the treated samples showed insignificant deviations from the control sample ranging 0.05- 0.1 units. The mass concentration of titratable acids in the treated wine samples decreases between 0.18-0.45 units. The mass concentration of volatile acids in the control sample is 0.64 g/L, after treatment with Vegecoll this indicates a decrease of 0.03 g/L, with ErbiGel 0.02 g/L, and in the case of treatment with Clear-V volatile acidity increased by 0.02 g/L. Regarding the pH value, the values are approximately the same with unsignificant deviations.

The results of the wine samples organoleptic analysis demonstrated that the average notes obtained by the wines treated in laboratory conditions vary depending on the clarification agent used (Figure 1).

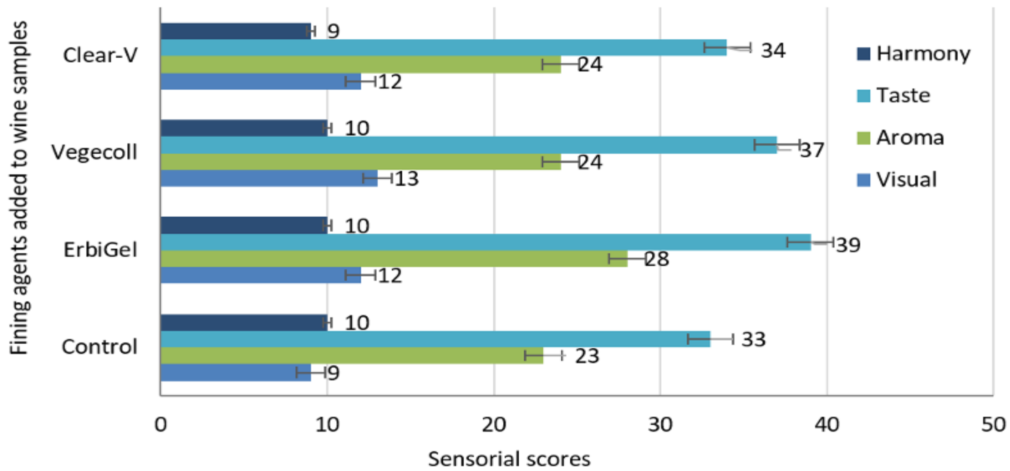

Figure 1. The sensorial scores for the Rara Neagra wine samples.

From the above diagram it can be concluded that visually, the highest score was given to the wine sample treated with Vegecoll (13 points), the lowest score to the untreated control sample. Clarifying agents have proven their effectiveness by significantly improving wine clarity. The highest score for the aromatic profile was given to the sample treated with ErbiGel (28 points), also the highest score was given to the sample treated with ErbiGel (39 points) for the taste parameter. The lowest score for harmony descriptor was given to the sample treated with Clear-V (9 points), the other samples accumulated equal scores - 10 points. Overall, the best score was given to the Rara Neagră wine sample treated with ErbiGel (89 points), and the lowest score to Vegecoll (79 points).

In order to evaluate the phenolic compounds features of Rara Neagra wine, the samples treated with vegetable and animal fining agents were subjected to UV-Vis analysis (Figure 2).

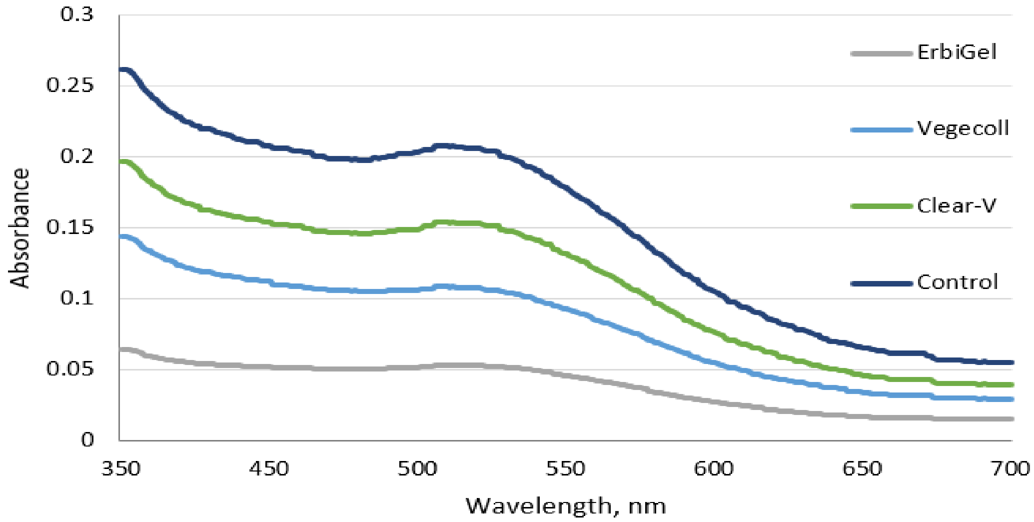

Figura 2. UV/VIS spectra of studied Rara Neagra wine samples.

From Figure 2 it can be observed that the maximum absorption of the analyzed samples is at the wavelength of 524 nm. The sample with the highest absorption is the sample where was used the pea protein fining agent (Clear-V), and the sample treated with ErbiGel (pork protein) recorded the lowest absorbance values.

The chromatic characteristics of the wines refer to the color of the wines, pointing out the intensity and hue of the color that reproduce the "appearance" and the brilliance of the color of the wine [39]. Based on the results obtained from the spectrophotometric analysis, the chromatic parameters of the wines were calculated: the color intensity and the color hue. The hue is related to the nature of the flavonic and anthocyanin pigments that give the wine its color, age and degree of technological treatments of the wine [10].

According to Ribéreau-Gayon, a young red wine's hue value ranges from 0.5 to 0.7, although it can rise to 1.3 as it ages [10]. In fact, the presence of anthocyanins in young wine gives it a vivid crimson color with violaceous reflections [6]. Because of increased content of tannins in an aged red wine, the color has an orange reflection [9].

Depending on the wine type, color intensity levels typically range from three to eighteen [10]. The anthocyanins in red wines have a strong correlation with the visible spectra of absorption. These anthocyanins contribute significantly to the wine's aging process by polymerizing with tannins [6,8,9]. We determined the values that characterize red wine color based on the absorption spectra, the obtained results were within the typical hue value range.

From the results shown in Figure 3, where the values of the chromatic parameters in dynamics is represented, we notice that during technological processing, both the color intensity and the color hue decrease. Color intensity ranges between 5.08-7.20, which is a lower content compared to other researches carried out on Rara Neagra wines [41-43].

Regarding the color hue, the values are in the range 0.6720-0.6943, being consistent with data provided by other researches on the color intensity of Rara Neagra wines [41-43].

Analyzing the average data obtained for the coloring intensity Figure 3, it can be observed that the reported differences between the applied treatment agents are insignificant from a statistical point of view (decrease by 3.2% for adjuvants of vegetable origin and 2.5% for gelatin). In case of color hue, the decrease is much significant: 34.4% for gelatin use and 29.5% for patatin and pea protein.

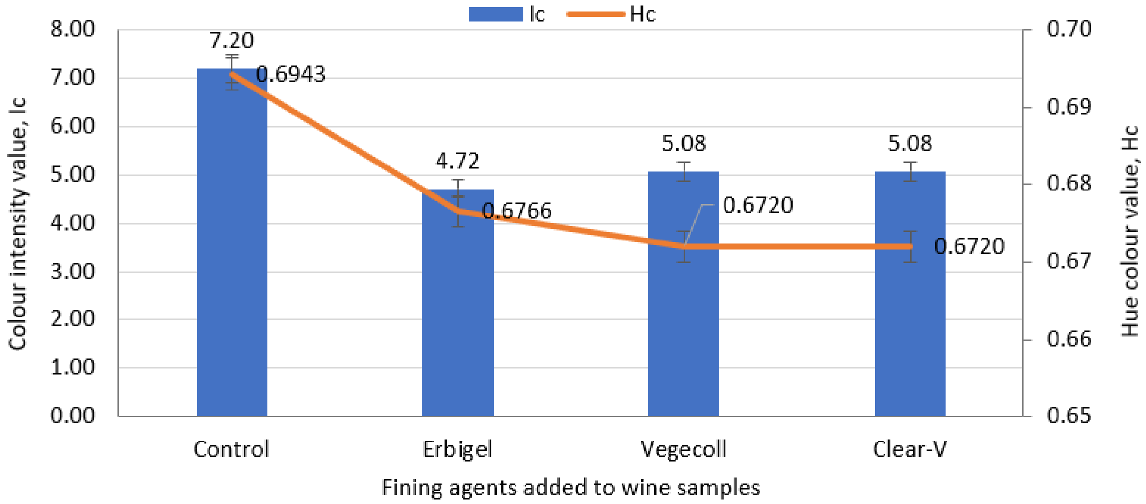

Figure 3. Variation of chromatic parameters I_c and H_c in dependance of fining agent.

Thus, it can be stated that fining treatment doesn't have significant influence on the color intensity, although an important decrease is noticed for the color hue, which mean that fining treatments reduced the compounds that give orange reflections to wine (i.e. flavones, tannins), giving the wine a bright red colour due to the anthocyanins. Our results confirmed previous findings that reported color parameters in Rara Neagra dry red wines [41-43], even though individual variations that could be attributed to the fining technique, selected dose and nature of fining agent.

The colour parameters were calculated according to Eq. 3-5, the results are illustrated in Figure 4. Yellow-colored components (A_{420}) varied between 44.09% for wines treated with vegetal protein, 44.92% for wines treated with gelatin compared to 45.00% in control sample (Figure 4).

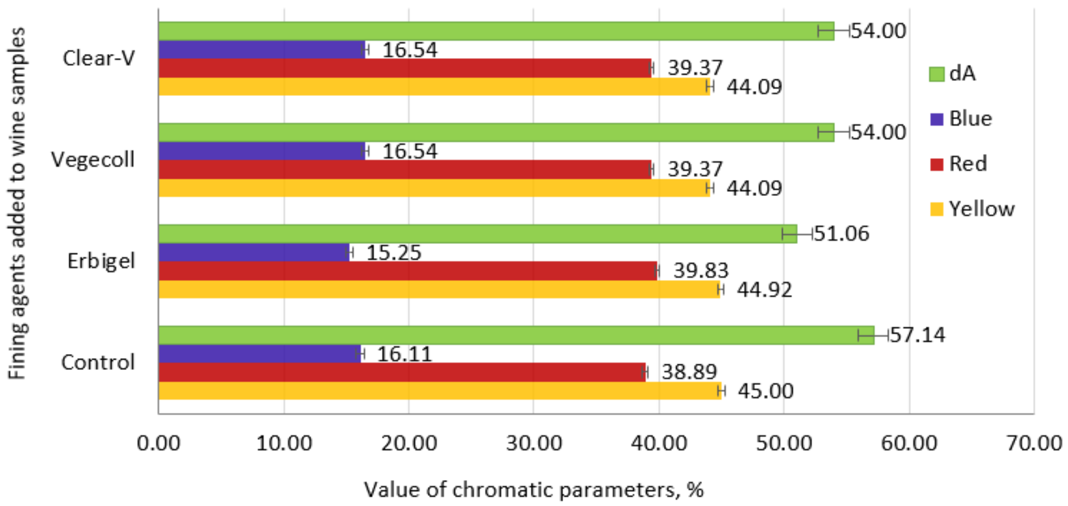

Figure 4. Intensity of yellow (A_{420}), red (A_{520}), blue (A_{620}) colours, and spectrum form (dA) of Rara Neagră wine with different fining agents.

The red-colored phenolic components (A_{520}) ranged between and 39.37% and 39.83% compared to the control sample – 38.89%, which demonstrates unsignificant statistical changes of red-coloured components (i.e. anthocyanins).

Regarding the spectrum form (dA), which describes the proportion of the red color, produced by flavylium cations of free and bound anthocyanins, from Figure 4 we can notice a 10.6% decrease in case of gelatin fining treatment and 5.5% lower values for wines treated with vegetal proteins. The spectrum form (dA) of red wines has values ranging between 40 and 60% for young wines, which implies that the higher the value, the more predominant the red color of the wine is. Normally, in red wines, the concentration of total phenolic compounds can be mostly up to 2.5 g/L [10]. More than 200 phenolic compounds have been found, and they are thought to be the fundamental ingredients of wines. Flavonoids and nonflavonoids are the two main phenolic groups found in grapes and wine [1,2].

From Figure 5 it can be observed that, through the Rara Neagra wine samples treated with different types of fining agents, the highest amount of total polyphenolic content is in the sample treated with pea protein Clear-V (787.37 mg GAE/L), and it decreased the most in the sample treated with gelatin ErbiGel (642.24 mg GAE/L).

The obtained results confirm a variation in the polyphenolic content amongst tested wines, which can be explained by the different technological treatment applied, as expected. Comparisons with other data of wines from Moldova and Romania is difficult since only phenolic content, antioxidant proprieties and chromatic characteristics of Rara Neagra wines are available [41-45], but no information regarding influence of plant derived fining agents on chromatic profile of the wines, except studies on other varieties [15-19,20,22,26]. However, the obtained concentration ranges are in agreement with the values reported in available literature [41-46], i.e., numerous factors, including variety, harvesting method, winemaking, meteorological and ecological factors, influence the variation in total phenol content.

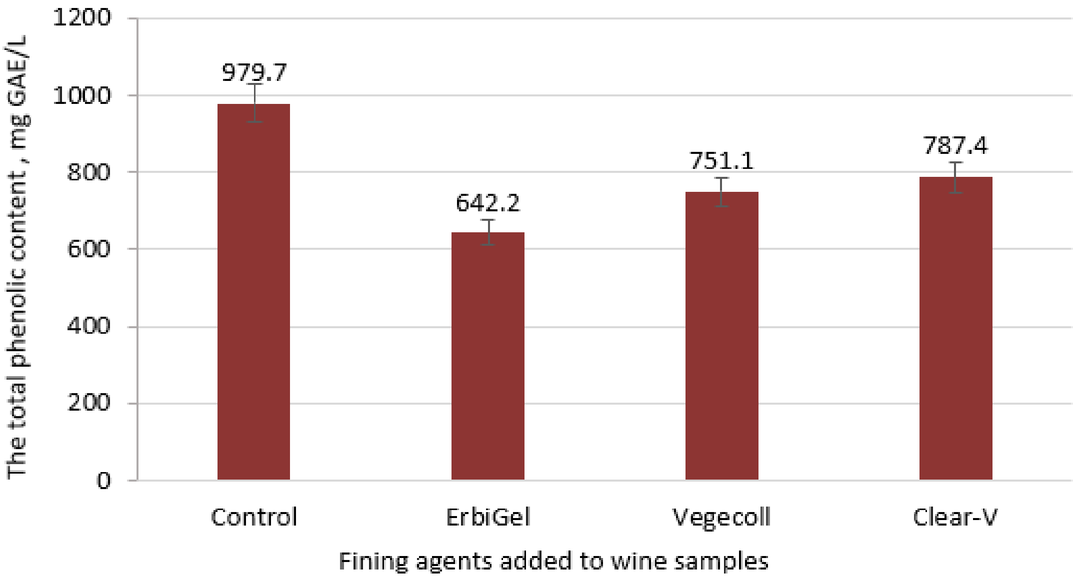

Figure 5. The polyphenolic content of Rara Neagră wine treated with different fining agents.

Analyzing the average data obtained for the total phenolic content in Rara Neagra dry red wine, the biggest difference is recorded in the case of the sample treated with gelatin -34.4%. In the samples treated with adjuvants of vegetal origin, there is a lower decrease: -23.3% in the case of patatin and -19.6% in the case of pea protein.

Gathering the color of red wines is a much more complex process compared to the process of the white wines; not only the flavones, phenolic acids and tannins participate in obtaining the color, but also important and diversified amounts of anthocyanins. The most important are the anthocyanins that give the red-blue color of the wines [10,12].

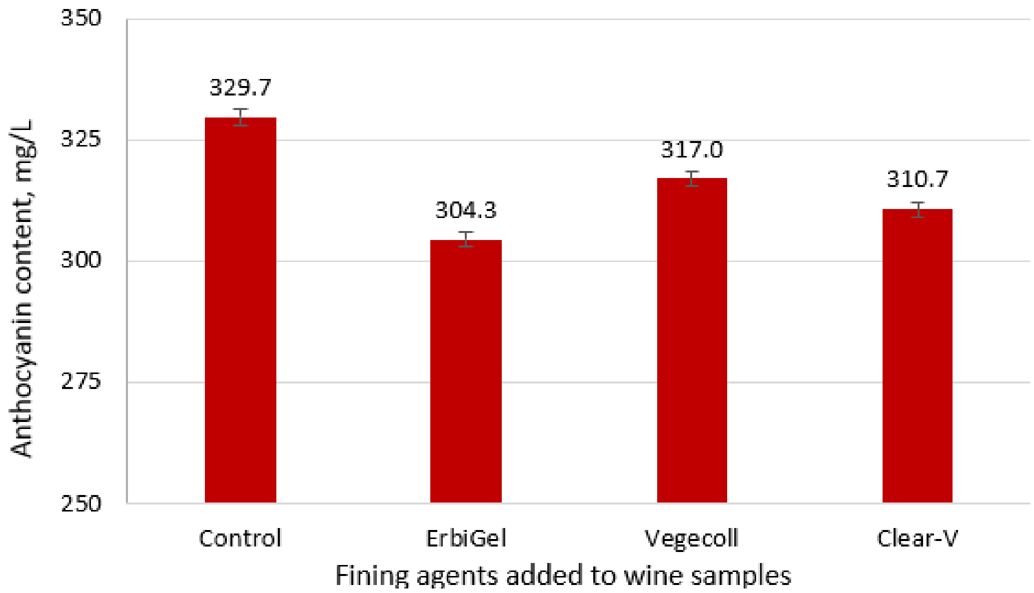

Figure 6. The anthocyanin content of Rara Neagră wine treated with different fining agents.

Anthocyanins, the visible phenolic compounds (pigments), accumulate in grapes skin and give the color of red wines. The Rara Neagra variety is characterized by an average containing of phenolic compounds which can be processed in order to obtain light red wines [44]. From the data presented in Figure 6, we can see that the most significant decrease in anthocyanins was recorded for the version where it was treated with gelatin - by 7.7% compared to the control, meanwhile the additives of vegetable origin showed a smaller decrease of anthocyanins: patatin - with 3.8% and pea protein - with 5.8%.

Previous studies reported Rara Neagra wine to have lower anthocyanins content than wines from other red varieties [41-45], nevertheless all values are within the normal range of anthocyanins (200-500 mg CE/L).

4. Conclusions

As a result of the experimental research, it was found that dry red wines obtained from the Rara Neagra grape variety are characterized by a relatively low content of phenolic substances and, although the wines are young, an intensive oxidation reaction took place with the accumulation of oxidized compounds. The treatment with protein agents of various origin had a positive impact on the structure of the polyphenolic complex, the treated wines being described by a more advanced content in anthocyanins and a lower content in oxidized and condensed compounds compared to the control sample.

The wine samples physicochemical parameters didn't show significant deviations and the organoleptic analysis demonstrated that the average scores obtained by the treated wines varied depending on the clarification agent used, the best score was given to the sample treated with gelatin, while the lowest score was given to the sample treated with patatin.

Potato and pea protein extracts were more protective in reducing the total polyphenol content of Rara Neagra wines compared to gelatin. Treatments with these two plant proteins showed a lower decrease of total anthocyanins content and slightly decreased the color intensity similar to other proteinaceous fining agents. Meanwhile, although it was stated that fining treatment doesn't have significant influence on the color intensity, an important decrease is noticed for the color hue, which mean that fining treatments reduced the compounds that give orange reflections to wine (i.e. flavones, tannins), giving the wine a bright red color due to the anthocyanins.

Overall, results showed that potato protein and pea protein could be used as effective fining agents alternatives to animal proteins such as gelatin, and their effectiveness should be researched in different variations depending on the chemical composition or variety of the wines.

Acknowledgments: The research was supported by Institutional Project, subprogram 02.04.05 Optimizing food processing technologies in the context of the circular bioeconomy and climate change, Bio-OpTehPAS, being implemented at the Technical University of Moldova.

Conflicts of Interest: The authors declare no conflict of interest.

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Citation: Vladei, N.; Arseni, A. Influence of Potato and Pea Protein Fining on the Chromatic Profile Features of Rara Neagra Wine. *Journal of Engineering Science* 2024, XXXI (3), pp. 117-129. https://doi.org/10.52326/jes.utm.2024.31(3).10.

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